Staff Meeting Bulletin
Hospitals of the » » »
University of Minnesota

Bile Acids

Volume IX | Friday, March 18, 1938 | Number 21

STAFF MEETING BULLETIN
HOSPITALS OF THE . . .
UNIVERSITY OF MINNESOTA

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INDEX

Published for the General Staff Meeting each week during the school year, October to May, inclusive.

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William A. O'Brien

I. LAST WEEK

Date: March 10, 1938

Place: Recreation Room
Nurses' Hall

Time: 12:15 to 1:45 P.M.

Program: Movie: "Forest Gangsters"

Announcements
1. Lecture
2. Lecture
3. Guest: Dr. A. W. Adson
4. Endocrinology

Roentgenologic Diagnosis of Brain Tumors
Harold O. Peterson
Clarence P. Truog

Discussion: Clarence P. Truog
Harold O. Peterson
William T. Peyton
A. W. Adson
J. C. McKinley
Carl J. Lind

Present: 110

Authors: Harold Oscar Peterson was born in Dalbo, Minnesota, attended high school in Minneapolis, University of Minnesota (B.S., M.B., M.D.) 1934, Internship at Kansas City General Hospital 1933-34, connected with Pathology Department Glen Lake Sanatorium, Oak Terrace, Minnesota 1928-33. Full time 1930-31. Appointment in Radiology Massachusetts General Hospital 1935-36. Appointment in Radiology Department, University of Minnesota January 1, 1937. Now Instructor of Radiology. Has been especially interested in Neuroradiology. Is administering affairs of Department during sabbatical leave of Head Radiologist Leo G. Rigler.

Clarence Peter Truog was born in Swanville, Minnesota and attended high school there. University of Minnesota (B.S. M.B., M.D. 1929). Internship at Minneapolis General Hospital 1928-29. In general practice at Lindstrom, Minnesota until December 1, 1936 when he was appointed Fellow in Radiology at the University of Minnesota.

Gertrude Gunn,
Record Librarian.

II. MOVIE

Title: "Hawaiian Holiday"

Released by: R-K-O

III. AUTHOR TODAY

Felix Hughes Crago was born in Wheeling, West Virginia, where he attended high school. He took his premedic work at West Virginia University and his medicine at Duke University where he graduated in 1935. He came to the Twin Cities for an internship at Ancker Hospital in St. Paul (1935-36). Since July 1, 1936, he has been a Fellow in the Department of Medicine. His contribution today is one of a series in the jaundice studies conducted under the supervision of Internist Cecil Watson.

IV. ST. PATRICK'S DAY IN THE MORNING

5:40 A.M. was the time selected by Patrick J. O'Brien to make his triumphant entry into the world. On the day of days for all Irishmen and those of Irish extraction there could not have been anything nicer, so Dr. and Mrs. William A. O'Brien, Bill Peg, and Kathleen join with Patrick in thanking you for your expressions of good will.

V. BILE ACIDS

F. H. Crago

Chemistry of Bile Acids

Human bile contains a mixture of different types of bile acids in which the cholic and deoxycholic acids predominate. The conjugated bile acids, glycocholic, taurocholic, glycodesoxycholic and taurodesoxycholic acids are combinations of cholic acid and desoxycholic acid with taurine and glycine. In addition to the above bile acids anthropodesoxycholic acid (chenodesoxycholic acid) and lithocholic acid may also be present. The bile acids are conjugate amino acids, the glycocholic acid yielding glycocoll NH_2CH_2COOH and cholic acid upon decomposition, whereas taurocholic acid gives rise to taurine $H_2N\text{-}CH_2\text{-}CH_2SO_2OH$ and cholic acid under like conditions. The principal cholic acids are (cholic acid $C_{23}H_{36}(OH)_3COOH$) (desoxycholic acid $C_{25}H_{37}(OH)_2COOH$) and (lithocholic acid $C_{25}H_{38}(OH)COOH$).

In human bile glycocholic acid predominates, while taurocholic acid is the more abundant in bile of carnivora. In the dog's bile it is present alone. The bile acids are present in the bile as salts of one of the alkalis, generally sodium glycocholic and taurocholic acid may be obtained in the form of crystals. When boiled with alkalies or acids these acids take up water and undergo hydrolytic cleavage, the reactions are as follows:

$$C_{26}H_{43}NO_6 = C_{24}H_{40}O_5 + CH_2(NH_2)COOH$$

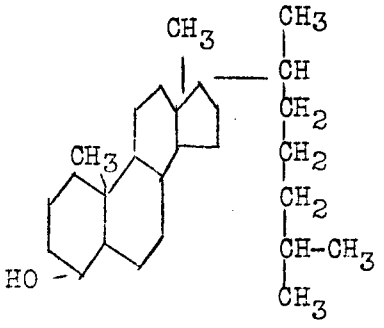
$$C_{26}H_{43}NO_6 + H_2O = C_{24}H_{40}O_5 + CH_2(NH_2)COOH$$

Glycocholic acid — Cholic acid — Glycocol(amino-acetic acid)

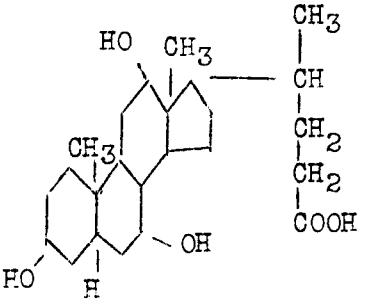
$$C_{26}H_{43}NS\,O_7 + H_2O \qquad C_{24}H_{40}O_5 + C_2H_4NH_2SO_2OH$$

Taurocholic acid — Cholic acid — Taurine(amino-ethyl sulphonic acid)

Cholic acid is a sterol derivative which is closely related to cholesterol. It may possibly be formed from cholesterol by the action of the Kupffer cells of the liver.



Cholesterol



Cholic Acid

Origin and Destruction of Bile Salts

Bile acids are formed solely by the liver cells. It has been shown that if the bile duct is ligated in birds, the bile formed is reabsorbed and bile acids may be detected in the urine and blood. If, however, the liver is completely extirpated, then no trace of the bile acids can be found in the blood or urine. No bile salts are formed in the body after total hepatectomy (Mann & Bollman). Tests for bile salts were made with liverless animals over a longer period of time than is necessary for bile salts to appear in the urine and

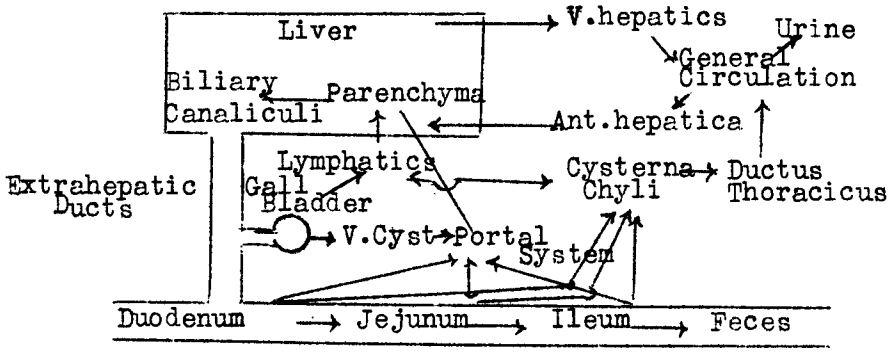
blood following biliary obstruction.

Whipple and Smith fed massive doses of bile salts to fistula dogs which increased the output in the bile markedly. Never, however, was the amount fed accounted for. They believe that there is some mechanism in the liver for destruction of bile salts. Ligation of the common duct results in excretion of bile salts in the urine in constant amounts. This is only one half the quantity excreted by dogs of the same size with bile fistulas. The normal animal must be able to destroy large amounts of these substances since when fed over a long period of time to normal intact dogs does not produce an appreciable increase in the urine and feces. Bollman injected bile salts intravenously into liverless dogs and recovered all in the urine. Nakagawa, Iimuro and Suzuke gave a 20% solution of sodium dehydrocholate intravenously to humans and found that bile salts appeared in the urine if liver damage was present but none of the liver was normal. They suggest this as a liver function test. Smyth and Whipple, and Bollman noticed a great decrease in urinary bile salt excretion after administration of hepatotoxic drugs. Thus we see that experimental evidence points to the liver as being solely concerned with the formation and destruction of the bile salts.

Entero-Hepatic Circulation of Bile

The production of bile acids is a specific function of the liver, but this organ utilizes the bile acids returned to it by the circulation. It is recognized that an additional function of the liver is the conjugation of the bile acids and that in hepatic disease this ability is markedly reduced (Schoenheimer, Andrews and Hrdina ,1932). Similar results were obtained by Doubilet & Colp 1936. The bile acids are resorbed from the intestine, carried back to the liver and partially resecreted in the bile in unaltered form. The introduction of glycocholic acid into the dog's intestinal tract by Weiss (1884), Brevost and Binet (1888) and others always led to the appearance of glycoacid besides tauro-acid in the fistula bile.

The major portion of bile is resorbed during passage through the small intestine. A small fraction, however, escapes absorption, leaves the enterohepatic circulation and escapes in the feces. None, however, escapes in the urine. The resorbed material passes through the villi of the intestinal mucosa, and reaches the portal circulation through the mesenteric veins. It is assumed to be practically completely absorbed from the portal blood flow when passing the polygonal liver cells. It is then resecreted into the biliary vessels together with newly synthesized bile acids. Bile acids, introduced experimentally share the fate of the resorbed bile. A part of the bile constituents may pass from the villi into the lymph spaces and the cysterna chyli, becoming a component of thoracic lymph, and may eventually join the general circulation. Bile acids, however, are returned to the liver under physiological conditions through the portal circulation and are not found in the thoracic lymph (Josephson and Ryding 1936). Bile acids under pathological conditions enter the general circulation and this takes place either through the lymph vessels or from the hepatic vein, when the liver fails to remove the biliary constituents from the portal blood. The rich supply of lymphatic vessels about the gall bladder wall allow bile acids partly to be resorbed together with the greater portion of the water during stasis of the bile in the gall bladder.



Entero-Hepatic Circulation of Bile
Sobotka - Williams & Wilkins Co.

Methods of Estimating Bile Acids

The literature is voluminous with various quantitative methods of estimating bile acids in blood, bile and other body fluids. By and large, the majority of these tests are based on the color produced by the Pettenkofer reaction. As a result of these researches it has been assumed that a positive Pettenkofer reaction given by specially prepared extracts of normal blood is an indication of the presence of bile salts. Aldrich and Bledsoe, on this assumption, found that normal blood contains 2-6 mgm% of bile salts. Perlzweig and Barron and Gregory and Pascoe using the same reaction but different means of isolation deny the presence of bile salts in normal blood. Mylius 1887 found 15 other substances that give a Pettenkofer reaction. However, it is recognized by most present day investigators that the Gregory-Pascoe reaction is specific for cholic acid but that deoxycholic acid, which may be present in large proportion in certain conditions, only gives one half to two thirds the color given by cholic acid.

Josephson (1935) devised a method using barium hydroxide and alcohol and again makes use of the Gregory-Pascoe reaction using the Stufenphotometer of Zeiss. He finds an average of 1.6 mgm% bile acids in normal blood.

Scott (1934) devised a method for the detection of bile salts in body fluids using a new color reaction depending on the action of levulose and pure hydrochloric acid.

Doubilet (1936) gives a differential quantitative analysis of the predominant bile acids present in bile. The total bile acids and percentage of free bile acid may be determined by this method.

Lichtman (1914) determined bile salts in blood, bile and urine based on the hemolytic property of bile salts on sheep cells.

Practically all methods given to date are laborious, time consuming and not generally applicable to clinical use. The question of their specificity in general is also doubtful.

Bile Acids and Metabolism

Cholic, deoxycholic and dehydrocholic acid depress the fasting blood sugar level and prevent hyperglycemia after injection of glucose experimentally (Brugsch and Horsters). Fuziwara and Misaki have shown that injection of bile salts lowers the glucose content of the blood and increases the glycogen content of the liver. Others have found that administration of bile salts lowered the renal glucose threshold.

Wisner and Whipple observed that

osteoporosis occurred in the bones of dogs who lose all their bile through a biliary fistula over a long period of time. Administration of bile salts also led to a greater output of calcium, phosphates and magnesium in the bile. According to Sekito (1930) hypocalcemia in fistula animals may be counteracted by administration of bile.

Experimental injection of cholic acid tends to reduce creatinine excretion. According to Okamura (1929) creatinine excretion is reduced in obstructive jaundice. There seems to be no relation or parallelism between bile salt and cholesterol content in the bile of dogs with biliary fistula.

Bradycardia

For over a century bradycardia has been recognized as a clinical symptom of icterus. Bradycardia is often overshadowed by tachycardia however, due to elevated temperatures in inflammatory conditions associated with icterus. The occurrence of tachycardia after injection of small amounts of bile acids has been reported by numerous investigators. There are two explanations for the bradycardia effect: 1. Possible action on the nervous system, and 2. A degenerative effect on the heart muscle. Wickham and Legg (1884) found an immediate effect of bile acids on the isolated organ and believed this effect was due to action on the heart innervation. For the second explanation Rywosch (1888) found muscles stimulated by dilute solutions of bile salts, while concentrated solutions coagulated the tissue. Schack claims loss of striation of the heart muscle, swelling of the sarcolemma and coagulation of myosin. These effects, however, would hardly play a role under physiological and pathological conditions since highly concentrated bile was used.

Night Blindness and Bile Acids

Visual purple, also called rhodopsin, erythropsin or visual red is the pigment of the rods. It was observed in 1876 that the layer of the rods in the retina during life had a purplish-red color which was bleached by the action of light. The pigment occurs chiefly in the rods and only in their outer part. A solution of visual purple in water which contains 2-5% crystallized bile, which is the best solvent for it, (Kuhne, Ewald and Ayres) is purplish red in color, quite clear and fluorescent. In diseases of the liver in which bile salts are believed to circulate in the blood in increased quantity, the visual purple may be dissolved out of the retina and therefore rod vision impaired. (Starling). No clinical evidence has been offered as to whether this does occur. If it does, this may be the explanation.

Bile Acids and Resorption

There is no doubt that the resorption of free fatty acids as well as that of neutral fat, also of phosphatides and of cholesterol is greatly enhanced by bile acids. The saponification, especially of saturated acids, as stearic acid, is essentially accelerated in the presence of bile salts, and also by a mixture of bile salts and oleate (Pflueger 1901). (Quoted by Sobotka) Bile acids also play a part in the prevention of the formation or precipitation of insoluble calcium and magnesium soaps which occur in the intestine in biliary obstruction and in certain conditions in children (Adler 1927). Fuerth and Minibeck (1931) advance the theory that the bile acids form an adsorption layer on the membranes which acts like a turnstyle admitting the fatty acids through the membrane. The consensus of opinion is that resorption of ingested cholesterol and of cholesterol excreted by the intestinal mucosa requires the presence of bile. Schoenheimer (1924) found the resorption of cholesterol hastened by ingestion of deoxycholic acid, as shown by analysis of the intestinal lipid residue in mice and by the appearance of lipemia in rabbits.

The resorption of fat soluble vitamin A and vitamin D has some bearing on bile fistula anemia. Seyderhelm and Tammann (1927) showed that bile acids are the essential substances which

prevent this type of anemia. However, large doses of vitamin D are likewise effective by making up for the impaired resorption. Greaves and Schmidt (1934) have shown that bile acids are indispensable for proper resorption of calcium.

It has been demonstrated by various investigators that the action of certain drugs, among which may be mentioned strychnine, amidopyrine, phenacetine, sodium salicylate, atropine and strophanthus glucosides, are enhanced greatly by their administration with bile. (Kolda 1926)

Bile Acids and Enzymes

Bile acids have an accelerating action on lipolytic enzymes. Rachford found an 80% increase with glycocholic acid and a 300% increase with bile for the hydrolysis of an oil emulsion with rabbit pancreatic juice. Rosenheim and Shaw obtained 700% increase of the action of pancreatic lipase on olive oil emulsions with cholate and glycocholate. In general this same action has been noted on the other digestive ferments.

The anti-coagulant effect of bile and of bile acids is of special clinical interest. Haessler and Stebbins (1919) found proportionality between clotting time of blood plasma and quantity of bile acids.

Final % of bile acids	0	5	7.4	7.7
Clotting time in minutes	3	14	52	no clot

This effect was not based on prevention of thrombin formation since addition of excess thrombin did not affect the anti-coagulant action of the bile. They believed that clotting time in icterus may depend on the presence of bile acids in the blood.

Deficiency of prothrombin is apparently one of the factors responsible for the hemorrhagic tendency in jaundice. This deficiency also occurs in biliary fistula animals fed on diets deficient in fat soluble vitamins. Most normal animals have a large excess of prothrombin in their blood. The liver may be concerned with the formation or destruction of prothrombin since hepatotoxins have been found to reduce this substance in the blood. Snell and Magath et al have isolated a substance, vitamin K, in crude form from putrified fish meal. This substance plus bile salts when given to icteric patients with increased prothrombin time promptly returns this prothrombin time to normal. Vitamin K alone does not do this. They postulate that bile salts must be present in the gut before vitamin K is absorbed.

Miscellaneous Effects

Extreme concentrations of bile are said to produce marked damage of liver cells (Von Dusch 1854) but Bratianu, Soloman and Bratianu (1933) by large doses of "decholin" in the rabbit produced no toxic effects on the liver cell itself but rather on the canaliculi and the connective tissue. This observation may shed some light on liver damage in obstructive and catarrhal jaundice where bile is being regurgitated. Itching which accompanies obstructive jaundice may be caused by the action of bile acids on sensory nerve endings.

The acute toxicity of bile and bile acids, especially after intravenous introduction, is apparently based on the paralyzing effect on the nerve centers of the heart and of the respiratory apparatus. Under experimental conditions the MLD for intravenous administration lies between 0.1 and 0.4 grams per kilogram of body weight in terms of bile salts or dried bile. The appearance of acute gastric ulcers after toxic oral or parenteral introduction of bile acids is well known. The action of bile acids is antagonized by phosphatides, cholesterol oleate and other lipoids. Tashiro (1931) suspected that a number of agents known to produce ulceration of the gastric mucosa in guinea pigs such as epinephrine, thyroxin and bacterial toxins, reduce the phosphatide content of the blood and thus remove this protection against the bile salts which he assumes to be

circulating in liberal quantities in the blood. Bayer (1908) showed that the bile salts in general have an affinity for the serum proteins in the blood. In obstructive jaundice bile salts would be expected to appear in the urine. This appearance, however, may not occur since they would be held within the circulation by the serum proteins.

Gallbladder Disease

Substance stimulating hepatic secretion are now known as choleretics. Those agents which cause emptying of the gallbladder are known as cholagogs. The most powerful choleretics are the bile acids and their effect is essentially and primarily choleretic. The effect, however, is not purely one of stimulation, but when reaching the liver they contribute starting material for the formation of the bile to be secreted. Whether they exert a cholagog action in addition to their choleretic properties, is not decided. Neubauer (1929), however, states that decholin relaxes the sphincter of Oddi and thus facilitates the bile flow.

Resorption of ingested cholesterol and of cholesterol excreted by the intestinal mucosa requires the presence of bile. Schoenheimer (1924) found the resorption of cholesterol hastened by ingestion of deoxycholic acid.

A rational medical treatment for gall bladder disease may then be based on the above observations. Finkelstein, using oleic acid as a cholagog and bile salts as a choleretic in a series of 25 patients, got a 72% marked or moderate improvement in symptoms. Some patients with previous non-functioning gall bladders by x-ray showed a return of function after treatment. Sterner and Bartle (1931) demonstrated that sodium dehydrocholate (decholin) was non-toxic when given intravenously and could be used with reasonable safety. Rewbridge reported good results and even disappearance of cholesterol stones in the gall bladder following bile salt therapy. He further states that cholesterol stone formation is due to a bile salt deficiency resulting from liver damage.

The oral use of keto-cholanic acid, the oxidation product of the natural bile acids, has been shown by Brown and Dalkart to be far superior to other forms of bile acids. They combine this medication with hourly feedings of milk and cream plus a diet of high smooth bulk in the treatment of chronic gall bladder disease. The high fat diet acts as a cholagog, the bile salts as a choleretic. This was found to be the most effective type of therapy both objectively - x-ray and subjectively.

In chronic gall bladder disease the administration of bile salts appears to have a rational and therapeutic place.

Duodenal and Gastric Ulcer

Spontaneous duodenal or gastric ulcer has been found to occur in dogs with external or internal biliary fistulas. Berg, Blanck, Hanke, Whipple and others report this condition occurring in 40-60% of such animals. Common duct obstruction with a subsequent hepatitis may also give rise to ulcers (Bollmann and Mann, Berg, Hosomi, Iesu and Ivy). Establishment of an Eck fistula often predisposes to the formation of ulcer. Peptic ulcer occurs frequently after partial hepatectomy or following the administration of drugs which damage the liver.

Schnitker, in a review of 100 cases postmortem of peptic ulcer and 100 control cases, found that there were more ulcer cases than control which exhibited evidence of liver damage.

It can be surmised then that although obscure there would seem to be a connection between liver function and peptic ulcer. Berg in a series of 32 patients with proven peptic ulcer found that 81.25% were definitely improved while 18.75% were not improved on administration of bile salts by mouth. Generally no other medicinal or dietary treatment was given. The mechanism of action does not seem to be a lowering of gastric acidity (Layne). The results, although not controlled, are en-

couraging and warrant further investigation.

Bile Salts and Jaundice in the Treatment of Arthritis

Hench (1933) observed a phenomenon experienced by 12 rheumatic patients who had become jaundiced. He noted that in the presence of clinical jaundice there was a marked remission in arthritic symptoms. This observation stimulated others to further investigate the relation of jaundice to arthritis. Thompson, Bernard and Wyatt, by using carefully calculated doses of pure bilirubin, were able to produce experimentally induced jaundice or hyperbilirubinemia not only in the laboratory animal but have applied the method also to man with safety.

The intravenous administration of bilirubin alone, even to a fair degree of jaundice, will not bring about a remission. The oral and intravenous administration of bile salts in large doses also fails to bring about the desired effect. However, when bilirubin plus bile salts (decholin-sodium dehydrocholate) are given, the bilirubinemia is slightly greater and definite remission is found to take place. Mild icterus has no effect. The effect of jaundice is probably quantitative rather than qualitative.

This investigation is still in its infancy and much remains to be learned concerning this therapeutic method which at best should be considered a crude temporary form of treatment. The high cost of bilirubin prohibits its general use. Further investigation may reveal a specific substance probably in the liver which may account for this action.

Impressions

1. Bile acids are formed and also destroyed solely by the liver.

2. Bile acids formed by the liver are excreted in the bile, reabsorbed in the small intestine and returned to the liver by way of the portal circulation. This constitutes the entero-hepatic circulation of bile acids. Bile acids are conjugated by the normal liver.

3. Methods of estimating bile acid concentration in body fluids are discussed. These methods in general are laborious and not generally applicable to clinical practice. The average concentration of bile acids in normal blood is about 1.6%.

4. Bile acids appear to have some effect on glucose metabolism, lowering the glucose concentration in the blood and reducing the renal glucose threshold. Experimental effects on calcium, phosphates, magnesium and creatinine metabolism are also discussed.

5. In chronic gall bladder disease the administration of bile salts has a rational therapeutic basis.

6. In the treatment of peptic ulcer bile salt therapy is encouraging but not conclusive. The relation of liver disease to peptic ulcer has not been adequately explained.

7. Further investigation concerning the effects of jaundice on arthritis is necessary before the specific substance causing remissions is found.

Bibliography

1. Aldrich, M. and Bledsoe, M. S.
Studies on the Metabolism of Bile.
J. Biol. Chem. 77:519, 1928.

2. Adler, E.
1927 Einwirkung v. Gallenss. auf die Loesung v. Ca-seifen.
Arch. Verdauungskrankh 40:174-196.

3. Bratiami, S., Solomon, E., et Bratiami
Action du dehydrocholate de soude sur la cellule hepatique du lapin.
Comptes rendus de la Societe de Biologie. 2: 384-386, 1933.

4. Bayer, G.
Dreislauf d. Galle-
Biol Z. 13:215-233. 1908.

5. Brugsch, H. and Horsters.
Ausscheidung d. Eisens durch d.
Galle. Cholerese und Choleretica.
Z. exp. Med. 38:362-366. 1924.

6. Berg, G. S.
To be published.

7. Bollman, J. L., Mann, F. C.
Influence of liver on metabolism of bile acids.
J.A.M.A. 101:1991 Dec. 1933.

8. Berg and Jobling.
Biliary and Hepatic Factors in Peptic Ulcer: An Experimental Study.
Arch. Surg. 20:997, 1930.

9. Berg and Johnson
Duodenal Ulcers in Dogs with Biliary Fistulas.
Proc. Soc. Exp. Biol. and Med. 25:334; 1928.

10. Blanck, E. E.
Peptic Ulcer: An Experimental Study.
Surg. Gyn. & Ob. 61:480- 1930.

11. Bollman & Mann.
Peptic Ulcer in Experimental Obstructive Jaundice.
Arch. Surg. 24: 126. 1932.

12. Bollman & Mann.
Chronic Duodenal Ulcer in Animals with Eck Fistulas on Certain Diets.
Arch. Path. and Lab. Med. 4:492, '27.

13. Brown, C. F., Dolkart, R. E.
Keto-cholanic acids in the Medical Management of Low Grade Gallbladder Disease.
J.A.M.A. 108:458-460. 1937.

14. Doubilet, H.
Differential Quantitative Analysis of Bile Acids in Bile and in Duodenal Drainage.

15. Doubilet, H. and Colp, R.
Differential Bile Analysis in Various Pathological Conditions.
Proc. Soc. Exp. Biol. and Med. 34: 326-329, 1936.

16. Finkelstein, R. and Lipschutz, E.
The Therapeutic Effect of Oleic Acid and Bile Salts in Diseases of the Biliary Tract.
Med. J. and Record 135: 440-442. 1932.

17. Furth, O. and Minibeck, H.
Gallensaueren im Darm und Fettresorption.
Biol. Z. 237: 139-158. 1931.

18. Fuziwara, K.
Bedeutung der Gallensaure im Kohlen Hydratstoffweschsel.--
Blutzucherkurv unter dem Einfluss non Gallensaure und adrenalin mit oder ohne milzextract bei splenectomierten Kaninchen.
Biochem Z. 256: 384-397, 1932.

19. Gregory, R. and Pascoe, T. A.
The quantitative Determination of Bile Acids by Means of a New Color Reaction, etc.
J. Biol. Chem. 83: 35-, 1929.

20. Horsters, H. und Rothmann, H.
Gallensaeuren und Zuckerstoffwechsel Diabetischer.
Naunyn-Schmiedeberg's Archiv der Pharmakologie und experimentelles Pathologie. 1929.

21. Haessler, H. and Stebbins, M. G.
Effect of bile on clotting time of blood.
Jr. Exp. Med. 29: 445-449. 1919.

22. Hanke, H.
Gastroduodenale Ulcerationem nach totaler Ableitung der Galle.
Ein experimenteller Beitrag zur Frage non Zusammenhangen zwischen veranderungen im Leger-
Gallensystem und Ulcus Arch. f. klin. Chir. 187: 675, 1937.

23. Hooper and Whipple.
Bile Pigment Metabolism.
1. Bile pigment output and diet studies.
Am. J. Physiol. 40: 332, 1916.

24. Hosomi, K.
Uber das sogenannte peptische Geschwur des Magens und Duodenums beim Hunde, das gelegentlich der Choledochusplastik entsteht.
Virchows Archiv. 267: 726; 1928.

25. Hawk and Bergeim.
Physiological Chemistry. 10th Edition.

26. Hench, P. S. and Thompson H. E.
1. Effect of Jaundice on Chronic Infectious Arthritis and on Primary Figrositis.
2. Experimentally Induced Jaundice.
J.A.M.A. 109: 1481-1484; 1937.

27. Iesu, G.
Ulcer duodenali consecutive ad esclusione sperimentale della bile.
Arch. Ital. di Chir. 46: 174; 1937.

28. Ivy.
Factors concerned in determining the chronicity of Ulcers in the Stomach and Upper Intestine.
Am. J. Surg. 11:531. 1931.

29. Josephson, B. and Rydin, A.
Resorption of bile acids from the intestines.
Bioch. Jr. 30: 2224-2228, 1936.

30. Josephson, B.
Determination of Cholic Acid.
Bioch. J. 29: 1935.

31. Kolda, J.
Bile et resorption de certains medicaments.
Comptes rendus de la Societe de Biologie, 1926.

32. Kuhne, Ewald and Ayres.
Untersuchungen aus dem physiol. Institut der Universitat Heidelberg, 1 and 2, and in Zeitschr. f. Biolog. 32.

33. Lichtman, S.
A new procedure for the estimation of bile salts in body fluids based on bile salt hemolysis.
Jr. Biol. Chem. 107: 717-730, Dec. 1934.

34. Layne, J. A. and Berg, G. A.
Unpublished data.

35. Mann, F. C. and Bollman. J. L.
The Physiology of the Impaired Liver.

36. Nakagawa, G., Iumuro, S., and Suzuki, Y.
Gallensaurebelastungsprobe zur Leberfunction prufung.
Klin. Wchnschr. 13: 1392-1394, Sept. 29, 1934.

37. Prevost et Binet, P.
Action des medicaments sur la secretion biliaire.
C. R. 106: 1690-1694, 1888.

38. Pettenkofer, M. J.
Notiz uber eine deue Reaction auf Galle und Zucker.
Ann. Chem. u Pharm. 52: 90, 1844.

39. Perlzweig, W. H., Barron, E. C.
New colorimetric method for the determination of bile acids in blood.
Proc. Soc. Exp. Biol. Med. 29: 233, 1926.

40. Rewbridge, A.
The Disappearance of Gallstone Shadows following the prolonged administration of Bile Salts.
Surgery 1: 395, 1937.

41. Rachford, B. K.
Influence of Bile on the Fat Splitting Porperty of Pancreatic Juice.
Am. J. Physiol. 12: 72-94, 1891.

42. Rywasch.
Ueber giftige Wirkund d. Gallensaeuren.
Arbb. Pharm. Inst. Dorpat 2: 102-140.

43. Schnitker and Hass.
A histologic study of the liver in patients affected with peptic ulcer.
Am. J. Digest Dis. and Nutrition 1: 537, 1934.

44. Sobotka.
Physiological Chemistry of the Bile.
Williams and Wilkins Co.

45. Sekitoo, T.
Einfl d. Gallensaeuren auf d. Calciumstoffwechsel.
J. Biochem. 11:391-406, 1930.

46. Schoenheimer, R.
Resorptionsbeschleumig ung d. cholest. durch Deoxycholsaeure.
Bioch Z. 147: 258-263, 1924.

47. Sederhelm, R. Tammann
Blutmauserung. L. Gallen fistel-anaemia d. Hundes.
Z. exp. Med. 57: 641-656, 1927.

48. Schack.
Ueber d. Wirkung d. Galle auf d. Herz.
Thesis Giessen. 1869.

49. Starling.
Human Physiology, 7th Edition.
Lea & Febiger, Philadelphia.

50. Scott, L. D.
A new colorimetric method for the determination of Biliary Acids in Body Fluids.
Jr. Lab. and Clin. Med. 19: 523-530.

51. Snell, A. M.
Proc. Staff Meetings Mayo Clinic, Vol. 13: p. 65.

52. Tashiro, Shea et al.
Studies on Bile Salts.
Med. Bull. Univ. Cincinn. 6:40-156, 1931.

53. Whipple, G. H. and Smyth, H. P.
Bile Salt Metabolism 4. How Much Bile Salts Circulate in the Body.
Jour. Biol. Chem. 80: 697-707, 1928.

54. Wickham. J. and Legg.
Folgen d. Diabetesstiches nach Zuschnucren d. Gallengaenge, Naunyn.
Schmiedeberg's Archiv der Pharm. und experimentellen Path. 1874.