THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Leo Pacci Bell final oral examination for the degree of Master of ~~Science~~ Surgery in ~~We~~ recommend that the degree of Master of ~~Science~~ Surgery in be conferred upon the candidate.

Minneapolis, Minnesota

May 23 1911

W B Bistrowark
Chairman

F. C. Mann

Wm Carpenter MacCarty.

H. E. Robertson

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Leo Pacci Bell for the Degree of Master of Science in Surgery. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science in Surgery.

W. E. Lahmann
Chairman

J. C. Mann

Wm. Carpenter MacCarty

H. E. Robertson H. E. R.

7-20 2M

T H E S I S

A Study of the Pathologic Phases Encountered During and Following the Relief of Experimental Obstructive Jaundice as Compared to Similar Pathologic Conditions Encountered in Man.

Leo Pacci Bell

Submitted to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the Degree of Master of Science in Surgery.

May 1921.

In reading scientific literature or textbooks written by surgeons, medical men, or laboratory men, the lack of experimental confirmation of the pathologic phenomena of the human body in reacting to disease becomes manifest. The pathologic or clinical conditions as they exist are described in detail but never sufficient explanations as to the underlying cause is offered. Authors of textbooks deal with each branch of a science as a separate entity. Surgeons describe an operation, pathologists a picture of a pathologic condition, clinicians a clinical syndrome, all on one pathologic condition, with little effort toward the correlation of the known experimental facts that may be known to each group. In making this study, I have endeavored to piece together the fragments of experimental knowledge gleamed from literature and my own experiments bearing on the subject of experimental obstructive jaundice and the repair of liver tissue following the relief of obstruction. I have endeavored to correlate the known experimental facts from the biologist, chemist, physiologist, pathologist, anatomist, surgeon, and clinician. I shall endeavor to explain pathologic phenomena as seen in man from these assembled facts.

It is the purpose of this thesis to produce an obstructive jaundice and relieve the obstruction, studying the phases of the pathologic changes by bile poisoning on the animal as a whole and the repair in the liver which takes place following relief of obstruction. Comparisons of the pathologic conditions produced experimentally in the dog in obstructive jaundice and those existing from similar causes in man will be dealt with. Since the metabolic activity of the liver in obstructive jaundice is of the most importance, a careful pathologic study will be made alone on it.

With this purpose in mind the common duct has been ligated

and divided in a series of dogs with the expectation of producing a biliary cirrhosis comparable to that found in man. After a varying period of time cholecysto-duodenostomies were performed to relieve obstruction. A study of the pathological changes in the liver during obstruction and following the relief of obstruction was made.

Cirrhosis of the liver as classified by W. J. Mayo falls into two groups, portal and biliary. He points out that the numerous pathological classifications are simply varying patterns or degrees of two separate clinical conditions of portal and biliary cirrhosis. In dealing with cirrhosis of a biliary origin, it is necessary to review both the literature of cirrhosis and experimental cirrhosis of both portal and biliary types. This is necessary due to the fact that most authors in presenting their conclusions have obtained the results from studies on both portal and biliary cirrhosis as it exists in man and as it is produced experimentally. (1)

Kretz defines cirrhosis as seen postmortem in humans as essentially a combination of focal or localized recurring chronic degenerative processes with regeneration. He believes that the pathological pattern as seen postmortem in man represents the amount of destruction to liver tissue whether bacterial, toxic, or mechanical in origin, plus the amount of repair on the part of the liver to the injury. In a pathological section of hypertrophic cirrhosis, will be seen new degenerative foci, old scars, and new regenerative processes. He considers this picture to be due to numerous attacks or succession of injuries from which the liver has more or less completely recovered. (2)

McCallum, in a careful study of cirrhotic livers has defined cirrhosis as a chronic disease in which the destructive processes, probably often repeated, result in a loss of the functional liver tissue immediately followed by the formation of a scar, the healing process, and later by an attempt at the restitution of the liver to normal by regenerative processes. (3)

Joannovics has worked extensively on experimental liver cirrhosis. He divides the experimental cirrhosis into groups. Group 1. According to the influence of the stimuli or irritation on the liver tissue. The procedures used in this group were tying common duct, portal vein, hepatic vein, injection of inflammatory materials into the common duct and liver parenchyma, and mechanical irritation to the liver surface. (4)

Group 2. Stimuli which are degenerative. Methods used: Poisons given subcutaneously, intravenous or by inhalation. They were namely, phosphorus, arsenic, alcohol, lead, toluylene-diamin, specific immune hemolysin, olieum-pulegii, cocaine, carbamine acid, ammonium carbonate and carbamine acids, chloroform. He found that ammonium carbamine acid, chloroform and toluylene-diamin to be best for the production of cirrhotic processes similar to man. He found that alcohol had no effect. He concludes that the degenerative changes must come in waves or or repeated phases. Destruction of parenchyma tissue is always followed by reformation of connective tissue and regeneration of parenchyma tissue. (4)

Fischler points out the great variety of results in the production of cirrhosis by various methods. He states that division and ligation of the common duct is most constant in the results. (5)

Porri working with the colon bacillus secured from a spontaneously jaundiced dog, produced a type of infective cirrhosis experimentally. He showed that bacterial infection and cirrhosis produces more marked parenchyma destruction than pure obstructive jaundice uncomplicated with infection and the regeneration was more marked in obstructive than infectious jaundice. In infective jaundice the liver destruction is due to deleterious action of bile plus the toxic action of bacteria. (6)

Isobe claims to have produced an alcoholic cirrhosis and a similar cirrhotic process with sulphate of potassium. (7)

Fiessinger and Rowdowska believe that it is impossible to produce an experimental cirrhosis similar to that found in man by ligation of the common duct. Fiessinger produces a cirrhosis by chronic intoxication of liver

tissue by repeated injection of chloroform in paraffin oil over a period of twelve to fourteen months. Toluylene-diamin was also used. He shows that cells ofparenchyma will not regenerate if chronically intoxicated. He also produced a moderate splenomegaly in these animals. (8 and 10)

Fiessinger also points out that biliary cirrhotic lesions are of two types, para and peri biliary. Peri-biliary is more constant and usually appears in ten days. This type of cirrhosis is annular, multilobular, and intralobular in appearance. The only point comparable to cirrhosis of man is the parenchyma tissue destruction. (9)

Lissaner finds that cirrhotic lesions produced by ligation of the common duct are not comparable to that of man. He considers that cirrhosis must be infection plus bile stasis in biliary cirrhosis in man. He believes the liver to have a very high power of resistence to infection and that many factors are included in human cirrhosis. (11)

Richardson has made a very careful study of the liver pathology in rabbits following ligation of the common duct. The studies are made at the times of twenty-four hours, forty-eight hours, one week, two weeks, and one month following ligation of the common duct. He believes he has produced a cirrhosis of biliary type quite unlike that of man. (12)

Malloy divides cirrhosis of man into five groups. 1. Toxic. 2. Infectious. 3. Pigment. 4. Syphilitic. 5. Alcoholic. He bases his classification on the pathological picture seen in livers of man postmortem. (13)

Pearce has produced a marked portal cirrhosis by the use of haemagglutmative serum injected intravenously. It always produces a definite and constant degeneration of the parenchyma tissue. This type of cirrhosis in the amount of destruction of liver parenchyma he considers comparable to experimental chloroform poisoning and acute yellow atrophy in man. (14)

Herterand Williams produced a cirrhosis by repeated inhalations of chloroform in dogs. (15)

In summarizing the data accumulated by these authors on their studies of cirrhotic liver of man postmortem and experimental portal and biliary in rabbits and dogs the following points are emphasized.

1. Many chemical irritants and mechanical means have been used to produce experimental cirrhosis. (24 different technics).

2. The response of the liver in the production of cirrhosis depends on the nature of the irritant or poison and the manner of application to the parenchyma tissue as a whole.

3. To produce a cirrhosis there must be parenchyma cell destruction either mechanical (ligation of the common duct) or toxic (introduced through the portal vein).

4. Experimental cirrhosis in animals is comparable to that found in man, in that there is parenchyma cell destruction, biliary duct proliferation, connective tissue proliferation, and regeneration of parenchyma tissue. The microscopic and macroscopic picture is otherwise dissimilar.

5. Cirrhosis as seen in man is due to a combination of factors. The factors have not been identified as yet experimentally.

6. Cirrhosis in man is due to the attempts at repair of damage done by successive waves of toxins producing parenchyma destruction to the liver substance through the blood stream and biliary apparatus. This chronic process or cell injury is assumed to cover a considerable period of time.

7. Atrophic cirrhosis in man is not due to connective tissue contraction. Compression of liver tissue in hypertrophic cirrhosis is due to new growth of parenchyma tissue.

Fat, immune (to distemper) dogs were chosen for experimental work. The animals were anesthetized with ether and high midline incision made, using sterile operative technic. The common duct was exposed and ligated, care being exercised that duct was ligated and divided below the point of entrance of

the left hepatic duct. (If the duct is not divided, the ligature will cut through and the duct reestablish itself.) Liver specimens were always taken for microscopic study when the abdomen was opened. The layers of the abdominal wall were closed with plain catgut No. 2; the skin with silk. The dogs were allowed to remain obstructed from four to seven weeks before cholecysto-duodenostomy was done. After five weeks any operative procedure is attended with considerable risk. At varying periods of four to seven weeks the abdomen was opened through a high right curved incision and the gallbladder exposed. A trochar was used to allow the bile to flow from the gallbladder. The usual type of cholecysto-duodenostomy as described by C. H. Mayo was done. Blood vessel silk (doubled) and blood vessel needles were used for the cholecysto-duodenostomy. The abdomen was opened at approximately monthly periods to procure specimens of the liver tissue for pathological study. The protocols of five dogs were selected. Microphotographs of the liver tissue at the time of ligation, the time of cholecysto-duodenostomy, one month and two months following relief of the obstruction, are shown on Plates I, II and III, IV, V, VI, and VII. Mallory connective tissue stains were used to show connective tissue changes soudan 111, for fat and heam and eosin for other sections.

1. Dog E 62 ligated twenty-eight days before cholecysto-duodenostomy.

Dog E 88 ligated twenty-eight days before cholecysto-duodenostomy.

Dog E 89 ligated twenty-eight days before cholecysto-duodenostomy.

Dog E 126 ligated forty-nine days before cholecysto-duodenostomy.

Dog E 149 ligated forty-two days before cholecysto-duodenostomy.

Dog	Group I		Group II		Group III		Group IV	
	operation date		operation date		operation date		operation date	
	date	weight	date	weight	date	weight	date	weight
E 149	12-17-20	11.5	1-28-21	7.6	3-2-21	8.4		
E 126	12- 3-21	13.1	1-21-21	9.6	2-25-21	10.		
E 89	11-12-20	8.	12-10-20	8.	1-21-21	10.3	3-2-21	11.4
E 88	11-12-20	9.1	12-10-20	9.3	1-21-21	11.6	3-2-21	10.9
E 62	10-29-20	7.8	11-26-20	6.8	1-28-21	8.	3-2-21	7.8

For convenience of assembling data the liver specimens taken at time of first, second, third and fourth operations on the five dogs will be referred to as group 1, 2, 3 and 4.

Group 1. The microscopic study of the tissue of the five dogs of group one was normal in appearance in staining.

Group 2. The following pathological changes were noted: (1) In a study of the five specimens of liver tissue taken at the time of cholecystoduodenostomy the parenchyma tissue shows varying processes from intoxication to marked destruction. These gradations were shown by mucleolars and cystoplasmic changes. These changes are more marked as obstruction progresses. One is struck with the lack of marked destructive changes in the parenchyma tissue as a whole. (2) Connective tissue proliferation is marked. Mitotic figures and excess of interlobular and perilobular connective tissue is a common picture. (3) Evidence of marked new formation of capillary network. (4) Marked evidence of proliferation of interlobular biliary capillaries. (5) New formation of parenchyma tissue from interlobular capillaries. (This process of budding of biliary capillaries and new formation of liver tissue will be shown with microphotographs, Plates IV, V and VI. (6) Biliary pigment in the peri-biliary spaces. (7) Petechial and massive hemorrhage into the parenchyma tissue.

Group 3. Studies of pathological sections were made on tissue taken from thirty-three to forty-one days following cholecysto-duodenostomy. The findings were as follows: (1) The liver parenchyma shows little evidence of previous destruction. (2) A moderate amount of connective tissue in the peri-biliary spaces. Most of the sections show no marked evidence of new formation of connective tissue. It is mostly older in appearance and e.t. cells smaller in size. (3) The liver trabeculae seems to be invading the peri-biliary tissue and surrounding the biliary capillaries. (4) Many interlobular bile capillaries are seen near the peri-biliary space entirely surrounded by new liver tissue. There is still considerable evidence of new formation of liver parenchyma. (6) Fatty degeneration is still present in the parenchyma tissue. (7) All parenchyma tissue seems to be of new formation surrounding the peri-biliary spaces. The nuclei stain deeply and the cytoplasmic outlines are clear. (8) Fibrous tissue stroma is still more normal in the intralobular spaces. There is little evidence of new formation of fibrous tissue except in the peribiliary spaces. (Note. All of the study on tissue following relief of obstruction cannot be completed due to the fact that this part of the experiment is not finished.)

Previous writers differ somewhat in their findings and interpretations of results obtained from experimental obstructive jaundice. Joannovics emphasizes the fact that biliary obstruction must be sufficient to produce parenchyma destruction to have connective formation in considerable amounts. He considers that factors not met experimentally are present in biliary cirrhosis of man. Parenchyma destruction of connective tissue and biliary hyperplasia is the essential picture.

Fischler points out the consistency of his pathological changes following obstruction.

Porri shows that parenchyma destruction is much greater in obstruction plus jaundice. He described the marked fatty degeneration of parenchyma tissue and shows it by staining.

Fiessinger and Rowdowska describe the pigment infarcts in microscopic sections which are stone-like in appearance. They consider later stages of biliary cirrhosis in a rabbit to be an annular or encystment form of cirrhosis. They consider the very early lesions of parenchyma due to toxicity of bile.

Richardson has made a most careful study of the findings in livers of rabbits under obstruction. The points emphasized in his paper are practically the same as those noted in my experiments before obstruction was relieved. So carefully has he described his work that I have not considered it necessary to repeat the early experimentation under one month in dogs.

In discussing the findings of my experimental data and the data of other men, it would seem that the parenchyma tissue goes through a process of destruction graded from a mild to severe cell injury with fatty degenerative changes before complete destruction takes place. Richardson, Porri, and others have emphasized this fatty change in liver parenchyma. Bell has pointed out the manner in which parenchyma cells are injured. (16) It would seem that the cause of parenchyma tissue destruction is bile pigment poisoning, increased secretory pressure in the interlobular biliary ducts, loss of secretory function of the liver parenchyma in bile production shown by ultimate destruction, as the chronicity of obstruction develops.

Parenchyma destruction depends on the length of time of obstruction. Connective tissue rapidly proliferates following parenchyma destruction and replaces the spaces occupied formerly by parenchyma tissue. This is demonstrated by the mitotic figures and embryonic appearance of the cells. It is considered by most authors to proliferate rapidly immediately following parenchyma destruction. The ability of connective tissue to regenerate apparently is not affected by continued obstruction. Mitotic figures are seen forty-nine days following obstruction. Bile pigment does not intoxicate connective tissue cells. It apparently acts as a stimulant to new growth by irritative action. The bile

pigment apparently acts in a toxic manner on endothelial cells of blood vessels. New growth of blood capillaries can be explained in part by the increase of blood supply to the new formed connective tissue and biliary ducts. This toxicity is manifest by petechial and massive hemorrhage in the parenchyma tissue. The blood as seen in the sections of liver tissue can be expalined as due to congestion of the liver and rupture of the endothelial lining of the walls of the blood vessels due to injury of endothelial cells. The interlobular biliary capillaries of the liver apparently are stimulated to growth by the bile pigment and the increased intrabiliary pressure. In addition there is an unexplained physiological factor which causes the change of interlobular ducts into liver trabeculae as metabolic needs of gland require or parenchyma destruction progresses.

Group 3. Following relief of obstruction complete regeneration takes place rapidly. The rapidity of the regeneration depends on the length of the previous obstruction. The new formed liver parenchyma tissue arising from the undifferentiated interlobular bile capillaries, is pushed outward from the vicinity of the per-biliary spaces and forms in trabeculae with the liver parenchyma undestroyed. (2) The connective tissue undergoes the shrinkage of age, becoming shorter, narrower and flattened. After two months there is little evidence except with high power study of excess of connective tissue in the interlobular spaces. In the peri-biliary spaces it is still present, but only in moderate amounts.

The bile capillaries in the peri-biliary spaces become converted into liver trabeculae. As this process goes on bile capillaries are frequently seen far removed from the peri-biliary spaces. They are completely surrounded by new formed parenchyma tissue in many cases. It is readily seen that thesebile capillaries before surrounded by the growth of parenchyma tissue form the peri-biliary spaces. After two months but moderate excess of biliary

capillaries is seen in the peri-biliary spaces. The blood vessels are also but little in excess. From this I conclude that complete regeneration following obstruction lies in a period of from two to four months following the release of obstruction. The factors controlling complete regeneration are the amount of destruction to liver parenchyma and the length of time obstructed. (Further study following the release of obstruction is yet to be made since my dataisas yet quite incomplete.)

Thompson shows the liver in a 2.5 mm. embryo to be a median ventral outgrowth of the entodermal tube. It is described as the median hepatic diverticula. (17) Bremer describes the hepatic diverticula in a similar manner. (18)

Lewis and Stohr state that the liver first appears in human embryosas a diverticulum of ventral wall of the foregut near its junction to the yolk sac. The hepatic diverticulum is entirely of entodermal origin. Very early the liver becomes divided into two parts. First, a somewhat rounded diverticulum proper, lined with columnar cells having pale protoplasm. Second, a mass of anastomosing cords or trabeculae, composed of deeply staining cells with round nuclei and abundant granular protoplasm. The anastomosing trabeculae later form the parenchyma tissue of adult life while the diverticulum proper forms the gallbladder and common ducts. Interlobular bile capillaries are of uncertain origin, presumably formed by local modification of cell membranes of two adjacent hepatic cells. (19)

Scammon in describing the histogenesis of biliary capillaries and ducts in the selachian liver states that two forms of bile duct formation exist, that of envagination of the liver pouch, and that of transformation of preexisting cylinders of parenchyma tissue into bile ducts. The first and most primitive is more active in the selachians than in the higher vertebrates and forms the major ducts and the proximal parts of the minor ones. The distal parts of the minor ducts are formed by cylinder transformations. (20)

Bensley studying the pancreas of guinea pigs points out that the islands of Langerhans are connected to and originate from the interlobular pancreatic ducts. He considers that the tissue lining the pancreatic ducts and interlobular ducts are of a low order of differentiation, which is capable under proper conditions of producing by differentiation and by mitotic divisions, islets, scini, and mucous glands. This function is also shared in part in the production of islets by the columnar cells of the ducts. (21)

In considering the histogenesis of the regenerated liver, it is necessary to review the work of the anatomic observers such as Marchand (22), Meder (23), Stroebe (24), Barbocci (25), and the experimental workers such as Podwyssozki (26), Ponfick (28), and Von Meister (27).

Repair, according to Marchand, takes place in three ways: First, by hyperplastic proliferation of the larger intact remains of the gland parenchyma. Second, by proliferation of isolated liver cell clumps or remains in the region of the most intense destruction. Third, by proliferation of the interacinous bile ducts which in conjuction with the latter form new liver strands.

Stroebe claims that repair takes place by an ingrowth of biliary sprouts from the interlobular bile ducts toward the central veins of the partially destroyed lobules. He believes that there is a gradual transformation of the epithelial bile duct cells into parenchyma tissue. He denies the views of Marchand on his first and second hypothesis.

Podwyssozki and Ponfick have shown experimentally that in regeneration of liver following destruction or removal of the large part of the parenchyma tissue is formed by mitosis of the parenchyma cells and from interlobular ducts of the biliary apparatus. True hyperplasia was observed on extrapation of large portions of the liver parenchyma by Ponfick and Von Meister.

Barbacci is of the same opinion as Stroebe concerning the views

Marchand. He believes that partly destroyed parenchyma tissue cells will not regenerate.

McCallum working on acute yellow atrophy as found in man finds that the regeneration of the parenchyma tissue takes place in two ways, namely: First, when scattered isolated lobules are left following extensive destruction the hyperplasia of this tissue takes place rapidly and the interlobular bile capillaries are not concerned in the regeneration of the parenchyma tissue. This supports the views of Ponfick on experimental functional hypertrophy. Second, when all, or nearly all, of the parenchyma tissue is destroyed, the bile capillaries rapidly multiply and their epithelium becomes differentiated into parenchyma tissue. This view is more in keeping with the experimental work of Podwyssozki, who finds that granulation tissue forms following removal of portions of liver tissue and that the bile ducts grow into the granulations and form parenchyma tissue by rapid differentiation. (29)

Both Pearce and Richardson have similar conceptions concerning the regeneration of liver tissue.

In studying the repair of liver tissue during and following release of obstruction produced by ligation of common ducts in dogs studied in serial sections, I find that a large amount of repair of parenchyma tissue has taken place in dogs ligated twenty-eight days. There is not the extensive destruction described in early stages by Richardson in his work on obstructed rabbits. There is practically no evidence of parenchyma tissue mitosis in animals obstructed over such length of time as I am describing. The bile pigment poisons the parenchyma tissue to such an extent that regeneration by mitosis is not possible. Richardson found no mitotic figures after twenty-four hours of obstruction in parenchyma tissue in rabbits. The regeneration of parenchyma tissue as seen from periods of twenty-eight to forty-nine days of obstructions is entirely from the undifferentiated cells of the interlobular biliary capillaries. The destruction

of parenchyma tissue takes place from the peri-biliary spaces toward the center of the lobule. Bile capillaries rapidly proliferate and form many buds which push into this destroyed or injured tissue. The differentiated cells form in masses which may form trabeculae immediately and push toward the center of the lobule. The trabeculae may be formed also by the biliary ducts growing outward and losing their lumen and becoming differentiated into the typical parenchyma tissue, which as regeneration continues, unites to other trabeculae to form liver tissue of normal appearance. It would seem that new formed parenchyma tissue has a resistance to cell injury from bile for some time following regeneration. I am in doubt as to the secretory activity of the new formed parenchyma cells. That new formed parenchyma tissue does not secrete bile until obstruction is relieved might account for the gradual loss of the power of the liver to secrete bile as obstruction progresses. It is shown that the new formed parenchyma cells do secrete bile following release of obstruction but I believe that they do not function to the extent that normal parenchyma does when unobstructed. It is, therefore, logical to believe that even though they appear to be normal in function, as seen micorscopically during bile stasis, their secretory powers are quite limited in bile production. The picture seen in some sections following long periods of obstruction is as if the new liver tissue surrounding the peri-biliary spaces were gradually being pushed toward the portal or interlobular spaces as it is formed the peri-biliary region. It appears that the greater part of the lobule had been replaced by new formed parenchyma tissue during obstruction lasting forty-two to forty-nine days. In considering the work of other experimentors it is shown that regeneration is very rapid if there is only one wave of intoxication. The source of repair as shown by McCallum depends on the amount of parenchyma tissue destruction. It is, therefore, probable that no highly specialized secretory cell can undergo mitosis when subjected to repeated or continuous injury. During parenchyma poisoning, therefore,

the regeneration can only be from the bile capillaries during obstructed jaundice. The process of rapidity of regeneration is definitely slowed up as the chronicity of the poisoning continues.

Howell states that the liver is the compound tubular gland. It has an internal secretion of glycogen and urea and an external secretion of bile. Recent observations with staining reagents tend to substantiate the accuracy of Kuppfer's observations and confirm the belief that normally the system of bile ducts begins within the liver cell in minute channels which connect directly with the bile capillaries. The characteristic constituents of bile are the pigments, biliruben in carnivorous bile and biliverdin in herbivorous bile, the bile acids, bile salts, the sodium salt of glycocholic and tanocholic acid. In addition there is present a considerable quantity of mucoid nucleo - albumin, a constituent which is not formed in the liver cells but is added to the secretion by the mucous membrane of the bile ducts and gallbladder, and small quantities of cholesterin, lecithin, fats and soaps. The inorganic constituents comprise the usual salts, chlorides, phosphates, carbonates and sulphates of the alkaline earths. Iron is present in a small amount, probably not in comparison with phosphates. The quantity of bile secreted is 8 to 15 c.c. per kilogram of body weight in man. It is proportionately greater in lower animals. The quantity of bile varies with blood supply and supply of material in the blood from which bile can be made. The quantity of bile is increased by reabsorption from the intestinal tract. Bile, when the common duct is occluded is reabsorbed through the lumphates into the blood stream. This mechanism of the flow of bile into the blood stream is questionable but it is considered to be a rupture of the bile capillary into the lymphatic space. (30)

Barbera shows that bile secretion during fasting is at a minimum; there is little change during hunger; meals of proteins markedly increase bile elimination from the liver. This elimination comes in thirty minutes and

lasts for fourteen hours. A meal of fat is followed by marked increase in bile secretion. It comes in one hour and ceases at the end of twenty-four hours. A meal of carbohydrate is followed by a slight increase in bile secretion which ceases at the end of six hours. He also showed that glucose solutions up to 10 per cent, when given subcutaneously, add no affect on bile secretion. (31)

In considering the mechanism of mechanical obstructive jaundice from a pathologic and physiologic aspect, I am inclined to believe the experimental explanation of Fiessinger,Lyonn and Caen (32) and Simpson and Herring. They believe the mechanics of this procedure to occur in the following manner. They think that during obstruction there is a communication between the terminal biliary filaments in the hepatic cells and the endothelial lymph spaces due to increased intrabiliary pressure and dilatation of the biliary filaments. The endothelial lymph spaces in turn communicating with the blood stream or larger lymphatic channels. The products of the hepatic cell secretion is emptied directly into the endothelial lymph space. (Some authors consider that a rupture of the filaments takes place into the endothelial lymphatic spaces.) During a catarrhal jaundice or cholangitis he believes terminal biliary filaments to be mostly affected and to become swollen, causing an emptying of bile in the subendothelial lymphatic space and thence into the blood stream. He considers that you must have cell destruction before jaundice is possible otherwise.

Herring and Simpson differ in their results obtained by injection of the liver through its blood vessels, from the conceptions of Fiessinger. They find the liver cells permeated by fine anastomosing channels. These channels undoubtedly receive plasma from the blood. They consider that the parenchyma lobule has no lymphatic ducts. They consider it possible that the cells of the lobule form a syncytium and lymph is thus able to pass from cell to cell and empty into the lymphatic at the periphery of the lobule. (33) Herring and Simpson in a later paper have shown that the average maximum pressure of bile

during obstruction is about 300 mm. of bile. Following obstruction the pressure rises rapidly at first but slows as a maximum is reached. The heighth attained is the pressure at which as much bile is secreted every moment as is taken up from the bile paths by reabosrption through the lymphatics. They believe that all obstructed bile leaves the liver by way of the portal lymphatics. I think that the real explanation is a combination of the ideas advanced in these papers- that there is a retrograde dilatation of the biliary filaments which communicate with the lymphatic system. The work of Herring and Simpson is rather conclusive in the structure of these anastomosing channels and their point of emptying into the lymphatic channel proper. (34)

I believe from my experimental data that following longer periods of obstruction the pressure in the common duct and gallbladder is much higher than 300 mm. of bile. This increased pressure is probably due to the precipitation of bile by the mucin of the gallbladder and formation of biliary infarcts as is shown by Rouse and McMasters. (35) They explain the "white bile of surgeons" as an excess secretion of mucin from the gallbladder wall, common duct, and hepatic ducts. As the gallbladder secretes, the bile in the common duct and hepatic ducts is forced back upward into the lesser channels, its place being taken by mucin and serous secretions from the gallbladder. Further explanatory experiments will be performed later to demonstrate the amount of gallbladder secretions as compared before and after cholecystectomy.

Fiessinger, Lyonn, and Caen classify icterus in three groups: 1. hemolytic icterus or icterus of supply of materials to the liver. 2. choledocic or mechanical icterus. 3. toxic icterus, which is a combination of hemolytic and choledocic. They consider icterus to be possible in its production both intrinsically and extrinsically to the liver.

That jaundice can be produced experimentally is shown by Whipple and Hooper. They injected hemoglobin into the blood stream of dogs in which the liver had been completely excluded from the circulation and were able to

prove that part of the free hemoglobin was changed rapidly into bile. They suggest that the endothelial cells of blood vessels, bone marrow, and muscle may have to do with this alteration of hemoglobin. (36) Whipple and Hopper have shown that the formation of bile and bile pigments is much less in an Eck fistula dog than in a normal animal, and consequently the icterus is much less intense. This, they assume, is due to a lessened activity of the liver cells because of decreased blood supply. They believe bile pigment to be formed from substances other than hemoglobin and further, that bile pigment formation normally may depend in part upon the functional activity of the liver cells rather than upon the amount of hemoglobin supply to it. (37)

The subject of experimental hemolytic icterus has been thoroughly reviewed by M'Nee. His experimental work has been confined to the use of AsH_3 on geese before and after extirpation of the liver, and on dogs with toluylendiamin. His work strongly supports the work of Whipple and Hopper in their conceptions of hemolytic icterus. (38) Fiessinger also calls attention to the fact that jaundice can develop following intraperitoneal injection of hemoglobin. He also points out that man clinically may become jaundiced following a hematoma in the abdominal cavity.

In considering the toxicity of bile to the organisms as a whole, King and Stewart have found that bile pigments are much more toxic than bile salts. Bile pigments injected into the circulation increase the vagus nerve tone which action can be abolished after the use of atropin. (39)

Meltzer and Salant believe that bile contains a depressive and an exciting element which are mutually anatagonistic, the total effect of both, when simultaneously present, being that of an algebraic sum, the depressive element being the stronger usually. (4) Bile salts seem to contain the tetanic element in a distinctly less amount than whole bile. (40)

In a study of my experimental data, it is shown that bile is toxic to parenchyma tissue of liver, increasing in toxicity as obstruction develops. It apparently has not this toxic action on new formed parenchyma tissue. It is a logical assumption, therefore, to believe that it has a toxic action on all highly specialized secretory tissue such as kidney, pancreas, and thyroid, depending on the blood supply to the gland and the regenerative ability of the gland. This toxicity might explain endothelial cell destruction and petechial and massive hemorrhage found throughout the body and liver during obstruction. King and Stewart also state that bile pigment in combination with calcium or sodium is less toxic than uncombined pigment, and that in experimentally produced jaundice the calcium content of the blood is increased while that of the liver, muscle, and brain are decreased. They consider calcium as a protective mechanism against the circulating pigments of obstructive jaundice.

King, Bigelow and Pearce conclude (1) in obstructive jaundice produced in dogs, there is a loss of calcium by the jaundiced animals, (2) the calcium is given up mainly by the bone to neutralize the toxic bile pigments circulating in the blood and tissues, (3) this neutralization affords protection to the body, but may lead to secondary disturbances such as bradycardia and change in the blood coagulation time, (4) these two latter signs of biliary toxemia can, perhaps, in some measure be explained by the disturbance of calcium, (5) the path of elimination of the increased calcium is mainly by the feces and only to a small extent by the kidneys. (41) Bile pigments are practically all eliminated through the urine.

It was my impression at first on studying experimental jaundice that there would be a marked diminuition in the functionsof internal secretions as well as external secretions of the gland. Experimental work preformed by Fitz (unpublished) on blood urea and total blood nitrogen would seem to point out that there is no marked increase of these constituents during obstruction except a

moderate fall in the cholestrin content of the blood as obstruction progresses. Mann and Williamson (unpublished) find no marked change in blood sugar in non-fasting dogs as jaundice progresses. It would seem that the regenerative liver tissue takes on the functions of the internal secretion at a very early date. Further work on this particular phase is being conducted at the present time.

Clinically, one is struck with the progressive anemia of chronic jaundice and the gradual gray, greenish color of long continued obstructive jaundice. The most logical conclusion arrived at to explain the anemia is that the blood forming organs and blood itself is in a state of intoxication and new blood is not formed in excess of the blood destruction. The fading jaundice is comparable to the work of Fiessinger on rabbits in that he shows that the secretory activity of the parenchyma cells in bile production ceases at about the end of two months. He thinks that liver cells secrete bile acids from then on and very little if any bile. He also advances the idea that the liver is an endocrine gland having an internal and external secretion and that during obstruction the power of external secretion is lost all together. The gradual fading of jaundice is a gradual elimination of bile pigments through the urine reabsorbed from the tissues of the body by the vascular and lymphatic systems.

The value of transfusions and calcium is being determined clinically at the present time in pre-operative preparations of patients. The value of carbohydrate diet in pre-operative and post-operative conditions met with clinically was suggested to me by the work of Opie, who believes that carbohydrate acts in a protective manner to prevent disentregation of body proteins when the individual is in a state of toxemia. He has shown this by a series of experiments on feeding of the carbohydrates, fats, and proteins to dogs before and after chloroform poisoning, the dogs being fed fat die first, protein second and carbohydrate third or not at all. (42)

CONCLUSIONS - EXPERIMENTAL

1. Bile acts as a toxic agent to parenchyma tissue of the liver during obstructive jaundice producing, first, intoxication of parenchyma which is later followed by complete destruction as obstruction progresses, This destruction of parenchyma tissue is from the periphery of the lobule toward the center of the lobule.

2. The regeneration of parenchyma tissue during and following obstructive jaundice is practically entirely from the undifferentiated cells of the interlobular biliary capillaries.

3. Trabeculae of new formation arise in two manners. A. By interlobular biliary capillaries budding from the interlobular ducts and becoming united to undestroyed parenchyma tissue and regenerated parenchyma of new formation. They then lose their lumen and go through a process of rearrangement of the trabeculae by anastomosis to form normal parenchyma tissue. B. By masses of undifferentiated cells formed along the border of interlobular biliary capillaries which almost immediately begin to assume trabecular form by anastomosis.

4. Regeneration of intoxicated or destroyed parenchyma tissue is, far advanced at the end of fifty days of total obstruction.

5. The return of the liver to normal following relief of obstruction is rapid. After two to three months following relief of obstruction there is little evidence of previous obstruction except from excess of old fibrous tissue stroma and moderate excess of biliary capillaries in the peribiliary spaces.

6. It would seem that parenchyma tissue loses to a great extent its external secretory function of bile production in later stages of obstruction.

7. The internal secretion of the new formed and old parenchyma tissue is still a matter of doubt. It would seem quite probable that they as-

sume internal secretory functions at an early stage following regeneration. This is assumed from the amount of parenchyma destruction which takes place and the lack of experimental data to substantiate their lowered function.

8. Bile acts on connective tissue as a stimulant to new growth in the nature of an irritant.

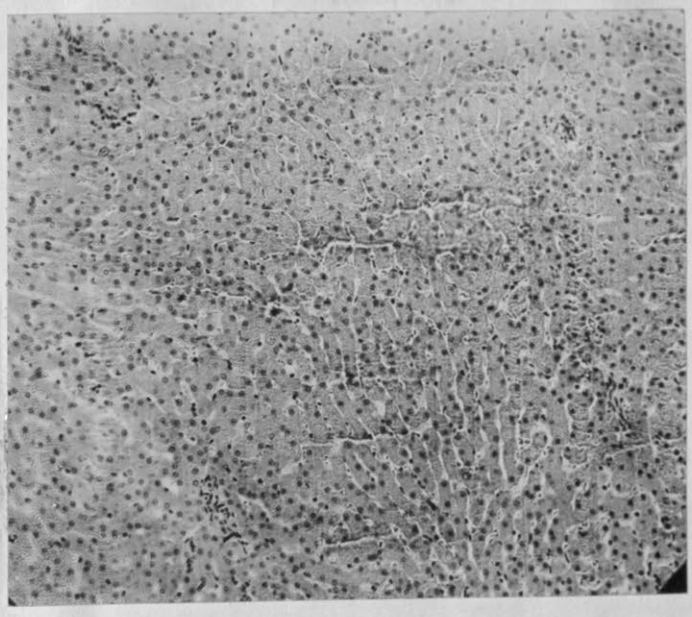
9. The interlobular biliary capillaries proliferate rapidly due to increased intrabiliary pressure and irritative action of the bile itself on the cells.

Therapeutic.

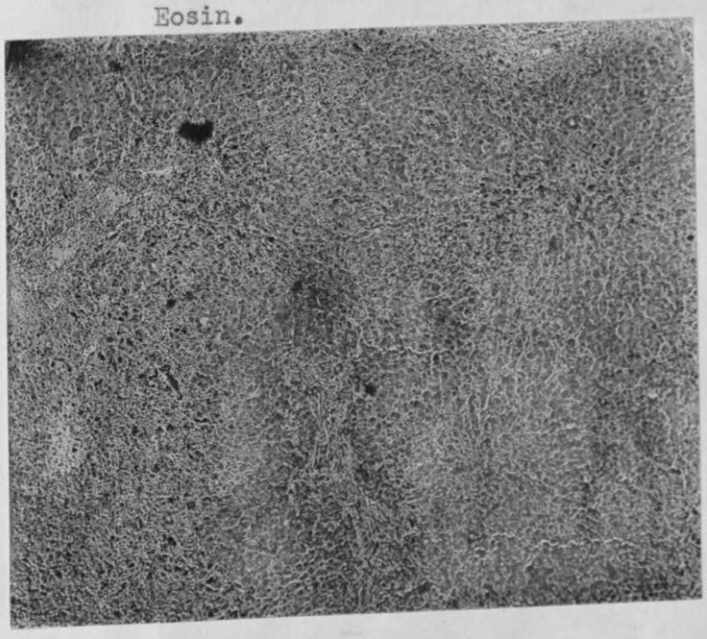
1. In attempting to make application in a practical manner of the data at hand, it occured to me to be a logical procedure to treat pre-operative and post-operative jaundice cases in the following manner: A. To put to rest and give 3,000 c.c. to 5,000 c.c. of water daily. B. A full diet consisting practically entirely of carbohydrate. C. To give intravenous injections of not more than $\frac{1}{2}$ gm. or $\frac{1}{4}$ gm. of calcium chloride, 5 per cent solution daily. Calcium lactate can be given by the mouth not in excess of 100 grains. D. Transfusions to be used if the anemia is marked. Transfusion should not be used if the cholemia is complicated by marked sepsis. E. Excess of glucose to be given daily into the duodenum through a Rehfuss tube and by rectum.

Note:- I wish to thank Dr. Mann for his courtesies and advice, without which the present work would not have been possible.

PLATE I.



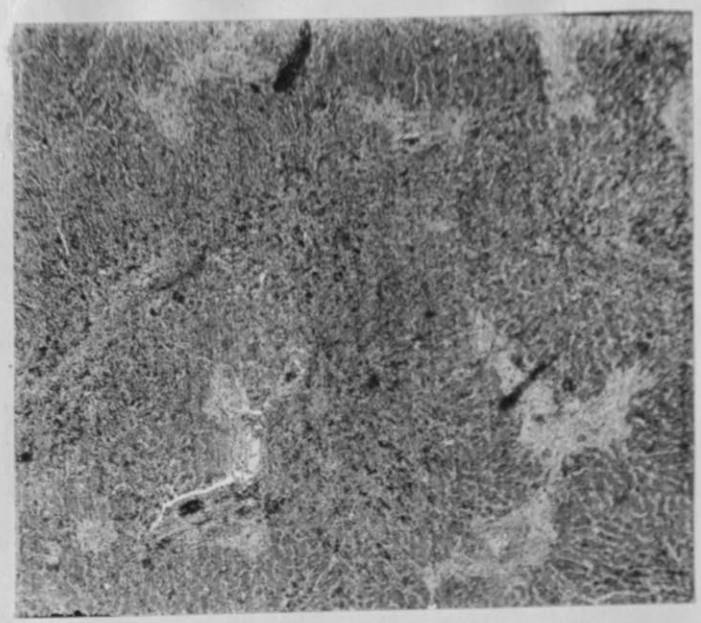
Group I. Dog E 88, 11-12-20. Normal Liver (x100). Heam and Eosin.



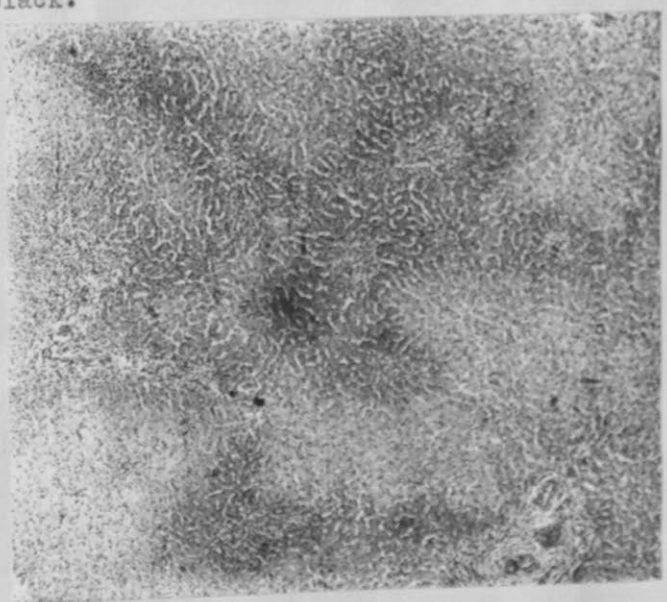
Group II. Dog E 88, Heam and Eosin, 12-10-20. (x60), obstructed twenty-eight days.

1. Showing marked parenchyma destruction.
2. Liver regeneration about peri-biliary spaces.
3. Bile pigment shown in black.

PLATE II.

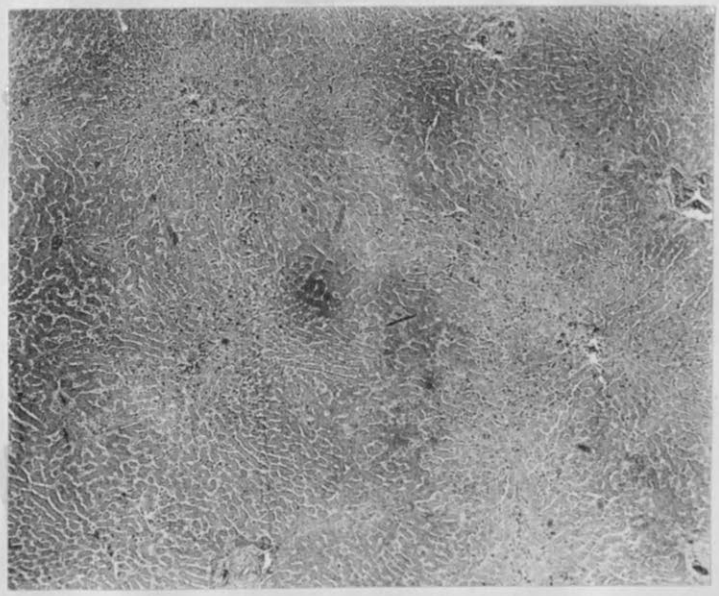


Group II. Dog E 88. 12-10-20. Mallory c.t. stain (x60). Obstructed twenty-eight days. Showing the amount of c.t. at twenty-eight days obstruction. 1. c.t. shown in light. 2. Bile pigment shown in black.

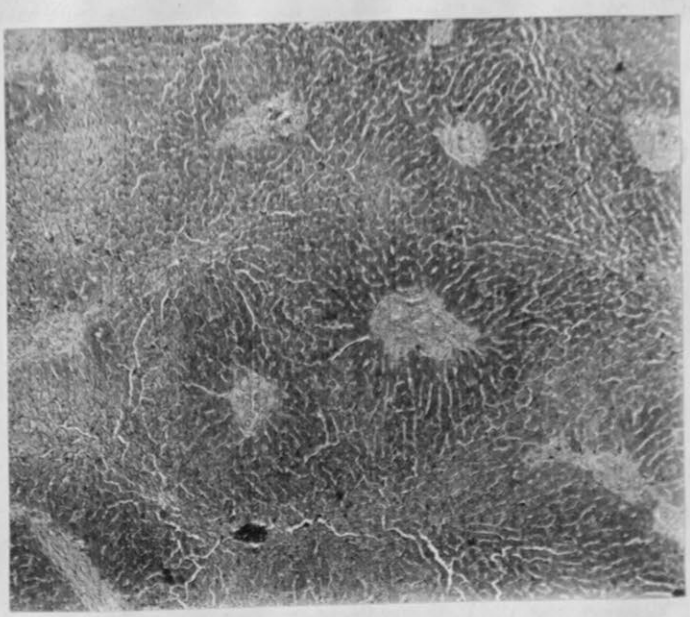


Group III. Dog E 88. 1-21-21. Forty-two days after cholecysto-duodenostomy. 1. New formed parenchyma tissue staining dark about peri-biliary spaces. 2. arrangement of new formed parenchyma tissue in trabeculae of normal appearance. 3. Numerous proliferating bile capillaries.

PLATE III.

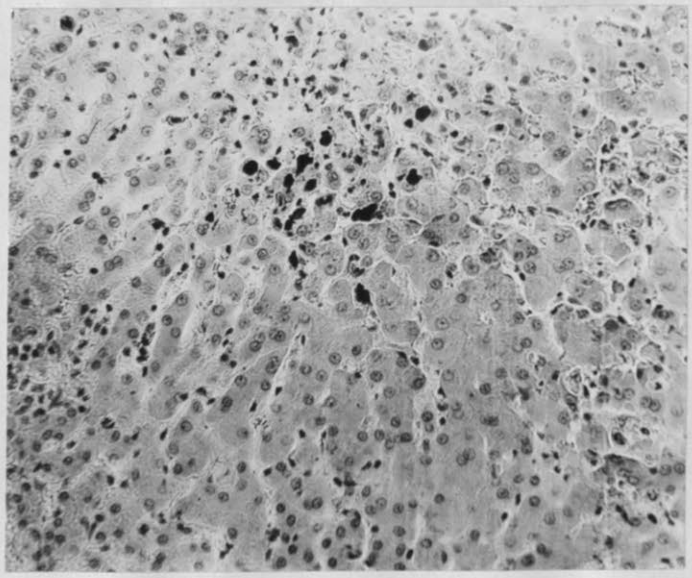


Group III. Dog E 88. Mallory c.t. stain. (x60). Forty-two days following cholecysto-duodenostomy, 1-21-21. Showing normal appearance of liver tissue. 1. C.T. shown in black in the interlobular spaces.

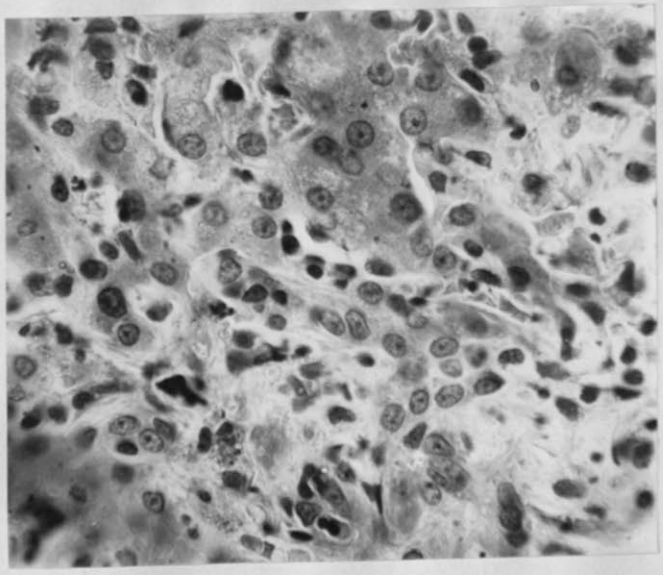


Group IV. Dog E 88. 3-2-21. (x60). Eighty-one days following relief of obstruction. 1. Parenchyma tissue normal in appearance and arrangement of trabeculae. 2. Evidence of peribiliary c.t. of old appearance. 3. Definite arrangment of capsule of Glisson with excess of fibrous tissue.

PLATE IV.

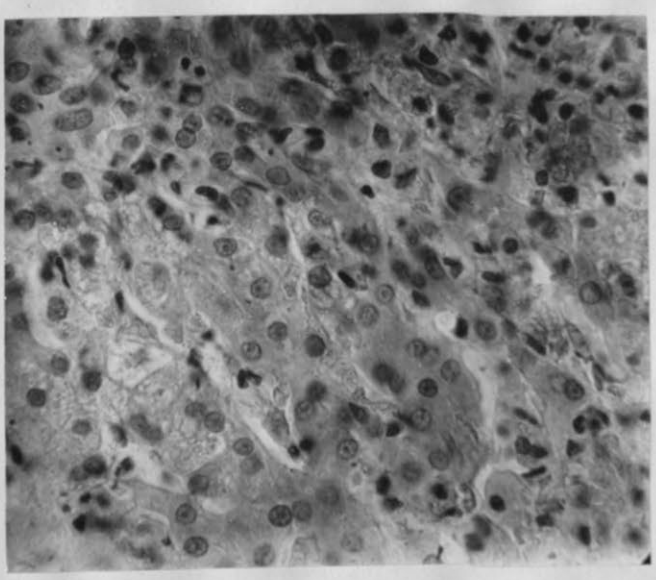


Group II. Dog E 88. Heam and eosin (x200). All parenchyma tissue is of newformation in section. Showing the grouping of new formed liver cells into trabeculae as they push away from peri-biliary spaces.

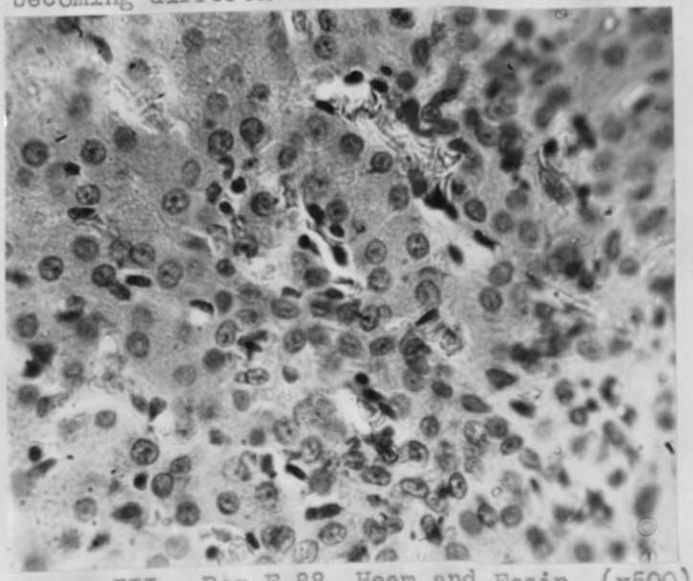


Group II. Dog E 149. Heam and eosin. (x500). Showing bile capillaries that have lost lumen becoming differentiated into liver trabeculae.

PLATE V.

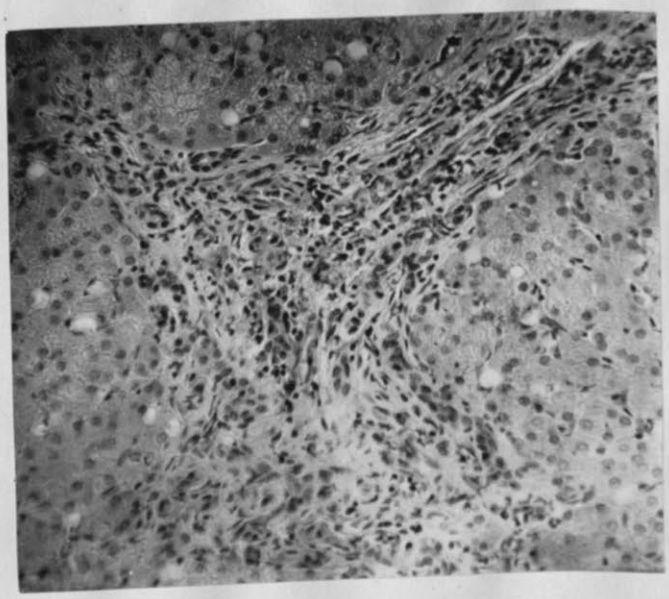


Group II. Dog E 149. Heam and eosin. (x500). Showing bile capillaries that have lost lumen becoming differentiated into liver trabeculae.

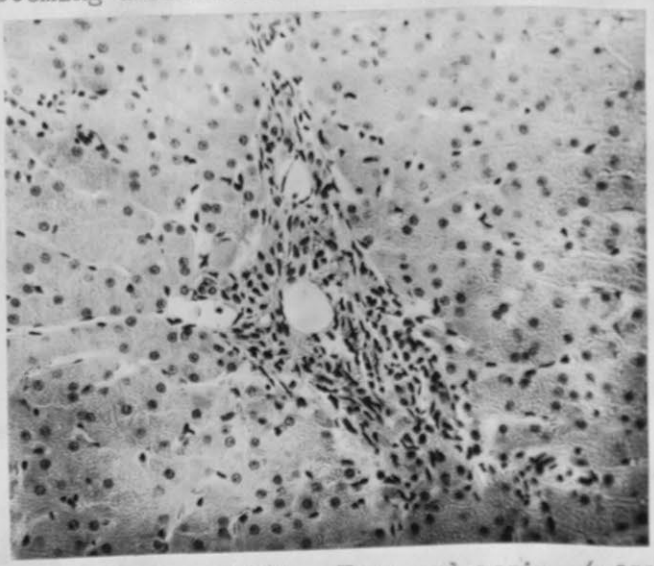


Group III. Dog E 88. Heam and Eosin. (x500). Showing bile capillaries that have lost lumen continuous with liver trabeculae.

PLATE VI.

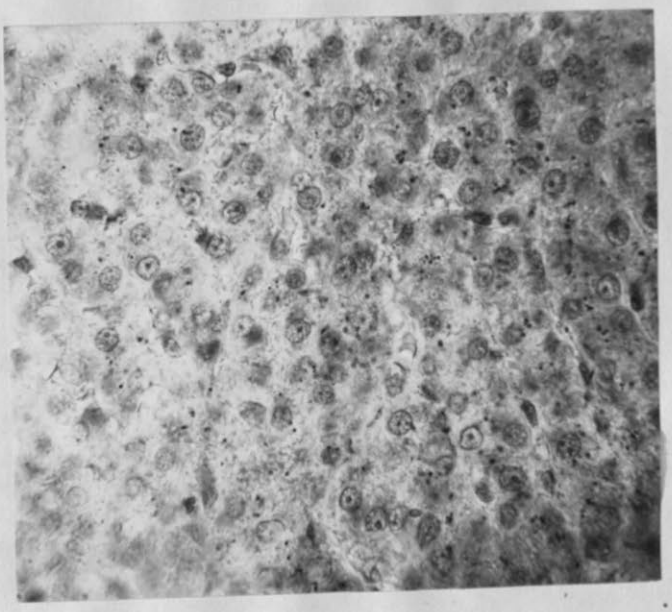


Group II. Dog E 126. Heam and eosin. (x200). Showing extensive biliary proliferation in peri-biliary space with biliary capillaries becoming differentiated into parenchyma.

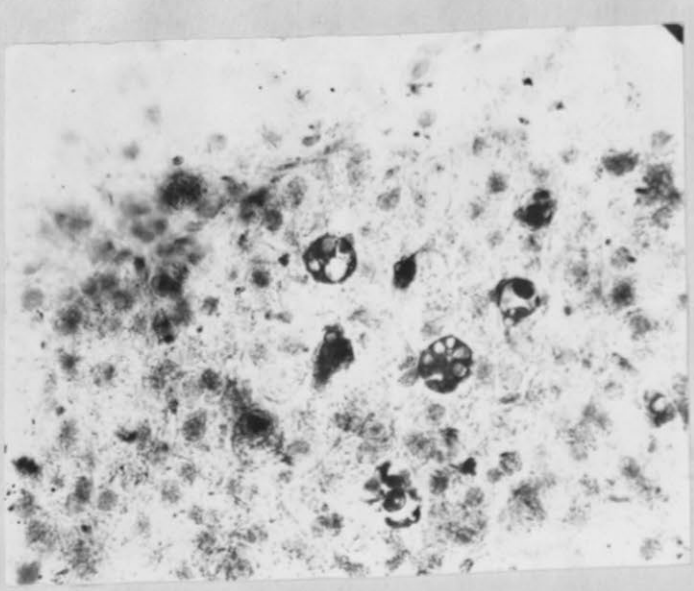


Group III. Dog E 126. Heam and eosin. (x200). Showing relatively small amount of peri-biliary space, old connective tissue and normal appearance of liver tubeculae, thirty-five days following relief of obstruction.

PLATE VII.



Group IV. Dog E 88. 3-2-21. (x500). Soudan III fat stain, eighty-one days following obstruction. Small fat droplets showing as black specks in cytoplasm.



Group III. Dog E 126. (x500). Soudan III fat stain. Fat droplets thirty-five days after relief of obstruction.

BIBLIOGRAPHY.

1. Mayo, W.J.: The liver and its cirrhosis. Jour. Amer. Med. Assn., 1918, lxx, 1361-1364.

2. Kretz: Ueber Lebercirrhose. Wiener klin. Woch., 1900, xiii, 271.

3. McCallum, W.G.: Jour. Amer. Med. Assn., 1904, xliii, 649-654.

4. Joannovics, G.: Über Experimentalle Lebercirrhose. 1904. Wiener klin. Woch., xvii, 757.

5. Fischler; Über Experimental erzengte Lebercirrhose. Deutsch. Arch. f. Klin. Med., 1908.

6. Porri, O.: Studio-anatomo-patholgico dell l'tterizia del cane. E. dell l'tteryia' da occlusione. Meccanico del Coledoce nuovo Ercolani. 1911, xvi.

7. Isobe, K.: Experimentellar Beitrag zur Entstehung der Lebercirrhose. Mitteilungen aus den Grengebreten der Medizin und Chirgie.

8. Fiessinger, N. and Rowdowska, L.: La Cirrhose Biliarre Experimentale Étude de Pathogenie et d'Histogenese générales. Arch. de Méd. exper. et d'Anatomie Pathologeque. xix.

9. Fiessinger, N.: Histogenese des processus de cirrhose toxique du foie. 1. Technique des intoxications chroniques Cirrhogenese. II. Cirrhoses Chloroformiques. Comp. rend. Soc. de Biol. 1908, lxiv, 597-649.

10. Fiessinger, N. and Rowdowska, L.: I. Dissemblance Anatomo-pathologiques de la cirrhose biliare de homme et de la cirrhose biliare experimentale l'ictère. (470). II La Cirrhose (524). Comp. rend. Soc. de Biol., 1913, lxxv.

11. Lissaner, M.: Die Experimentelle Lebercirrhose. Berliner Klin. Wochenschrift, 1914, L, 159.

12. Richardson, M.L.: Biliary Cirrhosis in the Rabbit. Jour. Exper. Med., xiv, 408.

13. Mallory, F.B.: Bull. Johns Hopkins Hospital, 1911, xxii, 69.

14. Pearce, R.: Experimental cirrhosis of liver. Jour. Exper. Med., 1906, viii, 64-73.

15. Herter, C.A. and Williams, W.R.: Experimental hepatic cirrhosis in dogs. Proc. Soc. of Biol. and Med. 1905, iii, 23.

16. Bell, E.T.: Jour. Amer. Med. Assn., 1913, lxi, 455.

17. Thompson, (cited from Keibel and Mall): Keibel and Mall, Human Embryology, ii, 403.

18. Bremer, (cited from Keibel and Mall): Keibel and Mall, Human Embryology, ii, 403.

7-20 2M

19. Lewis and Stohr: Text Book of Histology. 276.

20. Scammon, R.E.: The histogenesis of the Selachian liver. Amer. Jour. Anat., 1914-1915, xvii, 245.

21. Bensley, R.R.: Studies on the pancreas of the guinea pig. Amer. Jour. Anat., 1911-1912, xii, 297.

22. Marchand, F.: Ziegler's Beitrage, 1895, xvii, 206.

23. Meder, E.: Ibid, 1895, xvii, 143.

24. Stroebe, H.: Ibid, 1897, xxi, 379.

25. Barbacci, O.: Ibid, 1901, xxx, 49.

26. Podwyssozki,: Ibid, 1886, i, 259.

27. Von Meister,: Ibid, 1894, xv, 1.

28. Ponfick, E.: Virchow's Archiv., 1889, cxviii, 209, 1890,cxix, 193, 1895, cxliii, 81.

29. McCallum, W.G.: Johns Hopkins Hosp. Report, 1902, x, 374.

30. Howell,: Text Book of Physiology. Physiology of the liver. 187.

31. Luciani,: Human Physiology. Vol. ii, Internal secretion and digestion.

32. Fiessinger, N., Lyonn, L. Caen,: Journal de Physiologie et de Pathologie générale. xii, 1910. De rôle de la cellule hepatique dans la determination des ictérus experiments.

33. Herring, P.T. and Simpson, S.: On the relation of the liver cells to the blood vessels and lymphatics. Proc. Roy. Soc. of London, 1906, lxxviii, 455-493.

34. Herring, P.T. and Simpson, S.: The pressure of bile secretion and the mechanism of bile absorption in obstruction of the bile duct. Proc. Roy. Soc. of London. Series B. 1907, lxxix, 517.

35. Rouse, P. and McMasters, P.D.: A. Vicious activity of the gallbladder during biliary stasis. B. The determining factor in the causation of white stasis bile. Proc. Soc. for Exper. Biol. and Med., 1920, xvii, 159.

36. Whipple, M.D. and Hooper, C.W.: Icterus. A rapid change of hemoglobin to bile pigment in the circulation outside the liver. Jour. Exper. Med., xvii, 612 and 913.

37. Whipple, M.D. and Hooper, C.W.: Hematogenous and obstructive icterus. Experimental studies by means of Eck fistula. Jour. Exper. Med., 1913, xvii, 593.

38. M'Nee, J.W.: Experiments on haemolytic icterus. Jour. Path. and Bact., 1913, 1914, xviii, 325.

39. King, J.H. and Stewart, H.A.: The effect of the injection of bile on the circulation. Jour. Exper. Med., 1909, ii, 673.

7-20 2M

40. Meltzer, S.J. and Salant, W.: Studies on the toxicity of bile. Jour. Exper. Med., viii, 127.

41. King, Bigelow, and Pearce: Experimental obstructive jaundice. Jour. Exper. Med., 1911, xiv, 159-178.

42. Opie, E.L. and Alford, L.B.: The influence of diet on hepatic necrosis and tocity of chloroform. Jour. Amer. Med. Assn., 1914, lxii, 895.