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SOME PROBLEMS OF BILIARY CIRRHOSIS*

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Many of us have been puzzled at some time or other by a patient suffering from insidious, persistent jaundice. The subject, often a middle-aged woman, volunteers the information that her urine has been dark, and, on questioning, she admits that the stools are sometimes pale. But there is no history of pain, chills, or fever, and no particular aversion to fats is apparent. Physical examination, too, may be uneventful. The patient is well nourished and active, displays no striking physical signs, although jaundice is obvious enough. However, enlargement of the liver and spleen comes to light in a certain proportion of cases and progresses as the jaundice continues. Laboratory investigation, too, discloses much bile pigment in the blood serum; a high serum alkaline phosphatase level, steatorrhoea, and maybe an increase in serum lipids, so that clearly some disturbance of bile excretion has caused the trouble.

Nowadays liver puncture and direct microscopical examination of the organ confirms the diagnosis, and, armed with all of this information, surgical intervention is decided upon, for it now seems sure that the bile ducts are obstructed. Such proves to be the case in most instances, but every now and then we are confounded by the discovery that bile is certainly flowing along patent bile ducts despite the severe jaundice. And to make things even more difficult, the liver in these cases looks like that met with when there is complete blockage of the common bile duct by, say, a gall-stone or a carcinoma of the head of the pancreas.

It is with this problem that has puzzled me for many years that my remarks are concerned. Purely for my convenience I shall refer to the pathological state as biliary cirrhosis, recognizing full well that a host of changes may be met with in the liver according to the stage the disease has reached at the time of examination. But before I embark upon the troubled seas of conjecture let me recall what we encounter in the liver of biliary cirrhosis. Observation must always precede analysis; the latter, in its turn, may well demand experimentation and further breaking-down of the problem.

During the ten years from 1946 to 1955 we performed necropsies at University College Hospital on 58 cases which we feel confident are examples of biliary cirrhosis. In 50 the obstruction of the extrahepatic ducts was plainly responsible for the trouble, but in 8 no such obstruction was present and bile flowed uninterruptedly

from the liver to the duodenum. I shall therefore deal with the two groups separately, a plan adopted by other workers in this field (Rössle, 1930; Ahrens *et al.*, 1950; Albot, 1955). Moreover, I shall forestall consideration of finer details and classify all such cases into four groups: (1) cirrhosis from obstruction of the extrahepatic passages, the so-called secondary biliary cirrhosis; (2) primary cholangitic and cholangiolitic cirrhosis; (3) Hanot's hypertrophic cirrhosis; and (4) canalicular stasis. The fourth group concentrates attention upon the finest bile passages, the intralobular canaliculi where stasis occurs without much pathological change in the smaller and larger ducts or liver cells. But I must postpone the fuller discussion to a later section, since it is in many ways the most interesting though most puzzling variety of chronic biliary disease.

Cirrhosis from Obstruction of the Extrahepatic Passages

I shall say little about this condition, which is well known and has often been studied with great care (Gibson and Robertson, 1939). But I must first recall what happens when the common bile duct is blocked by a calculus for relatively short periods of weeks or a few months, without the complication of superimposed infection. With the introduction of needle biopsy of the liver we can make repeated observations on such cases, both before and after relief of the obstruction.

The outstanding features in the liver that have impressed me are three in number, and in this emphasis I agree with French workers whose findings are so ably summarized by Albot and Lunel (1955).

First of all, liver cells are singularly little affected, their vacuolation and acidophilic staining being, to my mind, of doubtful significance because they are so variable and are influenced greatly by fixation and handling of the specimen. Secondly, localized pear-shaped areas of necrosis at the periphery of the liver lobules are not uncommon. The third and most constant feature is marked increase of the smallest bile ducts around the lobules. This is due to proliferation of the canals of Hering and their supporting fibrous tissue. Such cases often present raised alkaline phosphatase and bilirubin levels in the serum, but other liver function tests are negative, although the colloidal red test is said to become positive when the fibrosis is progressing.

Prolonged obstruction of the extrahepatic ducts may in time produce a form of cirrhosis, especially when it is complicated by infection or hepatitis. This I shall label cirrhosis from biliary obstruction. In our 50 cases

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males and females were equally affected, the ages ranged from 35 to 83 years, and jaundice was severe in all. Obstruction was caused by carcinoma of the head of the pancreas (20 cases), tumours of the common bile duct and gall-bladder (10 cases), secondary carcinoma of the liver and hilar lymph nodes (7 cases), gall-stones (7 cases), carcinoma of the ampulla of Vater (4 cases), stricture (2 cases). The liver was considerably enlarged (mean weight 1,807 g., range 1,050–2,865 g.), green or yellow-green in colour, with a granular or finely nodular surface in about half the cases, smooth in the rest. The organ was firm, cut with resistance, and on section displayed greyish pink bands of connective tissue forming a network enclosing yellow or green nodules, some of which were certainly regenerative.

When fibrosis is advanced the liver may atrophy and portal hypertension with ascites then is common. Glisson's capsule may be thickened and adherent to the diaphragm and abdominal wall, bile ducts are often distended with green or even white glairy bile, and cholangitis may be apparent. The spleen is sometimes enlarged; in our material, however, it averaged 226 g. in weight with a liver:spleen ratio of 8:1, which is not far removed from normal.

Microscopically the liver lobules are atrophied and partly necrosed, but some may be enlarged through regeneration. Mild to moderate fibrosis of the portal tracts, especially around the interlobular and intralobular bile ducts, is associated with much hyperplasia of the tiniest ducts or canals of Hering and signs of biliary stasis. The latter gives bile staining of liver and Kupffer cells, distension of bile ducts and canaliculi, and numerous bile thrombi within the centrolobular canaliculi of many lobules. Collections of lymphocytes, plasma cells, and polymorphs—and, on occasion, eosophils—may occupy the portal canals close to the bile ducts and even overflow into their lumina. These may indicate the development of immunity to the infective agent or possibly to denatured liver protein. Such a picture, to my mind, represents the outcome of intermittent attacks of liver-cell destruction over a long period of time—our cases gave a history of jaundice lasting months to several years—with intervals of recovery, together with steady proliferation of the small bile ducts and a slow fibrosis.

Primary Biliary Cirrhosis with Inflammation of the Smaller Intrahepatic Bile Ducts

This is a most intriguing group, for enlargement of the liver and spleen with chronic retention (regurgitation) jaundice, lasting months to years, occurs without obstruction to the extrahepatic biliary passages. Some cases show persistent hyperlipaemia and xanthomatosis (Addison and Gull, 1851; Fagge, 1873; Weber, 1903; see Ahrens *et al.* (1950) for a review). Biochemical features include an increased serum bilirubin (mostly above 10 mg. per 100 ml.), high alkaline phosphatase level, a constant rise in serum cholesterol level without an associated increase in cholesterol esters, abnormal cephalin-cholesterol flocculation and thymol turbidity tests, rise of β -globulin, but no uniform change in the plasma proteins (Ricketts and Wissler, 1952). Steatorrhoea is constant, with soaps and fatty acid crystals in the faeces, and intestinal fat absorption is lowered (Ahrens *et al.*, 1950). A more or less similar biochemical pattern is found in the obstructive type of cirrhosis.

The liver is considerably enlarged, greenish, smooth or nodular, firm, and even tough. The spleen is less constantly enlarged in our experience—but according to Moschcowitz (1952) it is 400–550 g.; splenomegaly, when present, may

be fairly severe. Generalized enlargement of lymph nodes is common. As the disease progresses the liver becomes smaller and portal hypertension may set in. Death then results from gastric haemorrhage, intercurrent infection, or tuberculosis.

The main disturbances in the liver are again confined to the smaller bile ducts, especially the canals of Hering, and the bile canaliculi. Cholangitis and cholangiolitis are generally present, with destruction and acute inflammation of many of the interlobular ducts (MacMahon, 1948; Klemperer, 1937; Moschcowitz, 1952). Proliferation of the canals of Hering and intralobular bile ducts gives rise to many new but immature ducts projecting into the lobules from the portal tracts. These are elongated, often tortuous, and usually lined by pale cells like fibroblasts, with little or no lumen. The connective tissue of the portal tracts is increased and may extend into the lobules as finger-like processes or form annular bands around lobules. But the lobular architecture is disturbed only in the most chronic cases. More often the hepatic parenchyma is well preserved and regeneration nodules are present; the only regions showing degeneration or necrosis are those adjacent to abnormal bile ducts. Inflammatory cells collect in groups around and within such ducts but seldom involve the lobules. Bile thrombi are always present and are numerous when jaundice is severe.

Necrotizing cholangitis of the smaller bile ducts is met with occasionally, but extravasation of bile and bile granulomata are rare, although small or large abscesses are not uncommon. Xanthomas are rarely seen in the liver. Primary biliary cirrhosis thus differs from the secondary type in the absence of obstruction of the large bile ducts and the presence of relapsing chronic inflammation in and around the smaller bile ducts and canaliculi. Both forms, however, show much hyperplasia of the canals of Hering and the intralobular ducts, with fibrosis, the extent of which largely depends upon the presence of infection.

Our eight cases fall very well into this group. The liver weighed 1,280 g. on an average, with a range of 885–1,940 g. Half of our cases were of long standing and had passed on

Main Biochemical Findings in Eight Cases of Non-obstructive Primary Biliary Cirrhosis from 3,500 Necropsies at U.C.H., 1946–55

	Case							
	1	2	3	4	5	6	7	8
Serum protein:								
Total (g./100 ml.) ..	6.2	6.7	7.5	6.4	5.1	6.1	6.8	6.2
Albumin (g./100 ml.) ..	3.8	3.4	4.6	3.6	2.8	2.4	3.5	3.0
Globulin ..	2.4	3.3	2.9	2.8	2.3	3.7	3.3	3.2
A : G ratio ..	1.6	1.0	1.6	1.3	1.2	0.6	1.0	0.9
Serum bilirubin:								
v.d.B. reaction ..	D.	D.	D.	W.D.	D.	D.	D.	D.
Total (units) ..	12	24	3.6	14	38	20	10	28
Prothrombin concentration (%)	79	40	..	55	20	41	70	50
Plasma alkaline phosphatase (K.A. units) ..	30	52	..	45	31	59	48	56
Liver flocculation tests:								
Colloidal red ..	—	W. +	—	++	—	+	+	—
Cephalin-cholesterol ..	—	W. +	—	++	W. +	W. +	W. +	—
Thymol turbidity (units)	2	6	—	13	1	5	6	2
Urinary bilirubin ..	+	+	++	+	+	+	+	+

D. = Direct. W.D. = Weak direct. W. = Weak.

to the atrophic stage with much fibrosis. The mean spleen weight was 356 g. (range 190–850 g.), with a liver:spleen ratio of 3.6:1, which is well below the normal ratio. Ascites was present in four cases and generalized icterus was constant. The microscopical picture was dominated by small bile-duct proliferation, portal-tract infiltration with polymorphs and lymphocytes, pronounced fibrosis in half the cases, and numerous centrolobular bile thrombi. The accompanying Table summarizes representative biochemical findings in these eight cases. I wish to emphasize again that such findings differ in no way from those obtained from patients suffering from prolonged obstruction of the main bile ducts and variable degrees of liver fibrosis.

Hanot's Hypertrophic Cirrhosis

Hanot's (1876, 1892) own description of this condition is worth noting. The liver is constantly enlarged, weighing from 2,200 to 4,000 g. as against the average normal weight of 1,450 g. One lobe may be more hypertrophied than the other, but usually the whole organ is involved. The surface is generally smooth, though sometimes grained and yellow, brown, greenish, or very dark green in colour. Its consistency is increased and on section it may feel hard but elastic. Large grey bands of connective tissue enclose yellow or green lobules. Blood vessels and bile ducts are normal. Lymph nodes at the hilum are seldom enlarged and the gall-bladder is normal. *There is no obstruction to the flow of bile from the liver.*

In most cases the spleen is enlarged, weighing up to 1,300 g., and shows perisplenitis and cartilaginous plaques beneath its capsule. Chronic peritonitis is common, and the abdominal organs may occasionally be enclosed in fibrous adhesions. The peritoneal sac contains serous cloudy fluid which is variable in quantity but seldom abundant. No special lesions involve the alimentary canal or the mucosa of the ampulla of Vater.

Microscopically, there are five noteworthy features:

1. Connective-tissue bands course throughout the liver, forming large irregular islands in the portal spaces and anastomosing around the lobules. These bands also invade lobules between the liver-cell trabeculae, terminating at a variable distance from the centre of the lobules. They may ramify and anastomose, to break up the lobules into sectors and displace the centrolobular veins peripherally. They are often split by masses of small, round, deeply stained cells, especially near or within the bile ducts. Here, too, the connective tissue is much increased, and forms fibrous rings around the ducts (pericholangiolitis).

2. The flow of blood between portal and hepatic veins is not interrupted. Prussian-blue suspensions, for instance, pass from the portal vein into interlobular branches and hepatic venules. The hepatic arteries and lymphatics are normal.

3. The bile ducts of the portal tracts are lined by large cylindrical cells with a clearly visible nucleus which stains brightly with carmine. Often the lumen is narrow or absent, and sometimes is blocked by pigment masses. *The hyperplastic connective tissue is traversed throughout its entire length by a large number of fine elongated ductules which branch and anastomose to form networks.* Their origin is uncertain. The lining epithelial cells are closely packed together, but transverse sections disclose a small lumen occasionally filled with pigment. These cell groups spring from hyperplastic interlobular bile ductules (Cornil, quoted by Hanot, 1892). Some, however, originate in old hepatic cells and trabeculae as Klebs long ago suggested. Ackermann (quoted by Hanot, 1892) has shown that they can be injected by way of the main bile duct.

4. The liver cells in general are normal in size and arrangement or hypertrophied, rarely displaying fatty change or pigmentation. Where they come into contact with connective-tissue bands they lose their nuclei, break up, and disappear. Hanot especially stresses the great enlargement of liver cells and nuclei during the early stages of the disease. *He insists on the integrity of these elements as a characteristic of hypertrophic cirrhosis. The liver cells deteriorate only during the final stages of the illness.* Extensive connective-tissue hyperplasia contrasts with the hypertrophy of the liver cells. The liver enlargement is thus due to hyperplasia of the connective tissue, hypertrophy of the hepatic cells, and dilatation of the intra-lobular bile canaliculi.

5. Bile canaliculi show evidence of bile stasis with the formation of concretions, especially when connective-tissue hyperplasia is severe. *But there is no obstacle to bile flow along the large bile ducts.*

Hanot thus emphasizes (a) the abundant diffuse connective-tissue hyperplasia; (b) the perfect conservation of most of the hepatic cells; and (c) angiocholitis and periangiocholitis involving the small and medium-sized bile ducts. He thinks that hypertrophic cirrhosis belongs to the anatomical group of porto-biliary cirrhoses. The absence of ascites, the free flow of blood between portal and hepatic veins, the precocious development of jaundice, and the predominance of lesions around the bile channels suggest that the bile ducts are the centre of evolution of the disease processes. Of all the diseases of the liver it is the one

that is richest in new bile ducts; yet, strange to say, far from suppressing bile production, it seems to stimulate the organ to pour out bile into the intestine despite the jaundice. Hanot proposes for it the name of "biliary diabetes." He admits that no cause is known. The chief clinical manifestations are chronic jaundice, enlargement of the liver and spleen, pigmentation of the skin, and often a moderate undulating fever.

Little of note has been added to Hanot's classical description. On the contrary, much confusion has arisen over what is meant by Hanot's disease. Albot (1955) limits it to those cases with severe biliary stasis arising in the minute interlobular bile canals (canals of Hering especially) and huge islands of lymphocytes at the margin between lobules and portal spaces. Kupffer cells, too, are increased in number and may form small cellular aggregates.

I have seen two examples in the last 20 years that seemed to me to fit in very well with this description. Lymphocytic collections in the portal canals were very prominent and recalled similar nodules in the thyroid of Hashimoto's disease. Both cases showed much splenomegaly but no ascites. But I see no good reason for separating Hanot's cirrhosis from the group of primary biliary cirrhosis; the main uncertainty arises in deciding whether it represents an early stage of the disease or a variety in which the unknown stimulus falls particularly on the mesenchymal elements of the liver, or perhaps local immunity responses—possibly induced by denatured liver protein—have developed.

Canalicular Stasis

After excluding cases of obstruction of the extrahepatic bile ducts, cholangitis of medium and small bile ducts, with or without pericholangitis, there remain unexplained instances of acute and chronic jaundice of hepatic origin. They share one constant feature in the form of biliary thrombi located in the centrolobular zone and signs of biliary canalicular stasis. Inflammatory cells may be present within the portal tracts, but the bile ducts there are unaltered. Liver-cell degeneration is not prominent and may even be absent. More often the condition is associated with inflammation of the canals of Hering (cholangiolitis), when it may occasionally pass on to biliary cirrhosis. I have seen a number of these cases in recent years largely diagnosed by way of needle biopsies.

Some are caused by drugs such as arsenic, especially during arsenotherapy, *p*-aminosalicylic acid, and recently chlorpromazine. An allergic basis is suspected, but with very little evidence. Methyltestosterone in relatively large doses has been blamed (Wood, 1952; Almaden and Ross, 1954). The so-called hepatitis in adolescent girls may be an example (Popper and Schaffner, 1957).

Some Problems of Biliary Cirrhosis

This short survey should suffice to convince you that the pathogenesis of biliary cirrhosis is a complex one. How much more puzzling is the aetiology; for apart from those cases with gross obstruction to the large bile passages nothing is found to explain the cause. I doubt whether success will crown this vital inquiry until we have simplified our understanding of pathogenesis. Nevertheless, some progress can be claimed, and I shall try to convince you that the investigation of three problems may well disclose the secret of biliary cirrhosis. These problems revolve around the liver-cell necrosis, the fibrosis, and, above all, the bile-duct proliferation within the organ. They are, I believe, linked together by some disturbance of bile production and outflow, and apt to be complicated by the presence of infection. Complete obstruction to bile flow is not necessary, since primary biliary cirrhosis, Hanot's cirrhosis, and canalicular stasis can develop in the absence of any such obstruction. And we shall see that all such changes may be induced in the liver of experimental animals without jaundice or obstruction of bile outflow. It may

surely be significant that this latter state of affairs is also caused by the action of certain chemicals on bile-duct cells. Perhaps we have before us, for the first time, a valuable signpost for future research.

Bile Flow in Health and Disease

In searching for the cause of these structural changes in the liver we must, I believe, consider the bile flow from the normal organ and the manner in which that flow may be disturbed through abnormal conditions.

1. Dynamics of Bile Flow

Bile is produced in man at the rate of 15 ml. per kg. body weight in 24 hours, or 0.6 ml. per minute in a person weighing 60 kg. The pressure of secretion is between 200 and 300 mm. of water, although the sphincter of Oddi yields to lower pressures—for example, in man and dog, 100 mm. of water; in the rat 30 mm. of water; and in the rabbit 70–150 mm. (Sobotka, 1937).

Total bile flow is largely determined by the flow of blood through the liver (Brauer *et al.*, 1954). Most of our information comes from experiments on dogs and may not be applicable to man, although there is close agreement between the figures for rate of bile flow and secretory pressure in both species and both are influenced by several common factors. Animal observation shows clearly enough that bile flow increases with the portal blood flow, although sudden interruption of the latter decreases but does not stop bile flow. Dogs with Eck's fistula are said to secrete only one-half of the normal volume of bile. A raised head of pressure in the hepatic artery decreases bile flow, but occlusion of that artery does not necessarily prevent the formation of bile (Cameron and Mayes, 1930). Certain choleric drugs—for example, sodium dehydrocholate and cinchophen—increase the hepatic arterial blood flow (Grodins *et al.*, 1941) despite the fact that an increase in arterial flow to the liver is not essential for a choleric response to bile salts. Anoxia certainly decreases bile flow (Schnedorf and Orr, 1941) although it does not significantly alter the excretion of bile salts. It seems, therefore, that the relationship between blood flow and bile production is a complex one and clearly controlled by hepatic-cell function in the first instance. The studies of Brauer *et al.* (1954) with an isolated rat-liver preparation support these early conclusions and suggest that the capacity of the biliary system as an elastic reservoir is large enough to hold, at most, the volume of bile formed in a very short interval of time. For the rat, this interval is two minutes.

Bile flow is well known to be influenced by nervous factors, cholericotics such as bile salts and secretin, fatty diet, and infection. Infectious diseases decrease or even abolish the flow of bile, especially when they are associated with high fever. Once again we are forced back to a consideration of the part played by the liver cell in the secretion of bile, as distinct from the more or less passive removal by way of the bile ducts.

2. Passage of Dyes through Liver Cells to the Biliary Canaliculi

Much of what we know about the secretion of bile comes from watching the liver of animals after the administration of fluorescent dyes (Franke, 1931, 1936; Hirt *et al.*, 1938; Mendeloff, 1949; Grafflin, 1947; Hanzon, 1952). By viewing the edge of the organ with ultra-violet light, subject of course to the elimination of a number of artifacts, the passage of such dyes can be followed from the blood vessels through the liver cells to the bile passages. Hanzon, who has carried out the most elaborate studies with rats' livers, uses the dye uranin. Within 3 to 10 seconds of intravenous injection it appears in the blood plasma of the hepatic sinusoids, then in the Kupffer cells, and in traces within the liver cells after 15 to 32 seconds, increasing steadily during the next two to five minutes. The liver cells are strongly

stained in the centre of the lobules before the dye appears within the bile canaliculi, but after 5 to 10 minutes there is no difference in staining between central and peripheral liver cells. This agrees very well with the observations of Seneviratne (1949) on the removal of Evans blue from the blood stream by the central liver cells, and with my own limited studies with uranin.

Bile canaliculi contain the fluorescent dye 26 to 76 seconds after it reaches the sinusoids. By 30 to 60 minutes the liver cells are cleared of the dye, though some can be detected in the canaliculi as long as two to three hours after injection. A canalicular network along the liver trabeculae is shown up in this way, similar to that demonstrable in fixed preparations. We may therefore conclude that uranin is eliminated by way of the liver cells and not by direct filtration to the canaliculi with subsequent reabsorption of water by the liver cells as suggested by Pavel (1949). Perfusion experiments also teach us that uranin can be concentrated approximately 500 times in passing through liver cells, which suggests that transport is an active process. Transfer of uranin takes place at the cellular surfaces between sinusoid and liver cell, and liver cell and bile canaliculus, so that concentration is a two-stage mechanism which again suggests an active transfer.

On the other hand, transport of uranin through the cytoplasm must take place very rapidly, since fluorescent intensity does not differ in various parts of the cytoplasm, which suggests that diffusion or filtration is responsible at this stage. No accumulation can be detected in any organelle. Transport through liver cells thus seems to be by means of the active mechanism at the cell surfaces. The dye is driven in the cells from the surface of uptake to the elimination surface by the concentration gradient established at the surface of uptake. Uranin needs on an average 22.5 seconds to penetrate from sinusoids to liver cells and on an average 24 seconds to pass from liver cells to canaliculi. The contents of the canaliculi appear at all times to be fluid, with no evidence of local or generalized precipitation of dyes or other constituents. No contractile activity of canalicular walls has been discovered (Grafflin and Bagley, 1952). Concentration in canaliculi is first seen at the centre of the liver lobules, so that the liver cells here appear to possess greater secretory activity for uranin than those at the periphery of the lobules. Hanzon thinks that this may be due to a difference in the O_2 or CO_2 tension between periphery and centre, or to a competition between other substances and uranin for the secretory mechanism in the peripheral cells.

Since the concentration curves of uranin and bilirubin in blood and bile, after administration of identical amounts of these substances, are much alike and both are affected by similar factors, Hanzon suggests that the two compounds may be handled by the same secretory mechanisms.

Experiments with uranin, therefore, may be used to clarify our knowledge about the secretion of some of the normal constituents of bile, and to follow pathological disturbances of this function.

Two kinds of substance are present in hepatic bile. Some occur in concentrations that differ slightly from those maintained in plasma, and these no doubt represent a protein-free ultrafiltrate of plasma formed by liver cells. The chief examples are Na^+ , K^+ , Cl^- , creatinine, glucose, and cholesterol. Others, such as bilirubin and *p*-aminohippurate, are concentrated in bile many more times than in plasma and reach the bile by way of an active secretory mechanism (Cook *et al.*, 1950). In addition, bile acids are secreted after their manufacture by liver cells.

There is a sound experimental basis for this division, which we owe to Höber and his colleagues (see Höber, 1945). When certain highly diffusible acidic, and to a less extent basic, dyes are perfused through the isolated frog's liver they appear in high concentrations in the bile flowing from that organ. It can be shown that concentration is not due to water absorption during bile secretion. Such dyes

are absorbed easily and their secretion is inhibited by surface-active, dehydrating, or antidispersing compounds that are rich in polar groups. These in turn are antagonized by surface-active, dispersing substances that stimulate bile flow, such as sodium salts of higher fatty acids (detergents) and bile acids. In contrast to such dyes are substances, like glucose and inulin, that appear in the perfused frog's bile without change in concentration. All of these substances are poorly absorbable. Very large molecules, like starch, fail to pass through liver cells.

This concentrating action of the liver cells suggests that an active transport mechanism is concerned, as was first suggested by Höber and Titajew (1929) and later by Brauer and Pessotti (1950). Further evidence for such a mechanism is afforded by the work of Brauer *et al.* (1954) with the isolated-rat-liver preparation. When the temperature of the perfusate is varied, bile production ceases at approximately 25° C., and is greatest between 38 and 40° C. The Q_{10} averages 4.5 between 28 and 38° C. and 3.3 between 30 and 40° C. This suggests that bile formation must be an active secretion supported by metabolic energy for the performance of osmotic work. It is not based on a filtration-absorption mechanism, as some would have it. The maximal bile secretory pressure is determined by the critical pressure at which leaks appear in the biliary passages—in other words, the maximal pressure is a measure of the mechanical properties of the liver. Brauer *et al.* also agree that the rat's biliary system possesses an extremely limited capacity of functioning as a reservoir in the absence of a gall-bladder. They also show that bile can be formed under conditions in which the pressure gradient from blood to bile passages is the reverse of that required for a filtration mechanism. Clearly there is little support here for a hypothesis that attributes bile formation to filtration.

Other observers have noted the influence of temperature on bile secretion. Low temperatures, for instance, retard the transport of dyes from blood to bile and decrease their concentrations in liver cells and canaliculi (Jacoby, 1931; Reinhold and Wilson, 1934; Brokaw and Penrod, 1949; Hanzon, 1952). In the case of uranin secretion, Hanzon shows that the Arrhenius formula is obeyed between 25 and 44° C. in such a way as to suggest an oxygen-consuming biological process responsible for secretion. Hanzon thinks that the deviation at high and low temperatures from Arrhenius's equation may indicate that uranin secretion depends upon enzyme-controlled metabolic reactions rather than a change in permeability of the cell membrane for the dye. Injurious agents, such as chloroform, cyanide, sodium cholate, or strong irradiation with ultra-violet light, on the other hand, bring about a sudden increase of permeability of the cell membrane with a loss of functional polarity that may exist without any morphological change. Oxygen deficiency, however, reduces the volume of bile secreted and the concentration of eliminated substances below normal. Vacuoles storing uranin appear in the liver cells that are anoxic; they disappear rapidly when anoxia is relieved. These facts once again point in the direction of an active process of secretion, dependent upon an adequate supply of oxygen.

Finally, the excretion of bilirubin brings further support for a secretory mechanism in the liver cells. Bilirubin is produced by the action of cells of the reticulo-endothelial system on haemoglobin or its breakdown products, and is transported from these cells to the liver as a protein complex. Electrophoresis shows that bilirubin migrates mostly with albumin and to some extent with α -globulins (Snapper and Bendien, 1938; Gray and Kekwick, 1948; Martin, 1949; Klatskin and Bungards, 1956). Recent work by Lathe and his co-workers (Cole and Lathe, 1953; see Gray, 1957) suggests that bilirubin, like steroids, is conjugated with glucuronic acid during its passage through the liver. Such conjugation makes the pigment more soluble in water and therefore more readily excreted by the liver cells into the canaliculi. Here again we appear to be confronted with an active process.

3. Acute Obstruction to Bile Flow

When the common bile duct is obstructed, bile pigment reaches the lymph of the thoracic duct within 15 to 30 minutes (Saunders, 1803; Wertheimer and Lepage, 1897, 1899; Barron and Bumstead, 1928; With, 1954, review). It appears within the blood a little time later (Fleischl, 1874; Whipple and King, 1911; Mayo and Greene, 1929). If the bile passages of dogs are obstructed with pressures below 250 mm. of water neither blood nor lymph displays bilirubin (Shafiroff *et al.*, 1939). With pressures about 300 mm., about half the animals show bilirubin in both blood and lymph and the rest only in the lymph. With pressures above 400 mm. bilirubin is demonstrable in the lymph after 15 minutes and appears in the blood a couple of hours later in all cases. These experiments suggest that the special secretory mechanism for transfer of bilirubin from blood to lymphatics begins to function when pressure in the bile passages exceeds 250 mm. of water.

Recent electron microscopical studies of liver-cell structure by Braunsteiner *et al.* (1953) show that the bile canaliculi widen during bile stasis and become surrounded by a rich deposit of bile pigment. When pigment deposition exceeds a certain mass the cells near by become necrotic, and this is associated with or preceded by regurgitation of bile into the blood stream.

The fluorescence studies of Hanzon throw much light on biliary stasis. When the bile flow from the bile ducts is stopped in the rat, the intraduct pressure rapidly increases and reaches a maximum of 12.8 ± 0.05 mm. Hg (180 mm. of water) in 8 to 15 minutes. During this rise, which may be maintained for hours or days, uranin-filled canaliculi become slightly wider and fluoresce greatly. Here and there at the periphery of lobules, isolated vacuoles appear in liver cells. They fluoresce strongly and resemble those seen when the liver is suffering from anoxia. However, the secretion of uranin goes on normally in spite of impeded flow of bile until just before maximal pressure is reached, when concentrated uranin is suddenly seen flowing in one or several sinusoids throughout many lobules towards the central veins, where it is gradually diluted.

The source of the reversed uranin flow often lies at the extreme periphery of the lobules and occasionally may be traced to a vacuole or widened canalicular branch. As a rule it comes from isolated or small groups of strongly fluorescing liver cells. Such backflow, Hanzon suggests, indicates a leakage from the bile canaliculi to the blood, either between or through the liver cells. Probably all of the bile constituents pass direct into the blood in this fashion. For a time, more and more liver cells fluoresce around the leakage, but after 15 to 30 minutes backflow ceases, although the injured cells continue to fluoresce for a long time. Such leakage appears to constitute a safety-valve mechanism, since the biliary pressure remains raised all of this time.

Freeze-dried preparations of such livers show no changes suggestive of rupture of canaliculi, so that it is highly probable that the bile is regurgitated from the bile passages to the blood. Leakage most often develops in injured liver cells whose permeability has been altered because of the raised pressure in the bile ducts, but toxic action of a high concentration of bile acids may be important, especially where the liver cells are injured around the leakage. In this way the focal necroses so typical of obstruction develop. These may be seen within one hour of obstruction of the common bile duct in the rat (Cameron and Oakley, 1932; Cameron and Muzaffar Hasan, 1958) and many other animals at short but variable intervals. Man quite likely takes a longer time to develop necrosis than experimental animals, but few opportunities of checking this point have so far arisen.

With relief of obstruction, uranin secretion becomes almost normal in 30 to 90 minutes, even after severe stasis. Prolonged stasis (19 to 36 hours) gives serious functional disturbance, and uranin secretion then may be much

impaired. It seems as if the secretory mechanisms of the cells become overloaded and their activity falls off.

4. Prolonged Obstruction to Bile Flow

A very good imitation of the secondary biliary cirrhotic liver is obtained by deliberate ligation of the common bile duct of animals. The results of numerous experiments have been discussed by several workers, all of whom agree about the outcome. For some weeks following obstruction the extrahepatic and intrahepatic bile ducts dilate and show multiple bursting-points from which bile seeps out into the neighbouring lobules. The weak spots are the canals of Hering—the heel of Achilles, as Aschoff called them—and waves of necrosis ebb and flow round these canals so long as obstruction to bile outflow is maintained. But there must be some sealing-off mechanism available, for each little patch of necrosis is short-lived and seldom recurs. Necrotic cells, too, are effectively dealt with, for they seldom leave behind insoluble debris or induce fibrosis, so that the cirrhotic pattern that eventually evolves cannot be attributed wholly to necrosis.

By far the most interesting effect of prolonged biliary obstruction is the astonishing degree of biliary hyperplasia that sets in within a few days of obstruction. Indeed, division of duct lining cells may sometimes be detected within a few hours. Buds and syncytial masses of dividing epithelium grow out from the portal tracts around and into the lobules. They are supported by a modest proliferation of connective tissue-cells and blood vessels, which become pronounced if infection is present. In this way diffuse pseudo-lobulation of the liver results and the organ takes on a cirrhotic pattern. Since biliary stasis steadily increases as ducts proliferate and liver cells atrophy and disappear when bile ducts encroach on their territory, there comes a time when the fully developed picture of obstructive biliary cirrhosis emerges. Recent studies by Dr. S. Muzaffar Hasan and myself have also shown that functional disturbance unfolds more or less in parallel with the structural commotion. Clearly there can be little doubt that prolonged obstruction of bile outflow is a potent factor in inducing bile-duct proliferation and that bile stasis may be the underlying mechanism.

Bile as a Detergent

Detergency

Detergents are best known to us through the well-known properties of soaps and soap substitutes. In general, they consist of long aliphatic hydrocarbon chains, comprising 12 or more carbon atoms, with a water-soluble dissociable end-group. The common soaps are mostly alkali metal salts, which dissociate in water into anions—the long hydrocarbon chain—and Na or K ion, for example:

The $\text{R} - \text{COO}^-$ ions are strongly attracted to an oil-water interface, such as that which exists when a greasy surface contacts water, where they become orientated through the $\text{R} -$ tail portion dissolving in the oil and the COO^- , with a strong affinity for water, projecting into the water phase. Hence each greasy surface—usually an oil droplet—acquires one or more negative charges and will repel electrostatically other nearby droplets, reducing the frequency of collisions and resisting brownian thrust, so that they remain in suspension and show little tendency to coalesce as large oil globules. Such an emulsion is thus preserved from “breaking.” Detergents are also very effective in reducing surface tension, and this again inhibits coalescence of globules during collision. Detergents therefore possess three important properties: (1) they reduce surface tension of water and its interfacial tensions against oils; (2) they are good emulsifying agents for insoluble fatty liquids in water; and (3) they are good wetting agents. Washing, or the removal of greasy material—

“dirt”—from fabrics requires three processes: (1) the spread of the soap or detergent over the greasy surface, with wetting; (2) the detachment of the grease from the fabric; and (3) the subsequent removal of the grease as an emulsion, suspension, or foam.

In *wetting*, the attraction between the greasy surface and the water is increased by the orientation of the detergent molecules at the interface. The hydrophobic paraffin chain, $\text{R}-$, dissolves in the grease, the hydrophilic water-attacking portion in the water, *thereby anchoring water to grease*. Detergency consists in an alteration of the angle of contact between the grease-water surface and the solid surface being cleaned from 180 to 0 degrees, measured in the water. Any liquid making a contact of 90 degrees or less with a solid will suffice for some degree of wetting, and will flow into a capillary constructed of the same material without the application of an external pressure. For washing, the detergent must be able to enter the capillary spaces between the soiled fibres before the real detergent process can take place.

In washing, then, the detergent flows along the capillary spaces of the fabric, orientates itself at the interface between grease and water, anchors these, and thus attaches the water to the grease, with wetting.

After contact with the greasy surface the detergent *rolls up the dirt into small droplets* (up to 50 μ diameter) and thus *prises the dirt from the fabric*. It collects the dirt locally on the fibre, thus making it suitable for removal by mechanical agitation (Adam, 1937). At this stage two complications may arise. Some detergents react with certain polar components in dirt to form gelatinous complexes which are easily trapped by the fibre meshwork of the fabric, and rolled-up dirt may be re-deposited on the fibres when the detergent is rapidly and substantially absorbed.

Removal of the dirt is facilitated through aggregation of detergent molecules to form colloidal micelles. The nature of such aggregates is still highly speculative (Pankhurst, 1953; McBain and Hutchinson, 1955).

McBain thinks that the ions of each molecule are tightly packed in a condensed monolayer, with the hydrocarbon on one side and the ionized hydrophilic groups on the other. Such leaflets are arranged in pairs, with the paraffin chains in contact, like a sandwich.

Hartley prefers a spherical micelle, with upwards of 50 ions so directed that hydrocarbon chains are directed towards the centre and hydrophilic groups are arranged around the periphery. A number of oppositely charged gegen-ions reduce the overall charge on the micelle. The chaotic motion of the paraffin chains in the centre of the micelles makes them behave as liquid hydrocarbons.

Debye postulates a sausage-shaped micelle with Hartley spheres at the ends.

Micelle formation is influenced by concentration of the detergent, length of hydrocarbon chains, whether the chains are branched or not—branching produces smaller micelles—presence of electrolytes which increase the stability of micelles, and the temperature, which influences kinetic energy of the ions and so modifies micelle stability. The important thing about the micelles is that they solubilize fats, since the interior of the micelle—that is, the portion made up of free-moving paraffin chains—resembles the free paraffin itself, modified, it is true, to some extent by the sheath of hydrophilic groups and gegen-ions. In this way detergent micelles attach the grease to their internal portions, thereby removing them from the fabric.

There is one further important property of detergents that needs attention. They *denature proteins*. This leads to decrease in solubility of such compounds so that they precipitate more easily; they also lose their specific activity, especially when they are hormones and enzymes, and become more accessible to enzymic attack. Serum γ -globulin, for instance, becomes vulnerable to tryptic digestion. Many detergents—for example, ordinary soaps, gramicidin—are strongly bacteriolytic and cytolytic for this reason, hence their use in surgery.

Detergent Properties of Bile

Now it is generally agreed that bile acids in their normal neutral environment dissociate as their salts and act as detergents. Hence they promote emulsification and contact between water-insoluble and water-soluble compounds as in the digestion and absorption of fats from the intestines. Bile salts resemble the long-chain paraffin salts in possessing water-attracting (hydrophilic), and water-indifferent fat-attracting (hydrophobic) parts. The former includes $-OH$, $-SO_3$, $-COO$, or $COOH$ groups, the latter most of the nuclear $-CH_2$ groups together with the 3-methyl groups. At biological concentrations the bile-salt molecules form micelles similar to those formed by other detergents (Isaksson, 1954; Verschure and Mijnlief, 1956; Verschure *et al.*, 1956). The interior of these bile-salt micelles can dissolve fats, so that fatty substances exist in solution in micelles which are dispersed in aqueous medium because of their polar peripheral groups (Haslewood, 1955; McBain and Hutchinson, 1955). Though the solubilizing effect of whole bile is attributable mainly to the bile salts, a contributory part is also played by some bile acids, Na salts of fatty acids, long-chain fatty acids, lecithins, and perhaps related substances. Cholesterol and other steroids—for example, carcinogens, hydrocarbons (Ekwall *et al.*, 1951)—are partly dealt with in this way, partly through forming specific co-ordination compounds with bile acids. These latter are known as choleic acids, and they are generally water-soluble, which means that they can be readily absorbed from the intestine and no doubt just as easily transferred from the liver cells to the bile. In the pathway leading from the intestine to the liver cells, however, cholesterol is carried mostly as a macromolecular complex with α and β lipoproteins.

Bile constituents, like other detergents, can denature proteins and thus destroy bacteria and other cells. We are familiar with this property of bile through its well-known bacteriolytic and haemolytic action.

An interesting property of certain synthetic detergents is their capacity of forming synthetic fibres from proteins similar in many ways to natural protein fibres. Lundgren (1949) says that solubilized collagen is one of the proteins from which fibres are so formed most easily. It may be suggestive that some of the non-ionic detergents give liver fibrosis when painted on the skin of mice daily (Holsti, 1956). Holsti has also produced cirrhosis of the liver in rabbits by the frequent administration of desiccated bile. Obviously this field holds out promise of interesting developments.

Bile thus may be regarded as a detergent that launders the liver. It solubilizes greasy material and cholesterol that accumulates at the interface between the liver cell and the bile canaliculus, and flushes out the water-soluble components such as Na, K, Cl, creatine, glucose, and bilirubin complexes. Its surface-active properties facilitate its flow along the capillary spaces of the liver fabric—that is, the bile canaliculi—independently of any external force. Through its denaturing property it attacks protein materials that may have penetrated canaliculi, making them vulnerable to enzymic digestion and controlling the activity of hormones or enzymes that may have leaked through liver cells to the canaliculi. At the same time the bile salts carry a menace in their cytolytic property and their capacity to form fibrils with soluble collagen. The interaction with certain polar components in fats to form gelatinous components in the canaliculi cannot be ignored and may constitute the basis for bile thrombi.

Biliary Thrombi

Biliary thrombi (casts, cylinders, plugs) are found in many forms of jaundice, usually in dilated canaliculi and close to liver cells that show many pigment granules in their cytoplasm. But liver cells may be completely destroyed or be obviously degenerated in such localities, so that the origin of biliary thrombi is still uncertain. All observers agree that they first appear in the central zone of the lobule with

all types of jaundice, but that they are found in the peripheral zone when hepatitis is long-continued. Liver-cell damage, we have seen, is not an essential factor, for thrombi form regularly during the jaundice produced by certain drugs such as toluylenediamine, methyltestosterone (Werner *et al.*, 1950) and chlorpromazine (Menguy *et al.*, 1955). In none of these instances is there definite structural change in liver cells.

Alteration in the composition of bile may be important, and Eppinger (1937) assumes that abnormal occurrence of protein—albuminocholie—may follow cellular breakdown or altered permeability for protein and produce precipitation of bile components, especially bilirubin. The position is then analogous to that experienced with calculus formation, the cell debris or protein acting as a "nucleus" for precipitation or crystallization. Eppinger sees support for the idea in the association of protein in the bile and thrombus formation during toluylenediamine jaundice. Kühn (1947) has described thrombi composed of protein without bile pigment in the early stages of viral hepatitis, and these have also been figured by Altmann (1949) in livers of animals exposed to lowered atmospheric pressure. Weinbren (1952) suggests that when liver function is depressed or lost in necrosis hepatic cells do not secrete bile, so that the associated segments of canaliculi are empty. Adjacent still-functioning cells will continue to secrete, and, since there is no evidence of a gradient of bile flow in the canaliculi, quite likely some bile will pass towards the portal tracts and some away from it. The latter stagnates, becomes inspissated, and thus forms biliary thrombi, usually in the centrolobular zones. In fatal fulminant cases of hepatitis or necrosis few healthy liver cells remain, little or no bile is secreted, and bile thrombi are not formed (Wilson and Goodpasture, 1927; Mallory, 1947). In other words, bile thrombi are produced only when some hepatic cells are functioning.

It would seem, then, that loss of continuity of bile canaliculi due to destruction of liver cells favours the formation of bile thrombi (Movitt, 1947). Other writers suggest that the thrombi themselves favour obstruction of bile flow in the canaliculi leading to a type of jaundice that is partially obstructive (Hanger and Gutman, 1940; Dible *et al.*, 1943; Lucké and Mallory, 1946).

On the other hand, biliary thrombi have been detected in the liver some 24 hours before the onset of clinical jaundice (Mallory, 1947), and they are well to the fore in livers of children with neonatal haemolytic disease (Zolinger, 1946; Craig, 1950), which suggests that the primary factor may lie in disturbed liver-cell function, as was concluded from the study of jaundice due to certain drugs. Further support for this interesting suggestion comes from Smetana and Johnson (1955), who demonstrated giant liver cells and abnormal canaliculi of embryonal character distended with inspissated bile in a case of neonatal jaundice. Bile was being formed correctly enough in these cells, but there were no intercellular canaliculi to conduct it to the afferent channels.

Final Survey

I must now try to find out what we know for certain about biliary cirrhosis. A century of patient observation has taught us to recognize two clear-cut varieties, and to these I would add a third, as yet ill-defined, type which I have called canalicular stasis. To my mind this latter group holds many of the secrets of liver function, for it comprises disorders of bile production and excretion at the cellular level. All such functional upsets are a challenge to the researcher, no matter with what kind of cell he is concerned, but the reward is especially glittering when the discipline is applied to the diseased liver cell. We had come to a standstill in our search for the key to biliary stasis until our biochemical colleague, Dr. K. R. Rees, showed that bile can gravely disturb intracellular function, especially respiration.

Bile or one or other of its components must be locked up within the liver cell before it can suppress irreplaceable functions and so precipitate a cellular catastrophe. And it is not only in man that these singular anomalies may be initiated. Veterinarians are recording puzzling examples of non-obstructive biliary jaundice that can end in cirrhosis. I recall the disease of sheep known as geeldikkop that is an economic worry in South Africa, and the somewhat similar syndrome—facial eczema—that affects sheep and, to some extent, cattle in New Zealand. Distressing photosensitization symptoms tend to obscure the no less interesting liver disturbances that produce jaundice and serious hepatic failure. So far as one can judge from available accounts, these conditions lie midway between the biliary hyperplasias and canalicular stasis. Whatever may be decided eventually about their pathological status, there can be no question of the challenge they offer to the serious student of hepatic function.

With some relief I turn to the obstructive type of biliary cirrhosis, for here, it would seem, is a much simpler proposition. As I shall show, such an assumption is far from warranted, despite some encouragement from early analysis. Elsewhere Muzaffar Hasan and I have discussed four important questions that need answering if we are to understand how obstructive biliary cirrhosis is provoked, and I shall briefly recapitulate our arguments.

1. Since focal necrosis constantly develops soon after the extrahepatic ducts are blocked and continues in a cyclical fashion throughout prolonged obstruction it might well be thought that the ensuing fibrous-tissue repair and the associated bile-duct proliferation would stamp upon the organ the pattern of cirrhosis. But necrosis is extremely variable in extent and severity, and regional correspondence between it and fibrosis is so uncertain that I doubt its importance.

2. Secondary infection is no uncommon complication of biliary obstruction and plays a dominant part, as Oakley and I have shown, in fibrosis, though it cannot be blamed for the extensive bile-duct hyperplasia. All who have studied biliary obstruction have noted duct proliferation in sterile livers.

3. It is generally accepted that a prolonged rise in intraduct pressure seriously molests an organ whose duct has been obstructed. I need only recall the structural and functional distortion of hydronephrosis; a similar state of affairs is encountered in the liver, and indeed has been labelled hydrohepatosis by Counsellor and McIndoe (1926). Whether raised intraduct pressure acts by inducing ischaemia—such a factor apparently is not to be invoked in the early stages of biliary obstruction according to Dr. H. B. Stoner, who has measured blood flow in the liver at that time—or leads to a backflow of bile cannot be decided at present. Here is a fruitful field for investigation that might illuminate liver pathology.

4. That a chemical irritant may initiate bile-duct proliferation and fibrosis is an attractive idea. In recent years several such compounds have been discovered. Some, such as butter yellow, thiouracil, and thioacetamide, give abnormal proliferation of bile ducts and liver cells with fibrosis that may culminate in neoplasms of the liver. Others, such as α -naphthyl isothiocyanate, give hyperplasia, in rats, of small ducts alone (Lopez and Mazzanti, 1955). In our laboratory McLean and Rees (1958) have confirmed the Italian work; my colleagues also have shown that rats may be fed with this compound for as long as 16 months without developing neoplasms of the liver. Hepatic cells, after rather trivial focal necrosis, stand aloof from the proliferation. Recently McLean, Rees, and I have found that the duct hyperplasia is reversible, and we are now searching for the mechanism behind this extraordinary recovery.

There is, of course, a wide gap between such artificial experiments and human biliary cirrhosis, and so far no one has induced bile-duct proliferation and fibrosis by any component or derivative of bile, which is what must be done to consolidate the hypothesis. Holsti (1956), in Finland, has stated that he can produce cirrhosis by feeding rabbits for long periods with desiccated whole bile. The cirrhosis was portal in type, though a certain amount of bile-duct proliferation occurred. Until Holsti's important claim is confirmed and extended to, say, the rat, we must be cautious in applying it to our problem. Workers in my department are repeating and modifying the bile experiments in the hope of gaining new support for a chemical factor. Parabiosis experiments, especially those recorded by Moore *et al.* (1942) and Zeckwer (1949), support the chemical idea, since they show that ligating the common bile duct of one parabiotic rat leads to liver necrosis and bile-duct proliferation in its partner. The outlook, then, is encouraging, but the difficulties that lie ahead in pursuing this elusive will o' the wisp cannot be disguised.

I have here gone along many devious pathways and tried to surmount obstacles which I fear are insurmountable at present. To my mind, however, the prospect is hopeful, since the pitfalls along the way are clear enough and signposts beckon us onwards.

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REFERENCES

- Adam, N. K. (1937). *J. Soc. Dyers Col.*, 53, 121.
 Addison, T., and Gull, W. (1851). *Guy's Hosp. Rep.*, 2nd Ser., 7, part 2, p. 265.
 Abrams, E. H., Payne, M. A., Kunkel, H. G., Eisenmenger, W. J., and Blondheim, S. H. (1950). *Medicine (Baltimore)*, 29, 299.
 Albot, G. (1955). *Le Fole et la Veine Porte*, p. 204. Masson, Paris.
 — and Lunel, J. (1955). *Ibid.*, p. 155.
 Almaden, P. J., and Ross, S. W. (1954). *Ann. Intern. Med.*, 40, 146.
 Altmann, H. W. (1949). *Frankfurt. Z. Path.*, 60, 376.
 Barron, E. S. G., and Bumstead, J. H. (1928). *J. exp. Med.*, 47, 999.
 Brauer, R. W., Leong, G. F., and Holloway, R. J. (1954). *Amer. J. Physiol.*, 177, 103.
 — and Pessotti, R. L. (1950). *Ibid.*, 162, 565.
 Braunstein, H., Fellinger, K., and Pakesch, F. (1953). *Z. ges. exp. Med.*, 121, 254.
 Brokaw, R., and Penrod, K. E. (1949). *Amer. J. Physiol.*, 159, 365.
 Cameron, G. R., and Hasan, S. Muzaffar (1958). *J. Path. Bact.* In press.
 — and Mayes, B. T. (1930). *Ibid.*, 33, 799.
 — and Oakley, C. L. (1932). *Ibid.*, 35, 769.
 Cole, P. G., and Lathe, G. H. (1953). *J. clin. Path.*, 6, 99.
 Cook, D. L., Beach, D. A., Bianchi, R. G., Hamburger, W. E., and Green, D. M. (1950). *Amer. J. Physiol.*, 163, 688.
 Counsellor, V. S., and McIndoe, A. H. (1926). *Surg. Gynec. Obstet.*, 43, 729.
 Craig, J. M. (1950). *Arch. Path. (Chicago)*, 49, 665.
 Dible, J. H., McMichael, J., and Sherlock, S. P. V. (1943). *Lancet*, 2, 402.
 Ekwall, P., Lindström, E. V., and Setälä, K. (1951). *Acta chem. Scand.*, 5, 990.
 Eppinger, H. (1937). *Die Leberkrankheiten*. Springer, Vienna.
 Fagge, C. H. (1873). *Trans. path. Soc. Lond.*, 24, 242.
 Fleischl, E. (1874). *Ber. süds. Ges. Wiss.*, 26, 42.
 Franke, K. (1931). *Med. Klin.*, 27, 94.
 — (1936). *Z. klin. Med.*, 130, 193.
 Gibson, W. R., and Robertson, H. E. (1939). *Arch. Path. (Chicago)*, 28, 37.
 Grafflin, A. L. (1947). *Amer. J. Anat.*, 81, 63.
 — and Basley, E. H. (1952). *Bull. Johns Hopk. Hosp.*, 90, 395.
 Gray, C. H. (1957). *Brit. med. Bull.*, 13, 94.
 — and Kekwick, R. A. (1948). *Nature (Lond.)*, 161, 274.
 Grodins, F. S., Osborne, S. L., Ivy, A. C., and Goldman, L. (1941). *Amer. J. Physiol.*, 132, 375.
 Hanger, F. M., and Gutman, A. B. (1940). *J. Amer. med. Ass.*, 115, 263.
 Hanot, V. (1876). *Etude sur une Forme de cirrhose hypertrophique du Fole*. Baillière, Paris.
 — (1892). *La Cirrhose hypertrophique avec Ictère chronique*. Rueff, Paris.
 Hanzon, V. (1952). *Acta physiol. scand.*, 28, Suppl. 101, p. 228.
 Haslewood, G. A. D. (1955). *Physiol. Rev.*, 35, 178.
 Hirt, A., Ansorge, J., and Markstahler, H. (1938). *Z. Anat. Entwickl. Gesch.*, 109, 1.
 Höber, R. (1945). *Physical Chemistry of Cells and Tissues*, pp. 606-9. Blackiston, Philadelphia.
 — and Titajew, A. (1929). *Pflügers Arch. ges. Physiol.*, 223, 180.
 Holsti, P. (1956). *Acta path. microbiol. Scand.*, Suppl. 113.
 Isaksson, B. (1954). *Acta Soc. Med. upsallen.*, 59, 296.
 Jacoby, H. (1931). *Arch. exp. Path. Pharmacol.*, 161, 64.
 Klatskin, G., and Bungards, L. (1956). *J. clin. Invest.*, 35, 537.
 Klemperer, P. (1937). *J. Mt Sinai Hosp.*, 4, 279.

- Kühn, H. A. (1947). *Beitr. path. Anat.*, 109, 589.
 Lopez, M., and Mazzanti, L. (1955). *J. Path. Bact.*, 69, 243.
 Lucké, B., and Mallory, T. (1946). *Amer. J. Path.*, 22, 867.
 Lundgren, H. P. (1949). *Advanc. Protein chem.*, 5, 305.
 McBain, M. E. L., and Hutchinson, E. (1955). *Solubilization and Related Phenomena*. Academic Press, New York.
 McLean, M., and Rees, K. R. (1958). *J. Path. Bact.* In press.
 MacMahon, H. E. (1948). *Amer. J. Path.*, 24, 527.
 Mallory, T. B. (1947). *J. Amer. med. Ass.*, 134, 655.
 Martin, N. H. (1949). *J. Amer. chem. Soc.*, 71, 1230.
 Mayo, C., jun., and Greene, C. H. (1929). *Amer. J. Physiol.*, 89, 280.
 Mendeloff, A. I. (1949). *Proc. Soc. exp. Biol. (N.Y.)*, 70, 556.
 Menguy, R. B., Bollman, J. L., Grindlay, J. H., and Cain, J. B. (1955). *Proc. Mayo Clin.*, 30, 601.
 Moore, R. A., Hellman, L. M., and Jacobius, H. (1942). *Arch. Path. (Chicago)*, 34, 196.
 Moschowitz, E. (1952). *A.M.A. Arch. Path.*, 54, 259.
 Movitt, E. R. (1947). *Jaundice, its Pathogenesis and Differential Diagnosis*, p. 51. Oxford Univ. Press, New York.
 Pankhurst, K. G. A. (1953). *Lectures, Monographs and Reports*, No. 5. Royal Institute of Chemistry, London.
 Pavel (1949). Quoted by Albot (1955).
 Popper, H., and Schaffner, F. (1957). *Liver: Structure and Function*, p. 467. McGraw-Hill, New York.
 Reinhold, J. G., and Wilson, D. W. (1934). *Amer. J. Physiol.*, 107, 378.
 Ricketts, W. E., and Wissler, R. W. (1952). *Ann. intern. Med.*, 36, 1241.
 Rössle, R. (1930). In Henke and Lubarsch's *Handbuch der speziellen pathologischen Anatomie und Histologie*, Band 5, 1, 429. Springer, Berlin.
 Saunders, W. A. (1803). *A Treatise on the Structure, Economy and Diseases of the Liver*, 3rd ed., pp. 111-115. London.
 Schnedorf, J. G., and Orr, T. G. (1941). *Amer. J. digest. Dis.*, 8, 356.
 Seneviratne, R. D. (1949). *Quart. J. exp. Physiol.*, 35, 77.
 Shaffner, B. G. P., Doubilet, H., and Ruggiero, W. (1939). *Proc. Soc. exp. Biol. (N.Y.)*, 42, 203.
 Smetana, H. F., and Johnson, F. B. (1955). *Amer. J. Path.*, 31, 747.
 Snapper, I., and Bendien, W. M. (1938). *Acta med. Scand.*, 98, 77.
 Sobotka, H. (1937). *Physiological Chemistry of the Bile*, p. 96. Williams and Wilkins, Baltimore.
 Verschure, J. C. M., and Mijntjeff, P. F. (1956). *Clin. chim. Acta*, 1, 154.
 — de Wael, J., and Mijntjeff, P. F. (1956). *Ibid.*, 1, 511.
 Weber, F. B. (1903). *Trans. path. Soc. Lond.*, 54, 103.
 Weinbren, K. (1952). *J. Path. Bact.*, 64, 395.
 Werner, S. C., Hanger, F. M., and Kritzer, R. A. (1950). *Amer. J. Med.*, 8, 325.
 Wertheimer, E., and Lepage, L. (1897). *Arch. Physiol. norm. path.*, 9, 363.
 — (1899). *J. Physiol. Path. gén.*, 1, 259.
 Whipple, G. H., and King, J. H. (1911). *J. exp. Med.*, 13, 115.
 Wilson, J. D., and Goodpasture, E. W. (1927). *Arch. intern. Med.*, 40, 377.
 With, T. K. (1954). *Biology of Bile Pigments*. Frost-Hansen, Copenhagen.
 Wood, J. C. (1952). *J. Amer. med. Ass.*, 150, 1484.
 Zeckwer, I. T. (1949). *Arch. Path. (Chicago)*, 47, 424.
 Zollinger, H. U. (1946). *Helv. paediat. Acta*, Suppl. 2, p. 104.

SERUM GLUTAMIC OXALACETIC TRANSAMINASE IN DISEASES OF THE LIVER AND BILIARY TRACT

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LaDue, Wróblewski, and Karmen (1954) found that the enzyme glutamic oxalacetic transaminase was present in considerable quantities in the serum following acute myocardial infarction. A large number of studies have proved the diagnostic value of this laboratory test in acute myocardial infarction. More recently (Wróblewski and LaDue 1956b) it has been demonstrated that parenchymal hepatic diseases are also generally accompanied by marked elevation of the transaminase activity (T.A.) in serum. The more severe the disease the higher the activity in serum. In diseases of the biliary tract, on the other hand, this elevation is moderate and inconstant.

Karmen (1955) has worked out a spectrophotometric method for serum transaminase determination which is now extensively used in clinical practice. All our analyses were performed by the Karmen method without modification. In this paper we have restricted ourselves to discussing serum glutamic oxalacetic transaminase (S.G.O.-T.) determinations in hepatic diseases.

In many cases we have also determined the activity of serum glutamic-pyruvic transaminase (S.G.P.-T.). The latter has been reported (Wróblewski and LaDue, 1956a) to be of some value in the diagnosis of hepatic disease, but our experience in this respect is still limited and our results are not quite consistent.

The present series comprises 151 patients suffering from disorders of the liver and biliary tract—41 with acute hepatitis, 41 with cirrhosis, 42 with diseases of the biliary tract, and 27 with primary and metastatic carcinoma of the liver.

Acute Hepatitis

The activity of serum transaminase was determined repeatedly in 41 patients—adults and children—with acute hepatitis. These 41 were arbitrarily divided into two groups according to the severity of the disease: 16 mild cases (icteric index a maximum of <35 units and duration of jaundice <21 days) and 25 more severe cases (icteric index >35 units and duration of jaundice >21 days).

As is evident from Fig. 1, the icteric index and T.A. show a fairly parallel decrease in the mild cases, although the decrease of the latter is rather more abrupt, so that it reaches normal values sooner than the icteric index. In this group of mild hepatitis transaminase values of up to 400-500 units (T.U.)/ml. were found; this is about 10 times the maximum normal value. It will be seen that these rather high values were found at an early stage of the disease, almost coinciding with the onset of jaundice.

In the 25 more severe cases the T.A. reached much higher values, often 1,600 units/ml.

Fig. 2 gives the T.A. in a fairly severe case of acute hepatitis. This graph shows how the activity is constantly increasing in the early stages of hepatitis, reaching its maximum before the peak of the icteric index and returning to the normal range before the jaundice has subsided.

In addition to these patients, all of whom had been admitted with acute hepatitis during the days after the onset of jaundice, we had occasion to study the serum T.A. in the early prodromal phase of acute hepatitis and in very mild anicteric forms by following the course of an epidemic of hepatitis in a children's institution. This epidemic was fairly massive, 19 out of the 60 inmates—adults as well as children—getting hepatitis. Four adults and four children had fully developed hepatitis with jaundice, whereas 11 children presented the typical clinical appearance of anicteric hepatitis. The epidemic lasted three months, and there was evidence that the virus was transmitted from person to person via the faecal-oral route. The possibilities of food-

TABLE I.—Maximum Serum T.A. in 24 Cases of Proved Metastatic Carcinoma of Liver

Size of Metastases	No. of Cases	Serum T.A. Elevated	Normal	Range of Maximum Serum T.A.	Average of Maximum Serum T.A.
Smaller, limited	8	5	3	19-70 T.U./ml.	45 T.U./ml.
Larger, extensive	16	13	3	16-300 T.U./ml.	116 T.U./ml.

TABLE II.—Maximum and Average Serum T.A. in 41 Patients With Biliary Diseases

	No. of Patients	Maximum Icteric Index	Maximum T.A.	Average T.A.
Obstructive jaundice without inflammation of biliary tract:				
Cholelithiasis	14	10-125	<40-58	39.1
Cancer of biliary tract	6	40-200	<40-84	43.3
Obstructive jaundice with inflammation of biliary tract:				
Cholelithiasis and cholecystitis	17	12-110	42-228	105.8
Cancer of biliary tract	5	90-220	66-178	126.0