THE TOXICITY OF BILE

O. H. HORRALL

Department of Physiology, University of Chicago

This review of the literature has been undertaken to help the investigator and others interested in understanding the relation of bile to disease. An effort has been made to point out the principal contributions to this subject and to afford a list of references which may aid those interested in this kind of experimental work.

Much has been written concerning bile, even from the time of the earliest known writings. Hippocrates, Galen, Paracelsus and a multitude of others have sought to solve the most vexing of medical problems. There is today a very definite need for real intensive and extensive work in this field.

1. Bile acids and their salts. It is rather difficult to compare the relative toxicity of various constituents of bile, since all have not been isolated in chemically pure state. Many of the bile acids have been prepared in extremely pure form, but taurocholic acid has not. Bilirubin is the only pigment that has been isolated pure. Cholesterol, lecithin, glycocholic, taurin, and mucin have all been prepared in a very high degree of purity. Some substances have been purified in minute quantities but are not available for experimental purposes in sufficient quantities.

For almost a hundred years, there has been a controversy as to which substance in bile causes the toxic symptoms. In 1840 the toxicity was attributed by some investigators not to bile itself but to "impurities" which caused capillary thrombi. Bouisson (9) 1843, made parallel experiments using filtered and unfiltered bile. He reported that filtered bile was non-toxic. Then the whole question as to the toxicity was in doubt when Henle (38) 1847, wrote his Pathology. But in 1848 Strecker (97) discovered the bile acids and immediately investigators attributed all the toxicity to them.

Beginning with Frerichs (31) 1858, and followed by De Bruin (18), Bouchard (7), Lugli (50), Plaesterer (71), Bowler (10), King and Stewart (49), Prevost and Binet (73) all attributed the greater toxicity to biliru-
TOXICITY OF BILE

Practically all of these men worked with impure chemicals or drew the conclusion from indirect work, such as filtering bile, etc. The discussion of bilirubin will be given in a separate section.

Arrayed on the side of the bile salts are Röhrig (80), Feltz and Ritter (26), Legg (52, 53), Rywosch (84), Greene (33), Eibott (21), Quincke (74), Stadelmann (93), Emerson (22), V. Dusch (20), Biekel (5), Still (96), and Horrall (41-45). From the evidence, the writer believes we can conclude that bilirubin is non-toxic and that the bile acids are toxic.

The toxicity of bile varies directly with its salt content and specific gravity, so gall bladder bile has a greater toxicity than hepatic duct bile or fistula bile.

The relative toxicity of sodium taurocholate and sodium glycocholate cannot be compared scientifically. The former has never been purified, while the latter has. Commercial products of sodium taurocholate vary from 40 per cent to 80 per cent pure; this salt (or acid) breaks up and forms a gummy mass when purified to a much greater degree. So the diverse conclusions may be explained.

Stadelmann (93) considered sodium taurocholate ten times more toxic than sodium glycocholate. He found red blood cells were destroyed by a solution of the former while of the latter gave a comparative result. Feltz and Ritter (25) used the same method and concluded sodium taurocholate more toxic than sodium glycocholate.

Gillert (32) tested the toxicity by intravenous injections in rabbits and found sodium taurocholate slightly more toxic than sodium glycocholate.

Meltzer and Salant (63), in their experiments with frogs, found no difference in the toxicity of the two salts, differing with Rywosch on this point. Emerson (22) standing almost alone, believes sodium glycocholate is more poisonous than sodium taurocholate.

The most extensive work has been done by Rywosch (83, 84). He found the fatal dose for frogs to be sodium glycocholate 100 milligrams, sodium taurocholate 60 to 70 milligrams. Coagulation of blood is inhibited more readily by sodium taurocholate.

Samelson (87) reports coagulation is prevented more readily by sodium glycocholate. Rywosch considers sodium taurocholate acts more energetically on excised muscle of both warm and cold blooded animals. The lethal dose for frogs, determined by subcutaneous injection by Rywosch (83) is as follows:

...
Sodium chenocholate ............................................ 0.05
Sodium choloidinate ............................................. 0.07
Sodium cholate .................................................. 0.08
Sodium hyocholate ............................................. 0.10
Sodium glycocholate ............................................. 0.10

Toxicity determined by concentration necessary for complete solution of erythrocytes by Rywosch:

Sodium chenocholate .......................................... 1/700
Sodium taurocholate ........................................... 1/600
Sodium choloidinate ............................................ 1/500
Sodium cholate ................................................. 1/200
Sodium hyocholate ............................................. 1/200
Sodium glycocholate ........................................... 1/50

Still (96) determined the toxicity of bile salts by injection into the anterior lymph sac of frogs. His order of toxicity is:

1. Sodium dehydrocholate
2. Sodium cholate
3. Sodium glycocholate
4. Sodium desoxycholate
5. Sodium cholate.

Still concludes, from intravenous injections in dog, intra-peritoneal in rat, intra-lymphatic in frog, the order of toxicity is:

1. Choleic acid
2. Desoxycholic acid
3. Glycocholic acid
4. Cholic acid.

Macht and Grollman (59) find cholic acid very poisonous, and hyocholic acid and choloidinic acid less poisonous for the protoplasm of both plant and animal.

V. Dusch (20) assigned the toxicity to the cholate portion of sodium glycocholate and sodium taurocholate.

Bouchard and Tapret (7) measured the toxicity by intravenous injection in rabbits and reported that sodium cholate 0.54 gram is sufficient to kill, and sodium cholate 0.45.

Wieland (102) made comparative tests on excised frog's heart and found the relative toxicity as:

Cholic acid ...................................................... 1/800 solution
Desoxycholic acid ............................................. 1/6400 solution
TOXICITY OF BILE

Also subcutaneous injections of 10 per cent water solution showed desoxycholic acid 8 to 9 times more toxic than cholic.

Wieland also standardized them against red blood cells, observing complete solution:

Oleic acid .............................................. 1
Desoxycholic acid .................................. 1/25 as toxic
Cholic acid ............................................. 1/250 as toxic

Neubauer (66) placed the lethal dose for guinea pig subcutaneous:

grams per kilogram
Sodium dehydrocholate ........................................... 4.4
Sodium cholate ................................................... 0.5
Sodium desoxycholate ............................................ 0.5

Intravenous:

Sodium dehydrocholate:

For dog ...................................................... 3.6 (no toxic effect)
For man ...................................................... 2.0 (no toxic effect)

For hemolysis of red cells in salt solution:

per cent
Sodium dehydrocholate ........................................... 0.63
Sodium desoxycholate ............................................ 0.04

He concludes that in the blood stream the protective action of serum is so high that the hemolytic point would never be reached.

The relative toxicity of the bile acids and bile pigments is yet unknown, according to Bunting and Brown (12), 1911. Choleic acid enteroliths have been reported in seven cases, according to Hellstrom (37). Chemical analysis showed they were mainly made up of free biliary acid, choleic acid. He says they were formed in the intestines. Surely the toxicity of choleic in these cases was decreased in some manner or other.

There are a large number of bile acids which occur in very small amounts in man and other animals or are modified forms of known chemical substances. Their toxicity is unknown.

V. Duseh, Röhrig, and Leyden do not think that the activity of the bile salts is due entirely to the cholic acid part but is increased by the "pairing" process. Sodium taurocholate is four times more toxic than sodium cholate, and sodium glycocholate is four times less toxic than sodium cholate. Therefore, there is some influence due to the
combination of taurin and glycocol and cholic acid; otherwise, all three would have the same toxicity. Rywosch thinks that this combining process modifies the action but not entirely quantitatively nor qualitatively.

2. Taurin. Taurin is an important amino acid which combines with cholic acid, forming taurocholic acid. It may be found free in the bile, blood, or urine. Taurin was prepared by Schmidt, Adelung and Watson (89) from the abalone. They injected it subcutaneously and intravenously in rabbits and man. Taurin, 3 grams, 6 per cent in Ringer's solution was given to one man intravenously; to another they gave 10 grams subcutaneously, 10 grams intravenously, and then 10 grams by mouth. The doses were given three days apart. To another he gave 5 grams by mouth. Humans exhibited no toxic symptoms, of any kind, however the taurin was given. They also found that rabbits receiving large amounts intravenously did not exhibit any toxic symptoms. Röhrig (80), V. Dusch (20), Koloman Müller (64), and Frederickq (30) also found taurin non-toxic.

Salkowski (86), 1873, obtained what he called “taurin” from ox gall and found it somewhat toxic. He also gave 5 gram doses to rabbits and caused diarrhea. Rabbits are more sensitive to taurin than dogs and men.

Feltz and Ritter (27), 1875, injected taurin, 6 grams intravenously, in a 7-kilogram dog and found it non-toxic. Most of the taurin was eliminated by way of the kidneys without change, especially when given by mouth.

Macht, Grollman and Hyndman (59) found taurin very slightly toxic to plants and lower animals.

3. Glycocol. Feltz and Ritter (27) injected 12 grams of glycocol intravenously in a 5-kilogram dog and observed no toxic effects. This work has been confirmed by Röhrig (79) and Horrall and Carlson (43).

Glycocol was found very slightly poisonous for both plants and animals by Macht and Grollman. By comparison, cholic acid was very poisonous.

Discussion. Green and Snell (34) conclude that whole bile is more toxic than either of its major constituents and they state that this conclusion is at variance to that of Horrall and Carlson (43). More recent unpublished work by Horrall confirms the original conclusions. Whole bile is much less toxic than the same salt content in water solution. A given quantity that kills can be rendered less lethal by the addition of cholesterol.
Meltzer and Salant (62) think that the toxic effect of bile is not due to the sum of the effects produced by known components. It will be necessary, under this conclusion, to know all the constituents of bile, together with the quantity of all these substances; and also it will be necessary for the bile of each species to be tested, in order to obtain comparative results. This, of course, is something to be looked forward to in the future. It is even impossible at the present time to make a direct quantitative test for sodium taurocholate or even for the total cholates. Until recently have we been able to make very accurate tests of only one constituent of bile, namely, bilirubin, by van den Berg test. In man the only acids that we are interested in are the cholic acids and their salts, according to Gillert (32). To be sure, deoxycholic acid, lythocholic acid, and others are found in very small amounts in man. There does not appear to be a definite quantitative way to measure the toxicity of bile chemically. It would appear, from the work of Wieland (102) that merely calculating the cholic acid content of the bile would not give the total toxicity of the bile, for apparently the formation of a compound may cause markedly increased toxicity of the same molecular concentration. This change in toxicity is due to “paarling.”

4. Dehydrocholic acid. Most interesting recent investigations have been made, especially in Germany and Austria, with dehydrocholic acid (decholin). Neubauer (67) gave dehydrocholic acid 2 grams intravenously in a patient with bile fistula; it did not cause any toxic symptoms but did cause an increase in bile flow of four to five times. The dry content of the bile was increased, while the total bilirubin content of the bile was decreased. Neubauer (66) found that dehydrocholic acid is a cholagogue, causes an increase in the dry substance and decrease in bilirubin, increases the blood pressure, has no remote effects, and is a mild diuretic which is not constant in all cases.

Pohl (72) reported dehydrocholic acid as non-toxic. Hoesch (40) gave it to humans intravenously and found it non-toxic and of therapeutic value.

Düker (19) found decholin caused human bile from fistula to be markedly increased and also observed increased peristalsis of the gut.

Rahmlow and Ritterband (76) gave 2 grams in 10 cc. (20 per cent solution) as the usual dose for a human. In one case he gave 4 grams in a single dose. In another 2 grams once a day for three days intravenously. This caused diuresis in cases of decompensated heart. The heart was not affected and the urine increased two to three times.

Adler and Schmidt (1) found that sodium dehydrocholate is excreted
up to 95 per cent by way of the liver. They also found that dehydrocholic acid is much less toxic than deoxycholic acid and that it acts as a cholagogue by its action on the bile passages. They question the mode of action as a stimulus of the vagus nerve. The bile salts are increased while the bilirubin is diminished and it acts as a disinfectant by inhibiting the bacterial growth.

Hoesch (40), Neubauer (66, 67, 68), Pohl (72) and Lebermann (51) concluded that dehydrocholic acid is non-toxic and that it is useful as a practical therapeutic agent in man in the place of the common bile acids. It is also useful for physiological and pathological investigations.

5. Bilirubin. Bilirubin is the name applied by Stadeler, 1864, to the principal bile pigment. It was previously isolated and called "cholepyrrhin" by Berzelius (4), 1840. He worked out the empirical formula. It was later called "biliphaein" by Simon (91) 1845. Many chemists have done much work on this pigment.

Virchow (101), 1847, identified bilirubin crystals in an echinococcus cyst of the liver and noted the similarity of these crystals to crystals of hematoidin, thus connecting bilirubin with hemoglobin.

It was not until 1874, Tarchanoff (98), that bilirubin was injected intravenously. He injected bile pigment into dogs with gall bladder fistula but did not report whether or not the substance gave a toxic reaction. Nothing is known about the purity of his bilirubin.

Apparently Frerichs (31), 1858, injected intravenously bile salts and bile pigment but just what these substances were is not explained chemically. "Bile pigment is obtained by the action of alcohol upon dried blood." The crystals are unstable and cannot be re-crystalized, hence this mixture surely was not pure bilirubin or even bile pigments.

There has been much discussion concerning the toxicity of bilirubin and also the relative toxicity of bilirubin and the bile salts. Most of the conclusions were arrived at indirectly and very few workers used chemically pure bilirubin. King and Stewart (49) said "the cost precluded" its use, so they used logic instead. We have then a whole array of investigators arriving at conclusions that bilirubin is toxic and also that bilirubin is more toxic than the bile acids, as: Bouchard, De Bruin, Lugli, Frerichs, Plaesterer, King and Stewart, Prevost and Binet, and Bowler.

On the other hand, we find many who concluded that bilirubin was either non-toxic or relatively much less toxic than bile acids. Few of these investigators worked with chemically pure bilirubin, while many used fairly pure products. The following believe that bilirubin is
harmless or relatively much less toxic than bile acids: Köhrig, Legg, Feltz and Ritter, V. Dusch, Bickel, Rywosch, Leyden, Greene, Eilbott, Quineke, Stadleman, Still, Horrall and Carlson.

Bouchard, with Tapret, injected intravenously into rabbits bilirubin dissolved in a little soda and found that 0.05 gram kills. Then he says (7, p. 226) that 5 centigrams of bilirubin kills 1 kilogram of living matter. He does not state how much soda was used nor its concentration. From that, he calculates that bilirubin is ten times more poisonous than bile salts. He does not explain how the bilirubin was prepared, nor the degree of purity. He says that this quantity per kilogram when given intravenously kills “with safety.” From this he calculates that for a human weighing 60 kilograms the lethal dose would be well within three grams. Bouchard and Tapret say that 5 cc. of water solution of bilirubin for a 1-kilogram rabbit will cause death. This is a very interesting observation in view of the fact that bilirubin is practically insoluble in water. They also arrived at the conclusion that bilirubin is toxic because bile filtered with animal charcoal until decolorized has a greatly diminished toxicity, and they concluded that the only thing that is removed from filtered bile in this manner is the bile pigment. Bile thus decolorized is only one-third as toxic as that not decolorized. The present writer observed greatly diminished specific gravity and toxicity due to filtering with animal charcoal, bile or pure bile salts.

Lugli (58) removed the pigment from bile with animal charcoal and concluded that the colorless bile is four times less toxic than ordinary bile. He says that bilirubin is the most poisonous constituent of bile. He has not worked with pure bile pigment but draws his conclusions from the fact that charcoal removes “only” bile pigment and the deeper the color of the bile the more toxic it is.

He does not appear to notice also in his own tables that the specific gravity is increased in proportion to the increase in color of bile. Such a plain oversight is almost unpardonable and certainly brings a question upon all such work.

Bouchard says the tissues also play a protective rôle; they consume and transform the minute proportions of bile which have been absorbed, having penetrated the general circulation “they fix the bilirubin.” Bile in its entirety passes from the liver into the blood from the biliary cells to the blood vessels. Bile freed from coloring matter loses part of its toxicity; therefore, when the tissues take up the pigment they remove some of the toxic substance from the circulation; at the same time, bile salts escape by way of the kidneys or are “consumed in the blood.”
all bile secreted in eight hours were suddenly introduced into the blood it would produce "fatal nervous effects" (question of thrombosis and emboli). But if introduced slowly nervous accidents are averted. In black jaundice the absorption is slow and not poisonous, while in green jaundice the absorption is poisonous. With all the conclusions Bou- 

chard comes to, he does not give a single reference to the method of the action of the bile pigment on the tissues. He does show, however, how bile salts act deleteriously.

De Bruin (17, 18) tested bilirubin on an isolated frog's heart and concludes that its action is a little more intense than sodium taurocholate or sodium glycocholate. In other articles, he concludes that bilirubin is four to five times more poisonous than bile salts, while in the last article he tells of giving bilirubin intravenously to rabbits in quantities varying from 0.025 to 0.103 gram per kilogram and concludes that it is toxic. He thinks the action is principally on the central nervous system, causes convulsions, decrease of blood pressure, fall in pulse rate, dyspnecic breathing, increased salivation, and obstipation.

Lugli (57, p. 310) says that of the components of bile bilirubin possesses the greatest toxicity. This is proved by the decolorization of bile, with intravenous injection and by direct experiments with pure pigment dissolved in alkaline solution similarly injected.

Rywosch (84) gave bilirubin (Merk) subcutaneously 0.6 gram to a rabbit weighing 700 grams and observed a minimum disturbance (0.6 sodium taurocholate kills).

Greene and Snell (34) injected 2 mgm per kilogram intravenously into a dog and found a 0.1 per cent solution caused the blood bilirubin to increase, also the bile bilirubin and bile acid to increase. The greatest amount of increase appeared two to three hours following the injection.

Eilbott gave intravenously 0.07 gram in 108 different individuals. In six of the first 30 cases there was a slight reaction. This bilirubin solution was made up several hours before injection. On observing a change in color, they proceeded to make up the solution and inject it immediately. In the following 78 cases he did not notice any toxic reaction of any kind. He was using the bilirubin to test liver function. This was confirmed by von Bergmann.

Legg concluded that bile pigment is entirely harmless.

Quincke injected bilirubin in an alkaline solution 1 to 100 subcutaneously in dogs, rabbits, and mice and observed no effect except staining of the tissues. He concluded that bilirubin stains living connective
tissue cells and intracellular fibrils but in the dead tissues it stains muscle, fat, and blood vessel walls.

Verzar and Zih (99, 100) gave bilirubin by mouth 0.5 to 10.0 mgm. and observed no toxicity. Red blood cells and the hemoglobin were increased. They gave 3 mgm. intravenously to rabbits and observed no toxic effects. They gave bilirubin by stomach tube and observed no toxic effects.

Feltz and Ritter (27) injected intravenously in a 10-kilogram dog on the first day bilirubin 2 grams in alkaline solution; on the second day the same quantity; on the third day 3 grams; on the fourth day 4 grams. There was no toxic effect and they concluded the substance was non-toxic except that the temperature increased about one degree in one hour and was normal after six hours. The urine contained bilirubin, the animal was jaundiced, and there was an obstinate constipation. They (26) again injected pigment intravenously 3 to 4 grams in a dog and noticed no effect. The purity or method of preparation of their "bilirubin" was not given, but they state that they obtained the bilirubin from blood by alcohol extraction. Bilirubin is only slightly soluble in alcohol, so their product certainly was not bilirubin.

Plaestcrer injected bilirubin intravenously in rabbits, frogs, and mice in the same dose (0.1 to 0.004) as Bouchard used and found it was toxic. He also classed bilirubin as a toxic substance because of its action on the frog's heart. He obtained the bilirubin from ox gallstones. Autopsy revealed blood in urine and intestinal canal and thrombosis of gut vessels (very high grade).

Thus an intravitam coagulation of peripheral vessels due to strong sodium carbonate solution.

Naunyn (65) injected bilirubin, isolated from gallstones, into rabbits, 0.1 gram in a weak soda solution, by way of the stomach and by way of a fistula of the small intestine into the gastro-intestinal tract and concluded that bilirubin had no effect on bile or bile pigments in the urine. He does not mention any toxic symptoms. The purity of his product is questioned since he did his work in 1868.

Stadelmann (93) prepared bilirubin from human gall stones and injected this intravenously in doses of 0.2 to 0.4 gram each. Similarly, Rywosch (83) injected the same quantity in rabbits and also found it non-toxic even to 0.7 gram. Stadelmann (94) concludes from his researches and observations and daily practice with icterus, that the bile pigments are not very poisonous.
It would take an enormous quantity of human gallstones to yield that much pigment, as most human gallstones contain very little bilirubin.

The normal blood plasma in man has a yellow color, which is due to bilirubin. The blood plasma of the horse is very highly colored with bilirubin. The normal bilirubin content in the human serum varies from 0.2 to 1.0 mgm. per 100 cc. of blood serum.

Bauer and Spiegel (2) conclude that the bilirubin content of the blood in a normal condition varies according to individual differences but remains ordinarily fairly constant. They say that bilirubin may be increased in the blood serum due to obstruction of the outflow of bile, heart weakness, congestion of the liver, and traumatic hemothorax. There is a slight increase in kidney disease, tuberculosis, cachexia of carcinoma and in cachexia of inanition. They find that when the concentration of bilirubin in the blood is over 1 to 50,000 the bilirubin goes over into the tissues and urine (also 35). They report one family in which two girls had a fawn tint of the skin with high bilirubin concentration of the blood serum, averaging about 1 to 100,000 and they called this condition "biliary diabetes." The bilirubin content of the blood serum normally varies from 1.2 to 2.6 mgm. per cent, while the kidney threshold is about 2 mgm. per cent. In hemolytic icterus, pernicious anemia, and cirrhotic atrophy of the liver, the blood serum bilirubin may increase to 3 to 5 mgm. per cent.

Emerson (22) came to the conclusion that bilirubin is non-toxic by an indirect method. He claimed he removed 95 per cent of it from bile and injected the remainder intravenously. He obtained bilirubin from human gall stones dissolved in alcohol. He does not say whether he injected this bilirubin in alcohol solution or not, but according to Orn-dorf and Teeple (69) it is questionable as to how much bilirubin he would get into solution. There are too many loopholes in this technique to depend on the conclusion at which he has arrived. His conclusions are unwarranted.

McMaster and Elman (61) fed pure bilirubin 100 mgm. by mouth to dogs and also intubed it into the duodenum. They got an increase in hepatic bile but do not mention any bad effects. However, Blankenhorn (6) says that bilirubin is not absorbed from the intestines by the portal system and only small amounts by way of the lymphatics.

6. Erroneous idea of detoxification of bilirubin. Several investigators have assumed that bilirubin is toxic and inject calcium intravenously to detoxify the bilirubin. Bowler (10) concludes that calcium administered intravenously combines with and detoxifies the circulating bile
pigments. The idea that calcium detoxifies bilirubin probably came from the work of King and Stewart (49), who never worked with bilirubin because it was too costly. They arrived at their conclusions indirectly—from decolorized bile. Bowler states that jaundiced dogs required double the amount of calcium chloride given intravenously to cause death than the unjaundiced dogs required and that the blood calcium in each case was equal at death.

Bowler and Walters (11) find that the blood calcium in jaundiced animals is normal. Of course, this involves the question of available calcium.

The van den Berg test was the first test to give us an accurate idea as to what is going on in blood serum. The test appears to be almost specific for bilirubin.

As to just what happens in jaundice, there is some question. We know from the above tests and from clinical observations that there is an actual increase of bile pigment in the blood serum and body tissues, but clinically the appearance of the coloring matter does not seem to be parallel with all the toxic symptoms. In the work of Horrall, and Horrall and Carlson with mice, pups, rats, and dogs, with injections under anesthesia and without, of varying quantities of bilirubin, spectroscopically pure (see 90), intravenous subcutaneous, and intraperitoneal injections and also in heart-lung preparations in dogs, they arrived at the conclusion that bilirubin was entirely non-toxic. This work was supported by later investigations by Still.

7. Biliverdin, biliprasine, bilifuscine, and bilihumin. Rywosch (84) says that biliverdin (Merk) has no bad effect on the blood or heart.

Feltz and Ritter (27) gave bilifuscine and bilihumin about 4 grams intravenously and said it was non-toxic and similar to bilirubin. They also gave biliprasine intravenously to dogs 10 grams in one dose and found it non-toxic and having about the same effects as bilirubin. King and Stewart (49) claim to have worked with biliverdin but gave no test of purity.

These statements are particularly interesting because, as far as the writer is able to ascertain, biliverdin, bilifuscine, bilihumin, and biliprasine have not yet been isolated in chemically pure form. The writer has been unable to find a method for their preparation and has been unable to purchase them. I presume they used extremely crude products.

The beautiful coloring of egg shells is, in most birds, due to the bile pigments. In some birds crystalline bile pigments are excreted. If
these were toxic there should be some indication of their action along the passage.

8. Urobilin. The usual mode of formation of urobilin is from bile pigment in the intestines, but in disease it may be formed elsewhere, as in the liver cell, or in the bile passages, according to Wilber and Addis (104, 105). It is normally reabsorbed from the intestines and part of it may be used again in the formation of hemoglobin and part is excreted by way of the kidneys and liver. The latter channel admits it to the bile.

McMaster and Elman (61) fed 100 to 500 mgm. by mouth and noted no toxic effects. There is no evidence that urobilin is in itself toxic. Its appearance in unusual places or amounts indicates pathologic conditions somewhere.

This record is made to rule it out as one of the possible toxic constituents of bile.

9. Cholesterol. Cholesterine was the first substance discovered in bile. Conradi (l.c. 78) found it in 1775 but Chevreul (13) named it in 1816. It is difficult to get cholesterol in solution suitable for injection. It is insoluble in water but is soluble in hot alcohol, ether, chloroform, and some fats; all of these solvents are of themselves very toxic when injected intravenously. It is soluble in bile salt solutions, being present in normal bile in varying concentrations ranging from 0.5 to 50.0 per cent, Schafer, (88).

Austin Flint (29), 1862, thought that blood on passing through the brain took on two to three per cent cholesterin and gave it off in the liver to the bile. He believed that the symptoms of nervous disturbances were associated with jaundice and that the accumulation of cholesterin in the blood hindered the action of the brain, thus explaining the nervous disorders. He thought it was non-toxic. Injection of cholesterin, however, was not made by Flint, on account of the insolubility of cholesterin in the various media.

Röhrig (80), 1863, found that cholesterol had no effect on the heart and was non-toxic. Bouchard (7) criticized the cholesterin injections and pointed out that soap and water or potash mediums would of themselves kill. He thought cholesteremia induced experimentally by various processes is too questionable to be considered seriously. He found cholesterol in atheromatous abscesses which were wide open in the aorta of old people. The almost pure cholesterol content of these abscesses was several grams and there was no indication of poisoning. Cholesterin dissolved in glycerine caused toxic symptoms but Stadel-
mann (93) attributed these to the glycerine. He thought the injection of suspensions caused emboli, but Fasiani (23) injected slowly 1.75 grams in 175 cc. suspension without producing any poisonous effects.

Pages (70), 1860, injected cholesterol in soap solution intravenously in dogs and obtained negative results. In the course of sixteen days he injected 2.57 grams of cholesterol in one dog. In the first experiments he used ether as the solvent. Koloman Müller (64), 1873, concluded from the literature that bile salts or cholesterol could not cause cerebral symptoms and that they could not cause cholemic intoxication; nevertheless, he concluded that cholesterol is toxic. He made an emulsion of cholesterol, glycerine, and soap solution. He injected cholesterol 0.45 mgm. (8 cc.) intravenously into nine dogs, which caused coma and death in 56 hours. He concluded that the nervous symptoms were similar to those seen in jaundice and were due to hypercholesterolemia. Of course, his results are of no value for he injected solid particles into the blood stream and glycerine itself given intravenously in the same amounts would cause death.

Feltz and Ritter (27) injected cholesterol 0.025 to 0.8 gram into a dog in "Nouvelle" solution and repeated the injection six to eight times in a few days. They found cholesterol non-toxic. They thought emboli were formed, however, if a large amount of the solution got into the blood stream.

Chomjakow (14) dissolved cholesterol in oil of almonds, making a 5.0 per cent solution. Intravenous injections were made in cats; death occurred immediately from infarction of the pulmonary artery or the animal survived without any symptoms. Kausenstern (50) made a 3 per cent solution of stearin soap and then added cholesterol 0.5 per cent. Daily intravenous injections into dogs of 5 to 45 mgm. of cholesterol did not cause any toxic symptoms.

Cholesterol, 0.5 gram, was dissolved in "solvin" 2 cc. with water, same amount, and injected subcutaneously into a dog by Rywosch (83). Solvin is non-poisonous. He observed no effects. Danilewsky (16) applied cholesterol directly to the heart muscle of the frog at room temperature and found that 0.001 to 0.003 per cent in Ringer's solution caused stimulation. Atropine, muscarin, and curare do not prevent the action of cholesterol. The hemolytic action of bile salts is not influenced by cholesterol according to Bayer (3) and Roger (78) although it does prevent the hemolytic action of a number of substances. Pure cholesterol mixed with bile and bile salts caused slight diminution in the toxic effects of the bile acids. Intraperitoneal or intravenous injection
of the mixture likewise slightly inhibited the toxic action according to Horrall (45). Blood cholesterol is increased in icterus, due to retention, Hewlett (39). In Xanthelasmas there is probably a hypercholesterolemia, but no one has shown conclusively that it causes any of the toxic symptoms. It would appear to be the retention of a product that is normally excreted in the bile in quantities of 6 to 7 grams in twenty-four hours in man. A quantitative increase in excretion from the blood can be caused in normal rabbits by intravenous injections of cholic and deoxycholic acids according to the recent work of Yonemura and Fujihara (106). Cholesterol is interesting because of its association with gallstone formation and its intimate chemical relation to bile acids, Wieland (103). Almost pure cholesterol gallstones are frequently found in man, without causing any toxic symptoms, except mechanically.

There does not seem to be any definite positive evidence that cholesterol is toxic. The symptoms of hypercholesterolemia are indefinite and probably slight.

Mazzeo's (60) experiments led him to conclude that cholesterol neutralizes dysentery toxins in rabbits.

10. Lecithin. Fasiani (23) injected in the vein of a dog 2.0 grams of lecithin without any poisonous symptoms. Bayer (3) reports lecithin inhibits the hemolytic action of bile salts, but in the blood stream the quantity is never sufficient to be of any practical value. Opposing this view stands Danilewsky (15) who finds that lecithin acts as an outstanding stimulating agent on the heart muscle in quantities of 0.001 to 0.005 per cent solution. The writer observed diminished toxicity of bile salts when lecithin was added to the solution before intravenous injections.

11. Cerebrin. Cerebrin occurs in bile in very small quantities. It inhibits the action of bile salts on the erythrocytes in vitro, A. Bayer (3), but probably has no effect in vivo because of its diminutive amount.

Further experiments are necessary to elucidate the rôle of these lipoids in the bile in normal animals and then to determine their toxicity, if any, under pathologic conditions.

Bile constitutes a channel of exit for sterols and much research needs to be done to determine whether they are excretions or of some value in the biliary fluid; very little has been done with them under normal or diseased conditions. (Editorial, Journ. Amer. Med. Assoc., 1927 (88) 1322.)

12. Mucin and pseudo-mucin. Mucin is added to the bile after it leaves the liver and serves as a protection for the biliary passages and
gall bladder. This particular mucin is different chemically from that secreted elsewhere. Intravenous injection is difficult but when added to the bile salts it does not increase the toxicity, but actually decreases it. Mucin in the gut delays absorption of bile salts and prevents bile from acting on the intestinal mucosa, Naunyn (65). Mucin prevents hemolysis of red blood cells, according to Macht, Grollman, and Hyndman (59).

13. Other substances. There are a large number of other substances in the bile. Extensive lists have been compiled, which show marked variations in the constituents both qualitative and quantitative. The analyses for different species of animals show that bile contains many substances not found in the bile of other species. Even within the species, bile in the normal individuals varies considerably. Bile not only contains the substances previously mentioned, but also a large variety of other organic and inorganic substances. A large number of abnormal substances are excreted in the bile, such as bacterial toxins, poisonous metallic salts, and various dyes. Many substances occur in very small quantities and under normal conditions should not markedly modify the toxicity of bile.

Under conditions of extensive metallic poisonings, the toxicity of the bile is probably increased.

An attempt has been made to point out the most evident substances, rather than deal with an almost inexhaustible and yet scarcely known list of bile constituents.

14. White bile. The finding of white bile at an operation was supposed to indicate a fatal outcome; Ritter (77), Judd (48), Rous (81), and Hanot (36) think the term itself is a misnomer. The writer was fortunate enough to obtain “white bile” in two dogs with ligature of the common duct of several weeks’ duration. Injection of the so-called “white bile” did not produce any toxic symptoms.

Many other colors of bile have been observed in various animals. Practically all of these variations in color have been found to be due to variations in the type of bile pigment which is not toxic.

BIBLIOGRAPHY


(2) Bauer, J. and E. Spiegel. Über das Bilirubin im Blute und seine pharma-


(9) Bouisson, F. De la bile, de ses variétés physiologiques, de ses altérations morbidès. Journ. Soc. de méd.-prat. de. Montpel. 1841, iv, 352; 1842, v, 191; 1843, vi, 180, 254, 419.


(14) Chomjakow, M. loc. cit. 50.


(16) Danilewsky, B. Ueber die Wirkung des Cholesterins auf’s Froschherz. Pfliiger’s Arch., 1907, cxx, 181.


(24) Feltz, V. und E. Ritter. (Strausbourg). De l’action des divers prin-


(36) Hanot, V. La bile incolore; alcholie pigmentaire. Semaine Médicale, 1895, xxiii, 197.


*Has extensive bibliography.

PHYSIOLOGICAL REVIEWS, VOL. XI, NO. 2
(50) von KRUSENSTERN, V. Zur Frage über das Cholesterin. Virchow’s Arch., 1875, lxv, 410.
(54) LEYDEN. Leberkrankheiten. Berlin, 1861.
(55) LEYDEN. Beiträge zur Pathologic des Icterus. Berlin, 1866.
(57) LUGLI, A. La Tossicita della bile prima e dopo la legatura della vena porta. Instituto di Farmacologie Sperimentale, 1896, iii, 229.
(64)*MUeller, K. Ueber Cholasterämie. Arch. exp. Path. u. Pharm., 1873, i, 213.
TOXICITY OF BILE


(70) Pagès, H. De la cholestérine et son accumulation dans l'économie. These inaugs. de Strasbourg. 1869.


(73) Prevost et Binet. Recherches experimentales relatives a l'action des médicaments par la sécrétion biliaire et a leurs élimination par cette secretion. Revue Médicale de la Suisse Romande, 1888, viii, 249.


(85) Salkowski, E. Taurins. Chemisches Central-Blatt. 1872, iii, 646.


(92) *Stadelmann, E. Der Icterus und seine verschiedenen Formen. Stuttgart. 1891.


(101) Virchow, R. Die pathologischen Pigmente. Virchow’s Arch., 1847, i, 379.


