THE LIVER IN DISEASE

Part II.
CHAPTER 16.

THE LIVER IN OBSTRUCTIVE JAUNDICE.

Although the changes produced in the liver by obstruction of the bile ducts have been well studied in the experimental animal, the observations in man are few and poorly documented. Sometimes only an isolated operation or autopsy section is described and the larger series collected from the post mortem room have poor clinical correlations. Aspiration liver biopsy is obviously a suitable method not only of observing the state at any one time of the liver in obstructive jaundice, but by means of serial biopsies of following the changes during the course of the disease. The method has been previously used with success for this purpose (Roholm & Krarup, 1941).

Table 33.

OBSTRUCTIVE JAUNDICE:
Distribution of the case material.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of cases</th>
<th>Number of biopsies</th>
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<tbody>
<tr>
<td>Carcinoma of head of pancreas</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Carcinoma of bile ducts</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Carcinoma of Gall bladder</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Carcinoma secondary to stomach</td>
<td>5</td>
<td>8</td>
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<tr>
<td>&quot; rectum</td>
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<td>1</td>
</tr>
<tr>
<td>&quot; breast</td>
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<td>1</td>
</tr>
<tr>
<td>Gall stones</td>
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<td>Stricture of common bile duct</td>
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<td>1</td>
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<tr>
<td>Chronic pancreatitis</td>
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<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>25</strong></td>
<td><strong>31</strong></td>
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"Bile thrombi" lying in canaliculi.
Sinusoid with lining Küpffer cell is between the two large thrombi.
Methylene blue. X 950.
periphery of lobules but occasionally mid-zonal.

5. Occasionally increase in the number of Kupffer cells and round-celled infiltration of the sinusoids.

6. Preservation of the normal lobular architecture.

The accumulation of bile pigment is an early constant finding. This is most conspicuous at the centres of the lobules spreading outwards as the duration of obstruction progresses. Many of the central cells contain brown pigment diffused through the cytoplasm or aggregated into scattered granules. Bile thrombi, often branched or forked, are present in the canaliculi (fig. 92), and in the later stages may be seen extended into the sinusoids. The adjoining liver cells may show degenerative changes.

Another early feature is the increase in connective tissue in the portal tracts and with this the number of bile ducts multiplies. The bile ducts are elongated and tortuous. They have a wide lumen and are lined by high cubical epithelium. Monocytes also increase in the portal tracts. As the obstruction increases in duration the portal tracts send out strands of fibrous tissue which interlock with those from adjoining tracts so that eventually the lobule is enclosed by a band of connective tissue containing proliferating bile ducts. The essential lobular liver architecture is not disturbed.

Apart from the mild central degenerative changes the liver cell columns are well maintained. In the later stages there is sinusoidal distension and some
**Fig. 93.**  
**Case 1.**
Obstructive jaundice. Jaundiced 3 days.  
Preservation of the lobular architecture.  
Fibrosis and bile duct proliferation in the portal tracts. One small focal mid zone necrosis seen in bottom right hand corner of the field.  
Best's carmine stain. X 130.

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**Fig. 94.**  
**Case 1.**
Obstructive jaundice. Higher power view of central area. There are mild degenerative changes in the liver cells. The liver cells contain pigment granules. There is an increase in the number of Küpffer cells and of mononuclear cells in the sinusoids.  
Best's carmine stain. X 370.
FIG. 95.
Case 2. Biopsy 1.
Obstructive jaundice. Jaundiced 7 days.
Lobular pattern normal. Liver cells contain normal amount of glycogen; some show fatty change. Focal necroses are seen at the periphery of the lobule and in the mid zones.
Best's carmine stain. X 105.

FIG. 96.
Case 2. Biopsy 2.
Jaundiced 31 days. The portal tracts contain excessive connective tissue, proliferating bile ducts and round cells. Necroses are seen at the periphery of the lobule. One is joining on portal tract. Lobular pattern is well maintained.
Best's carmine stain. X 115.
FIG. 97.

Case 2. Biopsy 3.
Jaundiced 58 days. Very large necrosis of liver cells with central dense bile staining and surrounding ghost-like outlines of liver cells. The necrosis adjoins a portal tract.
Best's carmine stain. X 115.

FIG. 98.

Case 2. Biopsy 3.
Central area showing pigment deposition in hepatic cells and in the canaliculi. Some bile laden cells are undergoing necrosis.
Best's carmine stain. X 235.
the edge was palpable 4 cm. below the right costal margin. The gall bladder and spleen were not palpable. The serum bilirubin was 3.9 mg./100 ml.

The liver biopsy sections (figs. 93 and 94) show increased bile pigment at the centre of the lobules. The pigment is in the cells themselves and inspissated as thrombi in the canaliculi. The liver cells at the centre of the lobule show mild degenerative changes. Kupffer cell increases can be noted especially at the lobule centres. The portal tracts contain an increased amount of fibrous tissue and also proliferating bile ducts. An occasional small mid-zone focal necrosis is seen.

The next case is one in which the obstructive lesion was followed by serial biopsies. Portal zone bile-stained necroses were conspicuous.

Case 2. In January 1943 a 47 year old woman had a partial gastrectomy for carcinoma of the stomach. In July 1943 she was readmitted to hospital complaining of backache. On 27th August 1943 her eyes were noticed to be slightly icteric. A week later the serum bilirubin was 5.9 mg./100 ml and a liver biopsy was performed.

Liver biopsy 1. (fig. 95). The liver structure is well preserved. The portal tracts show only slight fibrous tissue increase. The liver cells contain their normal complement of glycogen, some show a little fatty change. Multiple focal necroses are
observed, usually in relation to the portal tracts, but occasionally in the mid-zones of the lobule. In the centre of the necrosis liver cells cannot be recognised but a fine lacework of reticulin can still be seen with here and there a pyknotic nucleus. The necrosis is not bile-stained. There is no surrounding cellular reaction and no attempt at organisation.

The jaundiced deepened and on the 31st day of icterus the serum bilirubin was 15 mg./100 ml.

Liver biopsy 2 (fig.96). Portal tract fibrosis and bile duct proliferation are more obvious. There is some increase of round cells in the portal tracts. The necroses are larger than those observed in biopsy 1. They are now solely in relation to the periphery of the lobule and in some cases they merge with the portal tracts. The centres of the necroses are bile-stained and through the lesion the delicate reticulin can still be seen. Apart from the necroses the liver cell columns are well preserved.

The icterus persisted at much the same level of intensity and on the 58th day of jaundice the serum bilirubin was 12.0 mg./100 ml.

Liver biopsy 3 (fig.97). Portal tract fibrosis has progressed. The lobular pattern is still well maintained. The peripheral necroses are larger and more numerous. Essentially they have the same structure as described previously. The centre consists of a mass of homogeneous orange-brown bile
and adjoining it is the loose bile-stained reticulin framework. In the meshes of the framework can be seen dense round nuclei, some belong to round cells, some are the degenerate nuclei of necrosed liver cells. Again there seems to be no surrounding cellular reaction to the lesion. The centres of the lobules show bile pigment both in the cells and in the canaliculi. Some of the pigment laden liver cells are undergoing necrosis (fig. 98).

Death occurred on the 60th day. At autopsy a large mass of secondary malignant glands was found in the porta hepatis. There was complete obstruction of the common bile duct. There were no neoplastic deposits in the liver substance.

The next case is one in which as the jaundice progressed the liver parenchyma showed a rather unusual degree of degenerative change.

Case 3. A 66 year old housewife was admitted to hospital suffering from carcinoma of the stomach. A few days later her eyes were noticed to be jaundiced and three days later the serum bilirubin was 7.2 mg/100 ml. and an aspiration biopsy was performed.

Liver biopsy 1. The sections show the usual obstructive features (fig. 99). There are central accumulations of bile. The portal tracts contain scanty fibrous tissue and proliferating bile ducts. Kupffer cells and monocytes are present in excess in the sinusoids.

Jaundice progressed and on the 25th day the serum

Obstructive jaundice. 3 days jaundiced. The lobular pattern is preserved. The portal tracts contain excess connective tissue and proliferating bile ducts. Sinusoids contain round cells and excess Kupffer cells.

Best's carmine stain. X 75.
Fig. 100.
25 days jaundiced. Appearances very similar to those described for biopsy 1.
Best's carmine stain. X 97.

Fig. 101.
Another field. Patchy glycogen staining. Occasional fatty change in liver cells. Some liver cells show necrosis. There are focal accumulations of round cells. Best’s carmine stain. X 175.
the serum bilirubin was 20mg./100 ml. A second liver biopsy was performed.

Liver biopsy 2. The histological picture varies from field to field. In some areas the picture differs little from that described in the first biopsy (fig.100). The trabecular pattern is preserved. The portal tract changes are minimal. Other parts show severe parenchymal changes (fig.101). The glycogen staining is patchy. Some of the cells show fatty change. Some of the cells are more obviously degenerate. Their nuclei may show pyknosis, the cell may be represented by only a faint outline. In some areas the cells have disappeared and their places have been taken by groups of round cells.

Such a severe change as this is unusual in obstructive jaundice. It was not constant throughout the section. The change was sufficient to be associated with an abnormality in the galactose tolerance test.

The next two cases emphasise the portal tract changes.

Case 4. A 62 year old man suffering from carcinoma of the ampulla of Vater. Progressive jaundice had been present for 42 days. The serum bilirubin was 9.1 mg/100 ml. A liver biopsy was taken at the operation of cholecystostomy.

Sections show the central lobular changes of obstructive jaundice. (Fig.102). The portal tracts appear oval in shape and are enlarged. This increase
**FIG.102.**

*Case 4.*
Obstructive jaundice. 42 days jaundiced. The centre of the lobule shows bile pigment and mild degenerative changes of the liver cells. The portal tracts are increased in size and are encroaching on the lobule. The lower and right hand tracts are being joined by a band of connective tissue containing bile ducts. H. & E. X 80.

**FIG.103.**

*Case 5.*
Obstructive jaundice. 23 days jaundiced. Great increase in connective tissue in the portal tracts. Proliferating bile ducts show cortical epithelium. There is fibrous tissue leakage between the portal tracts. H. & E. X 90.
has caused apparent shrinkage in the size of the lobule. The portal tracts contain excess fibrous tissue and distorted sinuous bile ducts. Some of the tracts show projecting bands of fibrous tissue which are interlinking with those from adjoining portal areas.

The next case shows a more advanced stage of the same portal tract changes.

**Case 5.** A 78 year old woman suffering from carcinoma of the gall bladder. There had been 23 days progressive jaundice. The serum bilirubin was 25 mg/100 ml. A liver biopsy was done at the time of exploratory laparotomy.

Sections (fig.103) show a very similar picture to that described in case 4. The portal tracts show a greater degree of fibrous tissue increase and are clearly joining with one another. The cubical epithelial lining of the proliferating bile ducts is conspicuous.

**Comment.**

Changes in the liver follow within a few hours of experimental ligation of a bile duct. Cameron & Oakley (1932), using the rat, record changes 1 hour after ligation of the common bile duct. Other workers, using different experimental animals, have recorded hepatic changes within 24 hours of bile duct ligation. (Richardson, 1911; Rous & Larimore, 1920; McMahon, Lawrence & Maddock, 1929). In man the classical
experience was that recorded by Eppinger (1902). Death occurred 36 hours after the accidental operative ligation of the common bile duct. Central zone pigmentation and biliary necroses were observed. In our series three cases were studied on the third day of icterus and four during the first week. All showed definite changes in the liver. The earliest is central zonal bile deposition. Small mid-zonal and portal cellular necroses appear about the same time. The bile duct proliferation in the portal tracts is also early but the portal fibrosis is at first scanty. After the first 2 weeks of jaundice no definite relation can be established between the histological picture and the duration. The liver in case 5, for instance, shows more advanced portal tract changes than that in case 4, yet the duration of jaundice (and of absent urobilin from the urine) was half that of case 4.

The causes of the hepatic changes are matters for speculation. The predilection of the bile pigment for the centres of the lobules has been variously attributed to easier discharge of bile from the cells nearest the bile ducts in the portal tracts, (Legg 1873) or to the lobule consisting of a central secretory and a peripheral absorbing portion (Bürker, 1901). It may be due to the cells at the centre of the lobule being able to produce a higher secretory pressure than those at the periphery. This may enable a little central secretion to continue. This, however, is pure theory. The large peripheral
bile stained necroses may be due to rupture of the small channels passing from the intralobular bile canaliculus to the branch of the bile duct in the portal tract, the canal of Hering (Heidenhain, 1883). Cameron & Oakley (1932) made reconstructions in rats and proved the relation of necroses to interlobular bile ducts. Herring & Simpson (1907) believe that the interlobular bile ducts are capable of allowing bile to pass out without any physical damage being present in the wall. They suggest this as the cause of the necroses. Cameron and Oakley believe that the total quantity of bile secreted is more important in the causation of the necroses than the pressure at which it is secreted. In our cases the pressure factor seems the more important. Focal necroses have been observed only in those cases where the obstruction is proximal to the entry of the cystic duct into the common duct or where the gall bladder has been non-functioning. It seems that the gall bladder can act as a safety valve on the pressure of bile produced by biliary obstruction (McMaster & Elman, 1926), and, if present, can prevent the pressure rising to the height necessary for the production of biliary necroses. The bile once spilt into the liver tissue is immediately toxic to the liver cells. It is surprising that surrounding cellular reaction is not more conspicuous and infection of the bile seems unlikely. The histological picture as a whole makes it doubtful whether infective
cholangiolitis plays any part in the production of the changes. The portal tract proliferation of bile ducts may be a response to the increased pressure within them (Steinhaus, 1891, Gerhardt, 1892, Rous & Larimore, 1920) or to irritation from static bile (Charcot & Gombault, 1876, Nasse, 1894, Ogata, 1913) or to a combination of these factors (Loeffler, 1927, Paolini, 1927, Lieber & Stewart, 1934). The diffusion of bile through the attenuated walls of the bile ducts stimulates the surrounding connective tissue (Richardson, 1911). The question of progressive fibrosis, liver cell regeneration and biliary cirrhosis will be discussed later.

Eppinger (1922) states that mechanically produced jaundice is due to tearing of bile capillaries by bile thrombi so that the bile enters the bloodstream by the perivascular lymphatics. Roholm & Krarup (1941) believe that the fault lies in the liver cells themselves which are wanting in their capacity to excrete bile. This seems the more likely. It is reflected in the high incidence of positive liver function tests in obstructive jaundice. The high proportion of cases showing impaired galactose tolerance and defective hippuric acid syntheses have already been discussed. The parenchymal damage existing in obstructive jaundice makes it difficult to discover laboratory methods to distinguish one type of jaundice from the other.

The hepatic changes which follow obstruction to the common bile duct show a constant pattern unrelated
to the cause of the obstruction. Our group contained a wide variety of obstructing factors (Table 33). The cases associated with gall stones, where a superadded infective process is often postulated, showed an identical picture to that of obstruction due to carcinoma.

The histogenesis of biliary cirrhosis.

The reported incidence of obstructive biliary cirrhosis has depended on the criteria adopted for the diagnosis of the disease. Rolleston & McNee (1929) use the term for fibrosis spreading from the bile ducts around the lobules of the liver and due to the obstruction of large bile ducts. This feature, as we have shown, is encountered in almost all cases of obstructive jaundice, even of quite short duration. Cases 4 and 5 show the spreading portal fibrosis quite clearly. If however the term cirrhosis is confined to lesions which include parenchymal degeneration, fibrosis and nodular parenchymal regeneration, then the incidence becomes much less. In a series of 244 cases of obstructive jaundice, collected from post mortem examination records, Gibson & Robertson (1939) found only 21 cases (8.6 per cent) which fulfilled these criteria. In our series of 25 cases there were 5 instances of true biliary cirrhosis. Observations on the experimental animal suggest that hepatic parenchymal regeneration does not occur in the presence of biliary stasis (Mann, Fishback, Gay & Green, 1931). In our group
**FIG. 104.**


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**FIG. 105.**


"Bile thrombi" present in large numbers at the centre of the lobule.

Methylene blue stain. X 105.
104.

105.
Fig. 107.
Cirrhosis with disturbance of the lobular architecture by bands of cellular connective tissue. Nodular hyperplasia of liver cells. Stained Best's carmine. X 145.

Fig. 108.
High power view showing a central vein (a) with clumps of bile pigment still present in the canaliculi (b) and scattered in the liver cells (c) Stained methylene blue. X 450.
nodular hyperplasia and disturbance of the lobular architecture have only been observed when the biliary obstruction has been intermittent or is released. This is illustrated by the next case.

Case 6.

A 37 year old soldier suffering from carcinoma of the head of the pancreas. On 22nd May 1945 he had been jaundiced 60 days. The serum bilirubin was 14.5 mg./100 ml.

Liver biopsy 1. Sections show the usual features of obstructive jaundice. (Fig. 104). There is some fibrosis and bile duct proliferation in the portal tracts. The centres of the lobules show accumulation of bile pigment (fig.105). The lobular architecture is well maintained.

On the 7th June 1945 the serum bilirubin was 12.5 mg/100 ml. The operation of cholecystostomy was performed. A liver biopsy was taken at operation.

Liver biopsy 2. Sections (fig.106) show a very similar picture to that shown in fig.104.

On the 10th July 1945 a second stage operation was undertaken. The gall bladder was anastomosed to the intestine. By the 3rd August the serum bilirubin was 0.5 mg/100 ml. A third aspiration liver biopsy was performed.

Liver biopsy 3. The sample was obtained with difficulty. As the trocar pierced the liver the organ felt very tough. The biopsy was in fragments,
a finding suggesting excess fibrous tissue.

Sections show a cirrhosis (fig.107). Fibrous tissue is passing not only around the lobule but has cut off nodules of hyperplastic liver cells. Even at this time (50 days after free drainage of bile) evidence of bile retention is seen at the lobule centres (fig.108). There appears to be no cellular reaction to the inspissated bile still present in the canaliculi.

It is important to note that parenchymal regeneration was observed only after release of the biliary obstruction. Three further cases have shown a similar picture of fibrosis with distortion of the lobular architecture and nodular hyperplasia of liver cells. In two the jaundice was due to gall stones, in the other there was a post-operative stricture of the bile duct. Intermittency of obstruction and of jaundice was a feature of them all. The cases which passed to a fatal termination without release of the obstruction have never demonstrated liver cell regeneration.

Karsner (1926) and other authorities have commented on occurrence together of biliary obstruction, obstructive jaundice and hepatic cirrhosis of Laennec type. That we are not dealing with two concurrent and unrelated lesions, namely obstructive jaundice and "portal" cirrhosis is quite clearly shown by the serial biopsy studies in case 6.
If intermittent obstruction to the common bile duct continues over a number of years progressive, irreversible changes are produced in the liver. This is illustrated by the next case.

Case 7. A 64 year old refugee doctor. 20 years previously he had his first attack of biliary colic and jaundice. A second severe attack was experienced in 1933 and in 1941 following another jaundiced episode the liver was first noticed to be enlarged. Since then until the present admission to hospital (April 1945) there have been recurrent attacks of jaundice. The icterus is preceded by pruritus and is associated with severe epigastric pain and fever. The urine becomes dark brown. The stools are a normal colour. Examination shows the liver enlarged 6 cm. below the right costal margin. The spleen is firm and palpable 4 cm. below the left costal margin. There is no evidence of portal vein obstruction or of finger clubbing. The urine contains excess of urobilin. The serum bilirubin is 0.5 mg per 100 ml. and the serum phosphatase 19 units per 100 ml. Cholecystograms show non-filling of the gall bladder.

Aspiration liver biopsy was easy but the liver substance felt very hard and "gritty" and the biopsy was very fragmented. Bacterial culture of a fragment of liver was sterile.

Sections show an extremely active cirrhosis (fig. 109). The fibrous tissue bands are very cellular.
Figure 109. Case 7.
Active biliary cirrhosis.
Stained Best's carmine. X 105.
Figure 110. Case 7.
Higher power view of a fibrous tissue band. Note proliferating bile ducts. Stained Best's carmine. X 220.

Figure 111. Case 7.
The cellular content of the fibrous tissue bands. Note polymorph leucocytes, fibroblasts and degenerating liver cells containing brown pigment. Stained Best's carmine. X 460.
They contain fibroblasts, leucocytes and proliferating bile ducts (figs. 110 and 111). The liver cells show some degenerative changes. There are a few "bile thrombi" suggestive of some bile retention.

While in hospital the patient had one of his attacks. The temperature rose to 102° and he became jaundiced. The serum bilirubin was 4.2 mg/100 ml. and the serum phosphatase 25 units /100 ml. A course of penicillin was later given with no clinical improvement.

Comment.

The two cases clearly illustrate the problem of biliary cirrhosis.

The first case (Case 6) demonstrates the histogenesis of the lesion and emphasises the fact that the true biliary cirrhosis with disturbance of lobular architecture and parenchymal regeneration is only present in intermittent obstruction. The second (Case 7) demonstrates the possibility of progressive cirrhosis developing in a case of intermittent biliary obstruction. The original lesion in this case was almost certainly gall stones and for the last 20 years recurring biliary obstruction has led to portal tract fibrosis followed by hepatic cell regeneration as the obstruction passes off. The end result is an irreversible and, we believe, progressive biliary cirrhosis. It is unlikely whether operative removal of the gall stones could now prevent
the progression of the lesion. Weir & Snell (1936) record latent icterus and abnormalities of the brom-sulphthalein test years after relief of the obstruction. The hepatic lesion is sometimes seen to progress after operation. Case 7 might be included in the group of cirrhosis described by Hanot (1872). There are various discrepancies however. The age of the patient, the absence of jaundice between attacks and the raised serum phosphatase are all unusual in Hanot's cirrhosis.

The Diagnosis of Obstructive Jaundice by Aspiration Liver Biopsy.

In obstructive jaundice the histological picture of aspiration liver biopsy sections are usually quite characteristic and a diagnosis is readily made from hepatitis whether acute or chronic.

The bile pigment accumulations are far in excess of those seen in hepatitis. Moreover they are located centrally in the lobule compared with acute hepatitis in which they are mid-zoned and the liver in cardiac failure jaundice in which they are peripheral. The proliferating bile ducts are lined by cubical epithelium and have a wide lumen, the bile ducts in hepatitis usually have a flatter lining and the lumen is slitlike. Parenchymal degeneration is a much more conspicuous feature in hepatitis than in obstructive jaundice. Diagnostic difficulties are only likely to arise when the obstruction is of short duration or where the hepatitis is healing. This is especially so where the
Figure 112. Case 8.
Obstructive jaundice. Jaundiced 35 days.
Portal tract fibrosis with some polymorph infiltration.
Stained H. & E. X 205.
obstructive case shows large numbers of round cells and Kupffer cells in the sinusoids (cf. fig. 104). This may resemble a healing hepatitis (cf. fig. 30). Difficulties also arise when the obstruction is intermittent.

**Case 8.** A 57 year old woman complained of progressive jaundice of five weeks duration. The liver was felt 8 cm. below the right costal margin. The spleen was also palpable. Serum bilirubin was 4.3 mg./100 ml., the serum phosphatase was 65 units.

Aspiration liver biopsy sections (fig. 112) show portal tract fibrosis together with some polymorph infiltration. Bile duct proliferation is not conspicuous. There is scattered bile pigment in the mid- and central zones. Bile necroses are not seen.

A diagnosis was made of hepatic cirrhosis. Jaundice subsided and she was discharged well.

Five months later she was readmitted to hospital. The liver now felt obviously nodular and neoplastic. At autopsy a diagnosis of ulcerating carcinoma of the ampulla of Vater was formulated. Obstruction to the common bile duct was incomplete.

This case gives an indication of the difficulties which may arise in the interpretation of such small samples, especially when the clinical features are atypical. Usually, however, aspiration biopsy provides a definite means of diagnosing primary hepatitis from the changes resulting from occlusion of the common bile duct.
Liver in Obstructive Jaundice: Summary.

The histological state of the liver in 25 cases of obstructive jaundice has been studied by aspiration and operation liver biopsies.

The essential pathological features are the central zone bile pigment accumulations and the portal tract fibrosis with bile duct proliferation. Midzonal and peripheral focal necroses of liver cells may be seen. The essential liver lobular structure is maintained.

The histogenesis of obstructive biliary cirrhosis is discussed. Nodular parenchymal hyperplasia seems only to occur in the presence of intermittent obstruction.

The use of aspiration liver biopsy in the diagnosis of obstructive jaundice from acute and chronic hepatitis is mentioned.
CHAPTER 17.

THE USE OF ASPIRATION LIVER BIOPSY IN THE DIAGNOSIS OF HEPATIC MALIGNANT DISEASE.

The accurate diagnosis of malignant disease is of great importance both in the consideration of prognosis and of treatment. Once hepatic malignant deposits have been identified the scope of any surgical procedure is much reduced and the outlook is virtually hopeless. Any method of definitely recognising hepatic malignancy is therefore to be welcomed. Four cases will be described in which specimens of hepatic malignant tissue were obtained by aspiration liver biopsy, the general application of the method will then be discussed.

Case I.

A 65 year old police pensioner was admitted to Hospital on the 12th January, 1944. He gave a history of 4 months generally failing in health. 5 weeks previously he had attended another hospital, an epigastric mass had been noted and a barium meal examination made. This latter showed only varying pressure deformity along the lesser curvature of the stomach. A lesion of pancreas or liver was suspected. 3 weeks before admission to Hammersmith Hospital the patient became jaundiced, the yellowness of the skin was said to vary in intensity. The urine was dark and the stools pale. There had been no change in the
bowel habit and he had never passed blood with the
motion. The ankles became swollen. Past health
had been excellent. The patient was a man of
moderate habits.

Examination showed a deeply jaundiced cachectic old man.
There was oedema of sacrum and legs. The abdomen was
distended and shifting dullness could be elicited.
Two distended veins passed from the umbilicus towards
the costal margin, the direction of blood flow was
cephalic. A huge knobbly liver could be felt filling
the upper abdomen. Rectal examination revealed no
abnormalities.

Urine examination. Specific gravity 1021. Acid. Albumen
present. Bile present. Urobilin absent. Microscopy
of the centrifuged deposit showed numbers of coarse
granular casts.

Haematology. 14.1.45. Erythrocytes 4.8 million per cu.mm
haemoglobin 80 per cent (Haden), colour index 0.84,
Leucocytes 12,000 per cu.mm., of which lymphocytes were
1320, monocytes were 120 and neutrophil polymorphs
10, 560.

Biochemistry.

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**Fig. 113.**

Case 1.

Hepatic malignant disease. Longitudinal section of liver showing widespread neoplastic involvement.

**Fig. 114.**

Case 1.

Hepatic malignant disease. Typical columnar celled adenocarcinoma.

Stained H.E. X 190.
Stools. Occult blood. 17.1.44. Trace. 18.1.44. Present. 19.1.44. Absent.

Liver Function Tests.

Intravenous hippuric acid test 0.12 g. excreted in 1 hour. Galactose time 98 minutes.

Aspiration Liver Biopsy. 17.1.44. Specimen appears whitish in colour. Histological examination shows a typical columnar celled carcinoma (fig.114). There is only a small fragment of liver seen and the liver cells show some compression and atrophy.

Progress. Steadily downhill and patient died on 28.1.45.

Autopsy. The relevant findings were a fungating carcinoma of the pelvi-rectal junction of the colon. The liver weighed 5, 300 g. and was heavily infiltrated with secondary growth (fig.113). Secondary growth had obstructed the common bile duct and there was some pressure on the portal vein in the porta hepatis.

Comment. The failure to diagnose the site of the primary lesion was due to its situation at the pelvi-rectal junction. It is well known that barium meals or enemas and rectal examinations are almost useless for this purpose. Had the patient lived longer sigmoidoscopy would have been performed. The degree of replacement of liver tissue by growth will account for the impairment of galactose tolerance. The low
serum proteins and the low hippuric acid excretion rather reflect the general cachectic state of the patient.

Case II

A 65 year old carpenter was admitted to hospital on 21st May, 1942. He complained of anorexia, epigastric discomfort and constipation of 3½ months duration. He had lost 4 stones in weight. Past health was uneventful.

Examination revealed a thin, pale, little man. The skin was lax and ineleastic. No abnormal masses were palpable in the abdomen and there was no hepatomegaly. Urine showed only a trace of Albumen.

Fractional test meal Histamine fast Achlorhydria.

Faeces.. Occult blood absent on 5 occasions.

Barium meal was reported as "no alimentary lesion discovered."

Intravenous pyelograms were normal.

Haematology. Erythrocytes 3.7 million per cu.mm.

Haemoglobin 60 per cent (Haden). C.I. 0.61.

Leucocytes 50,000 per cu.mm., of which 47,000 were neutrophil polymorphs, 500 eosinophil polymorphs, 2,000 lymphocytes and 500 monocytes.

Progress. A definite diagnosis could not be formulated. However on the 6th June 1942 a small firm nodule was felt in the lower border of the liver; aspiration liver biopsy was performed.
**Fig. 115.**

Case 2.

Hepatic malignant disease. Secondary hepatic squamous epithelioma with cell nests.

Stained Best's Carmine. X 100.

---

**Fig. 116.**

Case 3.

Hepatic malignant disease. Melanocarcinoma.

Liver tissue replaced by malignant tissue. Many of the neoplastic cells contain melanin pigment.

Stained H.E. X 100.
Liver Biopsy was easy. Sections showed the typical appearances of squamous celled carcinoma. Cell nests were conspicuous (fig. 115)

Comment. A source was sought for the primary growth. A gall bladder tumour was considered unlikely in the absence of jaundice. Renal pelvis carcinoma was unlikely with normal intravenous pyelograms. A small bronchial carcinoma seemed possible even though chest X-Ray had proved negative. A gastric neoplasm was unlikely with no occult blood in the stools, moreover a squamous carcinoma is very unusual in this site. The oesophagus was considered but rather excluded by the report of a normal barium swallow. This latter examination was however repeated. The report stated that the oesophagus showed considerable pressure deformity and marked irregularity of outline over such a wide area a malignant lesion was doubted. Oesophagoscopy was advised, the patient however died before this could be carried out.

Autopsy showed an extensive oesophageal carcinoma of a polypoid nature. The lesion had not produced an oesophageal obstruction. There were widespread carcinomatous metastases in the liver.

Case III

A 60 year old housewife was admitted to Edinburgh Royal Infirmary on the 12th March, 1943. She complained of loss of weight and fainting turns for
3½ months. The right side of the abdomen had become enlarged. The ankles had been swollen for 2 weeks. The urine was dark.

In the past the patient had suffered from rheumatic fever at the age of 20 and 2 years previous to this admission the right eye had been removed for a tumour of the choroid.

Examination showed a pale, ill-looking woman. The right eye was artificial. There was a fullness in the epigastrium and right flank, this was due to massive enlargement of the liver. The organ felt hard and nodular.

Urine darkened on exposure to air. The urine gave all the reactions for melanogen.

Biochemistry.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma urea nitrogen</td>
<td>23 mg./100 ml.</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>246 &quot;</td>
</tr>
<tr>
<td>Albumen</td>
<td>2.4 g/100 ml.</td>
</tr>
<tr>
<td>Globulin</td>
<td>3.2 g/100 ml.</td>
</tr>
<tr>
<td>Albumen/Globulin A/G</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Melanogen was not detected in the blood.

Laevulose Tolerance Test - marked impairment of function. A maximum of 28 mg/100 ml. blood laevulose was recorded.

Haematology.

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>4.22 million/cu.mm.</td>
</tr>
<tr>
<td>Haemoglobin (Sahli)</td>
<td>80 per cent.</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>10,800 per cu.mm.</td>
</tr>
</tbody>
</table>

Aspiration Liver Biopsy.

The specimen consisted of brownish black material. Sections showed that the liver tissue had been entirely
replaced by malignant material. The tissue consisted of cells containing melanin and groups of cells devoid of pigment (fig. 116) The diagnosis of secondary malignant melanoma was formulated.

**Progress.** The patient became steadily worse. She collapsed suddenly and died on 23.3.43.

**Autopsy.** Essential findings were a liver weighing 6920 g., almost completely replaced by secondary melanoma, and secondary deposits in heart, kidneys and suprarenals.

**Comment.** The history of ocular tumour, the massive liver and the appearance of melanogen in the liver were sufficient to make a diagnosis. Liver biopsy findings were of general interest rather than of diagnostic value.

**Case IV.**

A 57 year old woman was admitted to hospital on 13th October, 1943. She complained of abdominal discomfort and flatulence for one month. Her appetite was poor and she had lost weight. There were no other symptoms.

**Past Health.** In March 1936 the patient had had a panhysterectomy for metrorrhagia and menorrhagia. The uterus was large and contained fibroids. Histologically the endometrium was thickened and showing dilated glands. There was no evidence of malignancy.

**Examination.** showed a tall woman of average physique.
Case 4.

The abdomen was distended and the whole of the right hypochondrium was filled with a large irregular liver. There was slight pitting oedema of both ankles. Urine showed only a trace of albumen.

Radiological findings. Chest. No abnormality seen. Barium meal showed an elongated stomach displaced by a mass arising apparently from the liver. Barium Enema showed no colonic lesion.

Faeces. Occult blood absent on 3 occasions.

Biochemistry.

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum bilirubin (mg/100 ml)</td>
<td>0.5</td>
</tr>
<tr>
<td>&quot; phosphatase (units/100 ml)</td>
<td>13</td>
</tr>
<tr>
<td>&quot; albumen (g/100 ml)</td>
<td>3.1</td>
</tr>
<tr>
<td>&quot; globulin (g/100 ml)</td>
<td>2.0</td>
</tr>
<tr>
<td>A/G</td>
<td>1.55</td>
</tr>
</tbody>
</table>

Liver Function tests. Intravenous hippuric acid test 0.42 g. Galactose time 32 minutes.

Haematology. Erythrocytes 5.3 million/cu.mm.

Haemoglobin (Haden) 92 per cent. C.I. 0.87. Leucocytes 5,000 per cu.mm. of which 1,700 were lymphocytes, 200 were monocytes and 3,100 neutrophil polymorphs.

Aspiration Liver biopsies were performed on 11.10.43 and 2.2.44. The specimens were dead white in appearance. In neither instance did sections show any normal liver tissue. The specimen consisted of a mass of maturing neoplastic looking connective tissue with abundant reticulin formation. (fig.117) The diagnosis was fibrosarcoma of the liver.

Progress. 2 months after admission gross evidence of
inferior vena cava obstruction was observed. 4 months after admission the patient was noticed to be slightly jaundiced, bile appeared in the urine and the serum bilirubin rose to 3.7 mg. per 100 ml. Jaundice did not increase. There was a gradual failure of health and the patient died on the 14th March, 1944.

Autopsy. The essential finding was a very large liver (weight 5.5 Kg). The organ was invaded by massive malignant deposits. The growth had not caused compression of the common bile duct, a large intrahepatic bile duct however had been occluded. The inferior vena cava was compressed as it passed posterior to the liver and thrombosis had occurred below the site of compression. Some secondary deposits were found in the mesentery, lungs, kidneys, thyroid and vertebrae. Careful search failed to reveal a site for the primary growth.

Comment. Primary sarcoma of the liver is a rarity. It probably has its origin in the scanty amounts of connective tissue found in the portal tracts. Secondary sarcoma is also rare. The possibilities in the case described above are that the hepatic sarcoma was primary, or more probably that the lesion occurred secondary to a sarcoma of the uterus. The sarcoma was probably the result of malignant degeneration in a uterine fibroid. The microscopical examination of the uterus removed at operation is not against this
diagnosis. Unless serial sections are taken through the organ a malignant area can easily be missed. The long interval (7½ years) between the operation and the evidence of secondary deposits is unusual.

Discussion.

The majority of reports on the diagnostic application of aspiration liver biopsy have included instances of malignant disease recognised by the method. Tripoli and Fader (1941) identified metastatic carcinoma six times. Hatieganu et al., (1943) recommend the method for the early diagnosis of hepatic carcinoma. Baron (1-31) identified carcinoma in 12 out of 14 cases in which the lesion was eventually proved at autopsy. However, as is well-known, hepatic malignant deposits are localised and, in the early stages, small and few in number. Any biopsy method using a narrow bored trocar and cannula must of necessity rely on chance in the piercing of a deposit. With such a liver as shown in fig. 113 it would obviously be difficult to avoid obtaining a piece of malignant tissue, the identification of a malignant deposit at this stage however becomes a matter of academic interest. The proportion of cases in which a positive diagnosis of hepatic carcinoma has been made by aspiration liver biopsy is so linked with the laws of chance that statistical analysis seems of little moment. In our series, 19 cases, in whom a diagnosis of hepatic malignancy was confirmed by autopsy,
were submitted to liver biopsy and in 8 a specimen of the tumour was obtained. This proportion is not very encouraging to advocates of routine aspiration biopsies before operations in cases of malignant disease. The procedure should be reserved for the special case (as in case 2), where a hepatic secondary deposit is suspected and diagnosis is otherwise obscure; however failure to demonstrate the neoplasm will not exclude its presence.

**SUMMARY.**

Four cases are described in which aspiration liver biopsy made a positive diagnosis of hepatic malignant disease.

The application of the method to the study of hepatic malignant disease is discussed.
Amyloid disease can be one of the easiest conditions to diagnose or can present great difficulty. Two cases will be described. In one (case II) the complete text-book picture presented, and the diagnosis required little confirmatory evidence from the laboratory. The other (Case I) required all additional aids for diagnosis. In both cases tissue obtained by aspiration liver biopsy was used to demonstrate the amyloid material.

Case Report I.

A 26 year old clerk first came under observation in April, 1940. The main complaint was progressive diarrhoea of 8 months duration. The stools were fluid and brown. He never passed blood or slime. They occurred 2 - 3 hourly. The diarrhoea had been slightly lessened by a low residue diet. During the illness the patient had lost 2 stone in weight. He often felt hot and feverish. Appetite was good. He had been an inpatient in two other hospitals, in one of which a diagnosis of colitis was made and in the other tuberculosis of the intestine.

Past Health. was not relevant.

Family Health was good. No cases of tuberculosis in the
TABLE 34.

HEPATIC AMYLOIDOSIS.

Weight, liver size and laboratory findings in Case I.

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight (lbs)</th>
<th>Liver Size</th>
<th>Congo red % eliminated in 1 hour</th>
<th>Total serum proteins g/100 ml.</th>
<th>Serum Cholesterol mg/100 ml.</th>
<th>Urine Albumin</th>
<th>Max.Sp.Gr</th>
<th>Urea Clearance % normal</th>
<th>Hb.% (Haden.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/40</td>
<td>100</td>
<td>8</td>
<td>100</td>
<td>7.4</td>
<td>128</td>
<td>++</td>
<td>1008</td>
<td>-</td>
<td>68</td>
</tr>
<tr>
<td>11/41</td>
<td>112</td>
<td>8</td>
<td>100</td>
<td>7.8</td>
<td>200</td>
<td>+++</td>
<td>1010</td>
<td>49</td>
<td>61</td>
</tr>
<tr>
<td>2/42</td>
<td>118</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>1015</td>
<td>50</td>
<td>84</td>
</tr>
<tr>
<td>10/42</td>
<td>127</td>
<td>3</td>
<td>50</td>
<td>7.8</td>
<td>-</td>
<td>+</td>
<td>1018</td>
<td>82</td>
<td>110</td>
</tr>
<tr>
<td>11/43</td>
<td>122</td>
<td>0</td>
<td>50</td>
<td>7.7</td>
<td>190</td>
<td>0-Tr.</td>
<td>1015</td>
<td>83</td>
<td>98</td>
</tr>
</tbody>
</table>
family.

Examination. Thin pale man. Abdominal examination showed the liver to be enlarged 8 cm. below the right costal margin. The organ was smooth, moderately firm and slightly tender. Spleen and kidneys were not palpable. There was no ascites. Physical examination revealed no other abnormalities.

Investigations.

Laboratory data are tabulated (table 34)

Blood Wassermann - Negative.

Stools. Tubercle bacilli not isolated.

   Occult blood absent.

   Fecal fat content - total, solid and unsplit
   within normal limits.

Sigmoidoscopy One small superficial ulcer seen in upper rectum.

Radiological Examination Barium meal, barium enema and
x-ray of chest had been performed elsewhere and reported as showing no abnormal features.

Mantoux Reaction. 1:10,000 and 1:1,000 Negative.

Progress. Temperature rose to 99 - 100°F almost every evening. Diarrhoea remained uncontrolled. Patient gained 2 lbs. weight. Discharged home (devoted mother) to live under a "sanatorium regime."

Diagnosis. Amyloid disease of unknown causation.

Readmitted 4th November, 1941 for further investigation.
Following discharge from hospital the patient spent three months in bed. The diarrhoea was very severe and there was continuous pyrexia to 103°. Since August he has managed to be up and about but the diarrhoea has continued and there has been an occasional evening rise of temperature. Gained 12 lbs. in weight.

Examination. Liver still palpable 8 cm. below right costal margin. Visible peristalsis noticed in the hypogastrium.

Investigations.

Stools. No pathogenic organisms.

Occult blood present on one occasion. Slight excess of fat present (31% dried weight) fairly well split.

Radiological Examinations.

Barium Meal. An ileosigmoid fistula was demonstrated.

Barium Enema. The presence of ileosigmoid fistula was confirmed. The barium passed from the distal end of the large gut into a coil of small intestine.

Hippuric Acid Synthesis Test (oral method) 4 g. excreted in 4 hours (Normal)

Progress. With the finding of the fistula it was decided to submit the patient to operation.

Operation 19.11.41. The relevant details were:-

(1) Large smooth liver - biopsy taken.

(2) Indefinite mass in the pelvis chiefly in the right side involving two loops of the ileum.
Several small specks were seen on the peritoneum of the ileum and one of these was taken for section.

It proved impossible to deal directly with the ileosigmoid fistula and an ileocaecostomy was performed in the hope that this would relieve the diarrhoea and possibly lead to closure of the fistula.

Liver biopsy taken at Operation.
The liver shows extensive amyloid disease.

Nodule on the Visceral Peritoneum.
Shows chronic non-specific inflammatory changes with considerable fibrosis. No histological evidence of tuberculosis.

Progress.
Recovery was uneventful and the patient was discharged home on the 17th December, 1941.

Diagnosis. Ileosigmoid fistula of unknown aetiology.

Amyloid disease.

Readmitted 6th February, 1942, for closure of the ileosigmoid fistula. Since the ileocaecostomy the patient felt much better. He now has four motions per day. The stools are bulky and fairly well formed.

Investigations are tabulated. (table 34)

Operation 18th February, 1942. Essential findings were:

(1) Adhesions were found uniting adjacent coils of bowel. A large mass was also present and the pouch of Douglas firmly attached to the pelvi rectal
juncture of the sigmoid colon, posterior-superior aspect of the bladder, the pelvic floor and the sacrum. The consistency was that of chronic granulation tissue.

(2) The ileosigmoid fistula was located. On the ileal surface small translucent white nodules were present.

(3) Adhesions prevented closure of the ileosigmoid fistula.

(4) A thin portion of the ileum was removed for section.

Section of Ileum.

The only granulomatous lesion is a small patch of foreign body reaction in the subserous coat.

Progress.

Excellent recovery from operation. Discharged 20th March 1942.

Readmitted 12th October, 1942.

Following the last discharge from hospital the patient remained well until May, 1942. At this time he noticed that he was passing dark material in the urine. The material was black and had floating particles in it. Occasionally he passed bubbles. The colouration was not constant and at first only occurred once a week. On admission to hospital it was recognised 3 - 4 times a week. Otherwise he felt fairly well. The bowels moved 5 - 6 times in 24 hours. The motions were sometimes formed, sometimes loose and watery. He had gained over a stone in weight.
Investigations.

Urine. A dark coloured specimen was available. It was brownish black and on standing showed a flaky solid deposit. The odour was faecal.

Culture showed a growth of paracolon bacilli.

Cystoscopy. No cystitis seen. On the posterio-superior surface of the bladder a sharply defined depression was seen which was probably the opening of a fistula into the bowel.

Operation 27th October, 1942.

(1) Dense intra-abdominal adhesions again noticed.
(2) The fistula between large bowel and superior surface of bladder was demonstrated, the two viscera were separated and the fistula closed. Curettings from the fistulous track were taken for section.

Sections of the curettings show non-specific granulation tissue. Giant cells are seen but as there is no tubercle formation. Acid fast bacilli are not found.

Progress.

The patient made an uninterrupted recovery and was discharged on the 23rd November, 1942.

Readmitted 12th November, 1943 because of head injury.

On October 10th the patient had been involved in a bicycle accident. He was unconscious 4-5 days and there was retrograde amnesia. Since then there has been headaches and bad dreams. The diarrhoea has been much
Case 1.

Hepatic amyloidosis. Heavy infiltration of hepatic sinusoids with pale staining amyloid. Modified Van Gieson. X 112.
less. He now has four to five fairly well formed motions daily. There is no urgency and he is able to regulate the motions to regular convenient times. He has been on a normal diet. The weight has been fairly well maintained. On 1st June he was able to return to his work (clerical) and remained at work till the accident.

**Examination** The patient was well developed. Liver not palpable.

**Investigations.**

**Liver Function Tests.** Intravenous hippuric acid test 1.1 g. excreted in 1 hour. (Normal). Intravenous galactose tolerance test - galactose time 69 minutes (Normal)

Bromsulphthalein excretion test (5mg/kilo) No retention of dye in blood in 30 minute specimen.

**Barium Meal.** Appearance indicate ileosigmoid fistula. Most of the barium passes along the ileum to enter the caecum.

**Aspiration Liver Biopsy.** The liver showed still infiltration with amyloid disease although this was considerably less coarse than in 1941 (fig 118)

**Progress.** Discharged 20th December, 1943. Reported 18th January 1944 and was given permission to resume work.

**Final Diagnosis.** 1) Ileosigmoid fistula of unknown aetiology. 2) Atypical Crohn's disease. 2) Amyloidosis of liver.
Case II

A 29 year old housewife was first admitted to Hamp- 
smith Hospital on the 3rd March, 1941. A diagnosis 
of active bilateral upper lobe pulmonary tuberculosis 
was made and the patient was transferred to a san-
torium for further treatment. She was next admitted 
to hospital in February, 1944. She had been in a 
sanatorium until three weeks previously and had had 
gold therapy. 12 months previously she had first 
noticed borborygmi. 6 months ago attacks of vomit-
ing and diarrhoea started and these were associated 
with cramp-like pains in the epigastrium. Three 
weeks before admission she had noticed swelling of 
the legs and abdomen.

Examination showed a pale woman with a malar flush. 
The skin was waxy. Nutrition was good. There was 
bilateral finger clubbing. The abdomen was distended 
and a fluid thrill could be elicited. The liver was 
palpable 6 cms. below the right costal margin. Spleen 
could not be felt. There was ankle oedema.

Investigations.

Chest X-ray. Bilateral apical tuberculosis with 
cavitation.

Sputum. Acid fast bacilli present in small numbers.

Barium Meal Examination.

Irregular constriction and dilatation of the ileum, the 
lumen is rather of ragged outline and of varying
diameter. The appearances suggest ileitis.

Stools. Unformed, brown in colour and offensive. Acid fast bacilli present. Total faecal fat content 22.4 g/100 ml. dried faeces. Fat well split.

Congo red test.
60% eliminated from the plasma at the end of 60 minutes.

Intravenous Hippuric Acid Test.
0.84 g. excreted in 1 hour (low normal)

Intravenous Galactose Tolerance Test.
Galactose time 61 minutes (61 minutes)

Renal System.
Maximum specific gravity 1025.
Albumin constantly present.
Urea clearance (% normal) 133.

Microscopy of centrifuged deposit - occasional hyaline and granular casts.

Blood Biochemistry.

Serum Bilirubin 0.5 mg./100 ml.
Serum Cholesterol 250 mg./100 ml.
Serum Phosphatase. 10 units/100 ml.
Serum Albumin 1.9 g./100 ml.
Serum Globulins 2.0 g./100 ml.
A/G 3.9 g./100 ml.

Haematology.
Erythrocytes 4.5 millions/100 ml. Haemoglobin 75% (Haden), Colour Index 0.83, Leucocytes 7,000 of which 3,500 were lymphocytes, 280 monocytes and 3,220
Hepatic amyloidosis. Heavy infiltration of sinusoids with dark staining amyloid. Stained Congo red. X 205.
neutrophil polymorphs.

Aspiration Liver Biopsy.
The specimen showed heavy infiltration with amyloid (fig. 119).

Progress.
An attempt was made to control the pulmonary lesion. A left artificial pneumothorax was induced on the 26th February, 1944. This failed because of extensive pleural adhesions. On 27.3.44 a left phrenic nerve crush was performed. This measure failed to control the activity of the disease. Further chest x-rays showed extension of the lesions. The patient showed nocturnal pyrexia and general failure of health. The ascites required periodic paracentesis. On 8.5.44 the patient was transferred to another hospital where she died four days later. An autopsy was not performed.

Discussion.
The Diagnosis of Amyloidosis.
The laboratory method most often used in the diagnosis of amyloidosis is the Congo red elimination test (Bennhold, 1923), Stemmerman and Auerbach (1944) have subjected the technique to critical analysis. In 446
tests on 246 cases of amyloid disease there were 24.3% false negatives and 4.2% false positives. It is generally agreed that 90-100% absorption of the dye from the plasma is essential for certain diagnosis. Early cases with minimal amyloid deposition give results within normal limits. With this strict limit for positivity, test results in both the cases described alone would have been classed as normal. It seems essential to have a further diagnostic technique. It is believed that aspiration liver biopsy may provide such a method. It has previously been used for this purpose (Waldenström, 1928, Huard, May and Joyeux, 1935) The amyloid material is deposited in the walls of the hepatic sinusoids and in the walls of the hepatic arterioles. The staining reactions with methyl violet, congo red and iodine are quite characteristic. The hepatic involvement is in most instances diffuse. It is, therefore, evident that provided the aspiration biopsy is of the usual size, prepared sections will demonstrate the amyloid change. Occasionally there is unequal involvement of viscera (Rosenblatt, 1934) the spleen and kidneys may show gross amyloidosis, the liver demonstrating only a few focal deposits. It is in these cases that aspiration liver biopsy may give false negatives, In the presence of hepatomegaly liver biopsy will almost certainly reveal the amyloid change.
Liver Function in Amyloid Disease.

Liver function in amyloid disease is usually normal (Moschcowitz, 1936). In our two cases the liver function tests performed gave normal results. This may be due to the great reserve powers of the liver and to the chronicity of the condition. The liver cell columns become adapted to the pressure of the amyloid material, the bile canaliculi are but seldom compressed. Jaundice is, therefore, rare. Rosenblatt (1934) reported 100 cases without encountering an instance of jaundice. Spain and Riley (1944) describe a case of hepatic amyloidosis associated with jaundice. The icterus was believed to be related to the extremely heavy hepatic involvement, and to the consequent blockage of the bile.

Neither of our cases showed a raised serum bilirubin. The only biochemical abnormality possibly related to the liver was the very low serum proteins noticed in Case II. This we associated with a nephrotic syndrome. It cannot be related to the extent of the protein loss in the urine and its causation is still uncertain. The hyperglobulinaemia of experimental amyloidosis (Dick and Leiter 1937) is rarely encountered in man.

Aetiology of the Cases Described.

The most common conditions with which amyloidosis is associated are prolonged sepsis, surgical tuberculosis and pulmonary tuberculosis with cavitation.
especially if there is superadded secondary infection. Moschcowitz (1936) emphasised the rarity of the disease associated with non-suppurative maladies. It has, however, been reported in association with rheumatoid arthritis (Solomon, 1943). Case II presented the classical picture of advanced pulmonary tuberculosis and secondary amyloid disease. The diagnosis in Case I is uncertain. The intestinal change was thought to be tuberculous but attempts at confirmation by biopsy of the peritoneal and intestinal lesions failed. The more likely diagnosis is regional ileitis (Crohn's disease) the fistula formation with neighbouring viscera is particularly suggestive. Study of the literature has not revealed another instance of amyloidosis associated with this condition. Another possibility is primary amyloidosis of the alimentary tract (Golden, 1945). This seems unlikely as amyloid could not be demonstrated in the intestinal biopsy. Fistula formation and heavy amyloid deposits in the liver are also unusual in this condition.

Recovery from Amyloidosis.

Recovery from amyloid disease is rare. Trasoff et al (1944) quote 29 instances of clinical cure collected from the literature. The disease is usually fatal, 90% of cases are dead within two years of diagnosis (Cohen, 1943). Improvement is usually, but not necessarily, (see Grayzel and Jacobi, 1938, and
Rosenblatt, 1936) related to control of the primary disease. Hence the efforts made in Case II to control the pulmonary lesion by collapse therapy. Therapy was impossible, the disease progressed and the patient died. In Case I although eradication of the disease was not possible the provision of an ileocaecal anastomosis much lessened the diarrhoea. The improvement in the patient's general condition was marked by a gain in weight, a diminution in liver size, in the tissue absorption of congo red and in albuminuria. Urea clearance improved and the blood haemoglobin level rose. This case could be included in the group of "clinical cures." Liver biopsy shows that although amyloid is less it is still present. Clinical and histological cure are not synonymous. That resorbtion of amyloid material from the liver can occur is demonstrated by the beautifully illustrated paper of Waldenström (1928). Using the aspiration liver biopsy technique this author was able to show cure of amyloid disease in three instances and lessening of the extent of infiltration in others. Various therapeutic agents have been recommended for the treatment of amyloidosis. In particular powdered liver extract is in wide use for this purpose. (Whitbeck, 1932) It seems important to control the use of and assess the value of this and other therapeutic measures by serial aspiration liver biopsies in a large
number of cases.

SUMMARY.

The use of aspiration liver biopsy in the diagnosis of amyloid disease is described by illustration of two cases.

Liver function in hepatic amyloidosis is discussed.

The aetiology of the condition in the two cases and the question of clinical and pathological cure are mentioned.

The possible use of aspiration biopsy in assessing the value of therapeutic agents used in amyloidosis is discussed.
CHAPTER 19.

ASPIRATION LIVER BIOPSY IN THE STUDY OF
GLYCOGEN DISEASE.

Glycogen disease is a condition characterised by excessive accumulation of glycogen in the liver and other organs. It was first recognised in 1929 by Von Gierke from a post-mortem study of two cases dying from intercurrent infection. Since then reports of about 80 cases have appeared in the literature. Excellent reviews of the condition are those of Ellis & Payne (1936) and Van Creveld (1939).

The case to be described was not entirely typical of Glycogen disease; extensive laboratory investigations suggested associated pathology. Correlations were later established at autopsy. Aspiration liver biopsy has been mentioned as a method of diagnosing glycogen disease (Cazal 1943), the part the method played in the elucidation of this difficult case will the main point for discussion.

Case History.

An 18 year old girl was admitted to Edinburgh Royal Infirmary on 17th April, 1943. The main complaints were periodic attacks of abdominal pain and vomiting for the last 3 years. She had always been a poorly child and her mother stated that she had not developed beyond the age of 7. The attacks of vomiting had led
to her admission to many hospitals. In February, 1933, and November, 1937 she was under observation in Glasgow Sick Children's Hospital. Clinical notes were available from this hospital. At the age of six the liver had first been noticed to be enlarged.

**Dietetic history.** Marked craving for sweets and carbohydrates generally.

**Family history.** Mother and father alive and well. The mother was examined for hepatomegaly and none found. The patient was an only child. No history of liver disease in collaterals.

**Examination.**

**Mental State.** The patient was a bright nervous child. She had been so well investigated during her short life that she viewed doctors with suspicion.

Psychological examination gave her a mental age of 10 - 11. Her I.Q. by the Kent oral method was 79 and by the Terman and Merrill method 83.

**General Development.** The child was grossly undeveloped. Her general appearance and proportions were that of a child of 8 (fig. 120) The body measurements are shown in Table 35.

### Table 35.

<table>
<thead>
<tr>
<th>Year</th>
<th>Age</th>
<th>Height (in)</th>
<th>Weight (lb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1933</td>
<td>8</td>
<td>47</td>
<td>39</td>
</tr>
<tr>
<td>1937</td>
<td>12</td>
<td>48</td>
<td>45</td>
</tr>
<tr>
<td>1943</td>
<td></td>
<td>Physical Development compared with normal at age 8 &amp; 18. (see over)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patient aged 18</td>
<td>Normal aged 8</td>
<td>Normal aged 18</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------</td>
<td>---------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Weight</td>
<td>46 lbs</td>
<td>46-57 lbs.</td>
<td>106-129 lbs.</td>
</tr>
<tr>
<td>Height</td>
<td>48½&quot;</td>
<td>47-50&quot;</td>
<td>62-66&quot;</td>
</tr>
<tr>
<td>Span</td>
<td>47&quot;</td>
<td>46.5-49&quot;</td>
<td>62-67&quot;</td>
</tr>
<tr>
<td>Upper Measurement</td>
<td>23½&quot;</td>
<td>24-26&quot;</td>
<td>31-33&quot;</td>
</tr>
<tr>
<td>Lower &quot;</td>
<td>25&quot;</td>
<td>23-25&quot;</td>
<td>31-33&quot;</td>
</tr>
<tr>
<td>Chest</td>
<td>25&quot;</td>
<td>23.4&quot;</td>
<td>31&quot;</td>
</tr>
<tr>
<td>Abdomen.</td>
<td>23&quot;</td>
<td>20.8&quot;</td>
<td>25.5&quot;</td>
</tr>
<tr>
<td>Hips.</td>
<td>24&quot;</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

There was a conspicuous disproportion between the nutrition of trunk and limbs. The cheeks and body were plump, the limbs very thin. The skin and mucous membranes were pale.

Abdominal Examination was difficult as the muscles were held very rigidly. On one occasion the liver was palpated 6 cm. below the right costal margin. The organ surface and edge were smooth and there was no tenderness. Spleen and kidneys were never felt.

Investigations.

A very large number of investigations were performed during 1943. The relevant data will be summarised together with some data available from the 1933 and 1937 admissions to Glasgow Sick Children's Hospital.
Carbohydrate Metabolism.

1. Low fasting blood sugar.
   Average 8 readings 74.5 mg/100 ml. (Hagedorn & Jensen)

2. Glucose Tolerance test (oral)
   Flat sugar curves in 1933 and 1937.
   Delayed fall type of sugar curves in 1943.

3. Adrenalin tests.
   Blood sugar did not show significant rise compared with controls after 0.5 mg. adrenalin subcutaneously (1937 and 1943)

4. Marked sensitivity of blood sugar to 5 units soluble insulin subcutaneously.

5. Raised blood glycogen (1937 and 1943)

6. Normal laevulose and galactose tolerance tests.

7. Urinary diastase values within normal limits.

8. Almost constant ketosis in morning urine (Rothera and Fervic chloride (also noted in 1937 but not in 1933).

Fat Metabolism.

1. Hypercholesterolaemia.

2. Raised blood fatty acids.

   Increased total faecal fat and particularly of unsplit proportion.

Nitrogen Metabolism.

1. Acetonuria, especially in the morning specimen.
2. Low fasting blood sugar.
3. Raised blood glycogen.
4. Failure of the blood sugar to rise after adrenalin.
5. Delayed fall as the abnormality of the blood sugar curve following glucose.
6. In some cases rise of blood sugar following injection of laevulose.
7. Raised blood cholesterol.

Our case exhibited six of these seven features. However, in addition, there were findings not usually recorded in glycogen disease, in particular evidence of defective fat metabolism, evidenced by steatorrhoea, and of renal failure, evidenced by raised plasma urea and failure to concentrate urine. These made it very essential to be certain of the diagnosis of glycogen disease. For this reason it was decided to submit the patient to aspiration liver biopsy.

**Aspiration Liver Biopsy.**

The biopsy was done under light gas and oxygen anaesthesia. An adequate specimen was obtained. The surface was smooth and rather dull white in colour.

Histologically the most marked feature is the packing of the individual liver cells with coarse granules of glycogen. Many of the nuclei of the liver cells show glycogenic degeneration (figs. 121 & 122)

In addition many of the liver cells distended with fat,
Glycogen disease. The patient in May 1943 at the age of 18. Note normal proportions for a child or 8. The face is plump, the limbs are thin and spindly.

Length of rule is 39 inches.
**FIG. 121.**

Glycogen disease. Normal liver architecture. Hepatic cells packed with glycogen. Some show fatty change. Stained Best's Carmine. X 120.

---

**FIG. 122.**

Higher power view showing the granules of glycogen in the hepatic cells. The distension of some of the cells with fat and the glycogenic degeneration of the liver cell nuclei. Stained Best's Carmine. X 925.
FIG. 123.

Glycogen disease. The reticulin framework of the liver is normal.
Modified Font's stain. X 65.

FIG. 124.

Glycogen disease. Post mortem section of liver.
The liver cells show spaces occupied by glycogen.
Stained H.E. X 145.
the nucleus being compressed to one side. The fatty change is not distributed in any constant position in the liver lobule. The liver architecture is normal (Fig. 123) and there is no excess of fibrous tissue in the portal tracts or elsewhere.

**Diagnosis.** The excessive glycogen content of the liver persisting in the presence of acidosis, ketonuria and general anaesthesia suggested undue stability of liver glycogen and a diagnosis of glycogen disease was made. The causes of the fatty change in the liver, the steatorrhoea and the renal failure were not known.

**Progress.** The patient was discharged on the 7th August, 1943. On the 10th November, 1943, the child was re-admitted to hospital. She was in coma and having fits. The biochemical findings were:

- **Capillary Blood sugar** 98 mg/100 ml.
- **Plasma Alkali reserve** 18 vol. per cent.
- **Urea nitrogen.** 119 mg/100 ml.
- **Uric acid** 7.3 "
- **Phosphates** 10.0 "
- **Cholesterol** 367 "

The condition was clearly uraemic.

Death occurred on 13th November, 1943.

**Autopsy** was carried out 12 hours after death. Essential findings relative to this case report were:

1. Retardation of development. Configuration that of
Case of glycogen disease. The kidney sections show glomerular degeneration, thickening of Bowman's capsule, hyaline change in the arterioles, tubular disappearance and round cell infiltration.
Stained H.E. X 250.
girl of 8-10 years old. Absence of secondary sexual characters.

2. Liver weighed 2,100 g. and was soft in consistence. On section the parenchyma was very soft and friable and was extremely pale and mottled. 

Biochemical analysis of liver showed 15.5 g. glycogen per cent wet weight.

3. There was no abnormality of gall-bladder and bile ducts.

4. Spleen weighed 40 g. and presented no unusual features.

5. Kidneys were underweight and granular on the surface. The pelvis, ureters and bladder were normal.

6. A large cystic structure occupied the body and tail of pancreas. This contained thick yellowish green pus.

   Bacteriological culture of the pus revealed B. coli and a few slightly haemolytic streptococci.

7. Heart weighed 120g. and showed no abnormalities.

Histological sections.

1. Liver. The appearances were similar to those seen in the liver biopsy sections. The organ still contained a large complement of glycogen (Fig. 124)

2. Kidneys. The appearances resembled chronic glomerulonephritis. (Fig. 125)

3. Glycogen stains on the heart, kidneys and skeletal muscle failed to reveal glycogenic infiltration.
Comment. Clinicians hesitate to postulate multiple pathology in a single case where one diagnosis can be found to explain the findings. In the case described the clinical and biochemical picture was really only clarified by autopsy, at which diagnoses of glycogen disease, chronic nephritis and pancreatic abscess were made.

The possible pathogenesis of the small granular kidneys is outside the scope of this discussion. A third stage, Bright's disease seemed most likely. This caused the renal failure. It might be suggested that the defects in carbohydrate metabolism occurred secondary to the renal lesion. A high blood glycogen has been reported in nephritis (Ellis and Payne, 1936) Chronic renal disease in early life is associated with dwarfism, possibly due to a secondary hypopituitarism. Anterior pituitary deficiency per se causes increased resistance to the breakdown of glycogen to glucose in the liver (Houssay and Biassotti, 1931; Cope and Marks, 1934) However, it is known that the defect in carbohydrate metabolism was present in 1931 and at that time renal efficiency tests were normal. This mode of production of the syndrome therefore seems unlikely.

The steatorrhoea was not present in 1933 or 1937 and was almost certainly associated with the destruction of pancreatic tissue by the large pancreatic abscess. An attempt might be made to link the fatty change in the liver with the pancreatic lesion. Allan
et al. (1924) have reported fatty change in the liver of depancreatectomised dogs maintained with insulin. However, Whipple (1942) observed human cases in which the pancreas had been surgically removed from humans and was unable to confirm these findings. The fatty infiltration in our case seems more likely to be due to an intrinsic defect in fat metabolism associated with the carbohydrate defect. The case would fit into the group of "hepatomegalies polycorique" described by "Debré et al. (1934)."

In the presence of the associated renal and pancreatic pathology liver biopsy was very useful in demonstrating the glycogen excess in the liver, and so adding strong confirmatory support for the further diagnosis of glycogen disease. The essential feature of the glycogen in Von Gierke's disease is its stability (Schönheimer, 1929). In our case, 12 hours post mortem, 15.5 g. per cent glycogen could be demonstrated in the liver. This is an extremely high figure even for liver biopsies taken in vivo. Since the study of this case, other instances of presumed glycogen disease have been encountered. In these cases we have found it useful to place a small piece of the liver biopsy in normal saline solution for 24 hours before carrying out the Best-carmine staining technique. In normal livers the glycogen will all have been converted to glucose, whereas in glycogen disease the
glycogen is stable to this treatment. Another aid now in use is the diagnosis of this condition in the microchemical analysis for glycogen of the liver biopsy sample. This procedure is a matter of difficulty on such small specimens but has proved technically possible (Good, Kramer and Somogyi, 1933, modified Walshe, 1945)

Summary.

The use of aspiration liver biopsy in the diagnosis of glycogen disease is illustrated by a description of a case in which hepatic defects in carbohydrate and probably fat metabolism were associated with pancreatic and renal deficiency. The part played by liver biopsy in its elucidation is discussed.
CHAPTER 20.

ASPIRATION LIVER BIOPSY IN THE DIAGNOSIS OF KALA AZAR.

In the tropics splenic and liver puncture are commonly used in the diagnosis of Kala Azar. The specimens obtained are usually smears of blood which may or may not contain the parasite. Aspiration liver biopsy can not only demonstrate the Leishman Donovan bodies, but can also identify the characteristic liver histology which may itself be diagnostic. Of the three cases to be described, in one the parasites were clearly demonstrated, in another they probably existed in a degenerate form, and in the third no parasites were seen but the liver histology was so similar to the other cases that leishmaniasis was almost certain.

Case 1. A soldier aged 28 had served in the North African and Sicilian campaigns. On return to England he complained of rigors, malaise and occasional vomiting. These symptoms had persisted on and off for six months. Examination showed a well developed sallow man. There was irregular pyrexia of 100-102°. A few small rubbery lymph glands were palpable in the neck, the axillae and the groins. The spleen was palpable half way to the umbilicus; its margin was firm. The liver was palpable 3 cms. below the right costal margin; the liver edge was tender. The
### TABLE 36

**THE PATHOLOGICAL FINDINGS IN 3 CASES OF KALA AZAR.**

<table>
<thead>
<tr>
<th>Case</th>
<th>R.B.C. million/MM</th>
<th>Hb.% (Haden)</th>
<th>W.B.C. c.mm.</th>
<th>Polymorphs %</th>
<th>Platelets/c.mm.</th>
<th>Splenic puncture</th>
<th>Sternal puncture</th>
<th>L.D.Bodies</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3.3</td>
<td>57</td>
<td>2,850</td>
<td>15</td>
<td>134,000</td>
<td>No L.D.B.</td>
<td>No L.D.B.</td>
<td>Present</td>
<td>Typical histiocytic accumulations.</td>
</tr>
<tr>
<td>2.</td>
<td>58</td>
<td>1,500</td>
<td>44</td>
<td></td>
<td></td>
<td>No L.D.B. (4 punctures)</td>
<td>Degenerate forms</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>2.02</td>
<td>49</td>
<td>3,000</td>
<td>51</td>
<td></td>
<td>No L.D.B. (2 punctures)</td>
<td>Absent</td>
<td>&quot;</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 37

**THE BIOCHEMICAL FINDINGS IN 3 CASES OF KALA AZAR.**

<table>
<thead>
<tr>
<th>Case</th>
<th>Serum Bilirubin mg/100ml.</th>
<th>Serum Phosphatase units/100 ml.</th>
<th>Serum Cholesterol mg/100ml.</th>
<th>Serum Albumen g/100ml.</th>
<th>Serum Globulin g/100ml.</th>
<th>A/G ratio</th>
<th>Formal gel test</th>
<th>G.T.</th>
<th>H.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>less than 0.5</td>
<td>11</td>
<td>183</td>
<td>2.6</td>
<td>4.4</td>
<td>0.5</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>2.</td>
<td>do</td>
<td>15</td>
<td>189</td>
<td>4.1</td>
<td>2.9</td>
<td>1.7</td>
<td>-ve</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>3.</td>
<td>do</td>
<td>9</td>
<td>177</td>
<td>4.6</td>
<td>2.0</td>
<td>2.3</td>
<td>-ve</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

*G.T. = intravenous galactose tolerance test.*

*H.A. = intravenous hippuric acid synthesis test.*
urine showed no abnormal features.

Pathological and biochemical findings are shown in tables 36 and 37.

Progress. The fever failed to respond to mepacrine and to sulphathiazole, and a provisional diagnosis of Kala Azar was made. The patient received ten injections of Stilbamine (total 1.37 g.) He was referred to Hammersmith Hospital for liver biopsy.

Aspiration Liver biopsy. The liver cells contained their full complement of glycogen and appeared normal. Focal accumulations of large mononuclear cells were scattered throughout the liver and there were infiltrations of the portal zones with histiocytes. (Fig.126) When the sections were stained by Leishman's method, small bodies were seen lying within the macrophages. The bodies were about 2 μm in diameter and rounded in contour. Some contained two lilac staining chromatin masses, one larger than the other. The cytoplasm was a very pale blue (Fig.127) They were quite characteristic of the Leishman Donovan bodies described in Kala Azar. The liver cells themselves contained no parasites.

Case 2. A 26 year old soldier had served in the Sicilian and North African campaigns. One month after his return to England he was admitted to hospital with a history of cough and sweating of two weeks' duration.
FIG. 126.

Kala Azar. Histiocytic accumulations scattered through the liver.
Stained Best's Carmine. $X$ 145.

FIG. 127.

Kala Azar. Leishman Donovan bodies lying within the histiocytes.
Leishman's stain. $X$ 775.
For the next two months there had been intermittent pyrexia, the temperature rising as high as 105°. He had lost 17 lbs. in weight. Examination showed a well developed pale man. The liver was readily palpable 4 cm. below the right costal margin. The spleen could just be felt on deep inspiration. There were no enlarged lymph glands. The urine showed no abnormalities. Laboratory findings are tabulated (Tables 36 & 37).

Progress. The fever showed no change when mepacrine was given. A provisional diagnosis of Kala Azar was made and the patient given Stilbamidine (total 1.45 g) This medication had led to a steady improvement in the blood picture so that on admission to Hammersmith Hospital the erythrocytes were 4.5 million per cu.mm., the haemoglobin was 80 per cent and the leucocytes 5,000 per cu.mm. with a normal differential count; He was now apyrexial.

Aspiration Liver biopsy. The liver showed essentially the same structure as that described in the previous case. There were peri-portal cellular infiltrations and macrophage accumulations scattered through the liver. However, Leishman-Donovan bodies could not be definitely identified. There were a number of ill-defined bodies lying within the macrophages which might have been degenerate forms of the parasite.
Case 3. A 24 year old soldier had served in the North African, Egyptian and Sicilian campaigns. Three months after arriving home he was admitted to another hospital with a history of fourteen days sweating and shivering. For the next three months there was an intermittent pyrexia of 100 - 103°.

The patient looked pale and ill. The liver was felt 5 cm. below the right costal margin. The spleen was also palpable. There was no lymphadenopathy. The urine showed no abnormalities. The laboratory findings are tabulated (tables 36 & 37)

Progress. No response of pyrexia to mepacrine medication. The patient was transferred to Hammersmith Hospital for aspiration liver biopsy.

Aspiration liver biopsy: The same histological picture described in the other cases was encountered in this case. However, in this instance Leishman-Donovan bodies, whether easily recognisable or degenerate, could not be demonstrated.

Discussion.

The diagnosis of splenomegaly in patients returning from the tropics must always be a matter of some difficulty. In Kala Azar the parasites are engulfed by the cells of the reticuloendothelial system and leishmaniasis is essentially an infection of the reticuloendothelial system (De, 1927 & 1934; De & Tribedi, 1939). Kala Azar, therefore, falls into the
wide group of reticulosis. It would be expected that definite diagnoses would come from obtaining infective material from the members of the reticuloendothelial system, and in particular from the liver, spleen and bone marrow.

Splenic puncture has been the time-honoured method of demonstrating the Leishman Donovan bodies. Napier (1936), from his wide experience in Calcutta, reports five thousand punctures with no fatalities. In the hands of less experienced operators the puncture is not so safe. The dangers have been pointed out by Giraud and Gaubert (quoted Manson-Bahr, 1940). In the only case of the present series in which splenic puncture was attempted infective material was not obtained. Sternal puncture is the method of choice for the diagnosis of Kala Azar. The applicability of the method has recently been discussed (Chung, 1938) It is only in the rare instances in which marrow puncture fails to demonstrate the parasite that recourse must be made to liver puncture. In the three cases described sternal puncture, in two instances repeated, had failed to make a diagnosis. The material obtained from aspiration hepatic puncture may, as in Case I, be used to demonstrate the parasite. It has the advantage over that obtained by puncture of the sternum or spleen however in that sections can be cut and a picture of the general histology so observed. The reticuloendothelial proliferations of
Kala Azar are quite characteristic. This picture, with the clinical history and laboratory findings, enabled a diagnosis to be made in cases 2 and 3, although the Leishman-Donovan bodies were not demonstrated. The absence of Kupffer cell pigmentation eliminates malaria. It has been suggested that Kala Azar is associated with a hepatic cirrhosis (Rogers, 1919) Our findings did not substantiate this finding. There was no evidence of either hepatic cell necrosis or of hepatic fibrosis.

Summary.

Aspiration liver biopsy findings in three cases of Kala Azar are described. In only one were Leishman-Donovan bodies demonstrated. In all three the characteristic reticuloendothelial proliferations were seen. Kala Azar falls into the group of reticuloses; the histological picture in the liver is quite characteristic.
CHAPTER 21.

HEPATIC CHANGES IN INFECTION MONONUCLEOSIS

Little is known of the histological appearances of the liver in infectious mononucleosis. The condition is so rarely fatal that autopsy material is not available. Where death does occur it is usually attributable to secondary infection which tends to obscure the underlying pathology of the disease.

The present case is described because of the rarity in the literature of reports of the hepatic lesion and because the material was obtained from a case in which the histological picture was not complicated by the presence of either a secondary infection or jaundice.

Case report. A 20 year old civil servant was admitted to hospital with a complaint of 10 days general malaise. The illness had started with a transitory sore throat. There had been excessive sweating and a pyrexia of 99-100°F had been noted for a week, at which time a swelling appeared under the left jaw. Examination revealed a pale, well-developed man. The skin was warm and moist and there was a blotchy erythematous rash over the trunk. There was generalised lymphadenopathy, firm mobile glands being palpable in the neck, axillae and groins. The tonsillar lymph glands were particularly enlarged. The spleen was readily
palpable 3 cms. below the left costal margin. The liver was not felt.

Urinalysis showed only excess of urobilinogen.

Haematological findings at once suggested the correct diagnosis. Erythrocytes were 4.7 million per c.mm.; haemoglobin (Haden) was 92 per cent; the leucocytes were 14,000 per c.mm. and of these 1,260 (9 per cent) were neutrophil polymorphs, 12,460 (89 per cent) large mononuclear cells and 280 (2 per cent) monocytes.

Examination of the stained blood film showed the large cells to be of a rather atypical nature with deep basophilic cytoplasm and fenestrated nuclei. The cells were typical of those encountered in infectious mononucleosis.

Serological findings were a positive Paul Bunnell reaction in a dilution of 1:56 and negative Wassermann and Kahn reactions.

The serum bilirubin concentration was less than 0.5mg. per 100 ml.

Progress: 5 days after admission to hospital there was an exacerbation of fever, the temperature rising to 102°F. At the same time a tonsillitis developed with white exudate on the left tonsil. The exudate when cultured bacteriologically showed only the usual mouth flora. The sore throat cleared in 3 days. There were recurrent crops of petechiae on the palate. The spleen enlarged until it was palpable almost to
the level of the umbilicus. It had receded to just below the left costal margin on discharge. The Paul Bunnell on the 28th day was positive in a dilution of 1:112 and on the 40th 1:32. The patient was discharged on the 48th day of the illness feeling well. Aspiration liver biopsy was performed on the 14th day of illness (fig.128). The most marked change was a great increase in the cellular content both of the sinusoids and the portal tracts. In the sinusoids the cells resembled the large mononuclears described in the peripheral blood. There was an occasional mature neutrophil polymorph (fig.129). In the portal tracts there were large accumulations of similar round cell infiltrations. The appearance in the portal tracts was not unlike that of an early acute hepatitis. The architecture of the liver as a whole was preserved. The hepatic cells contained their usual complement of glycogen (fig.130). However, scattered here and there through the liver were focal necroses. The liver cells in these areas had completely disappeared. The necroses were not bile stained as are those seen in obstructive jaundice. Neither was there any surrounding cellular reaction.
infectious mononucleosis. Great increase in the cellular content of the sinusoids and portal tracts.
One small focal necrosis in lower left hand corner. Stained Best's Carmine. X 95.
Discussion.

We have found two other instances of aspiration liver biopsy being used in the study of infectious mononucleosis. Kilham and Steigman (1942) give a pathological report by Dible on one of their cases. The findings differed little from those described in acute (epidemic) hepatitis. The only unusual finding was the presence of excessive numbers of Kupffer cells and monocytes in the sinusoids. The biopsy came from a case which showed the typical blood picture and serology of glandular fever. At the same time there was an epidemic of hepatitis proceeding in the area, and it seems likely that the case was suffering from both conditions, thus confusing the histological picture. The other case was reported by Van Beek and Haex (1943) and showed a picture very comparable with that described in our case. The histology resembled that of a myeloid leukaemia. A repeat biopsy in 3½ weeks showed that the tissue had regained its normal condition. Du Bois (1930) obtained autopsy material from a fatal case and states that the portal spaces of the liver were infiltrated with large mononuclear cells.

The picture we have described is of a pattern similar to that seen in any generalised disease of the reticulo-endothelial system. The finding in the sinusoids of mainly large atypical mononuclear cells similar to those seen in the blood supports the diagnosis of infectious mononucleosis. That the disease process is
a widespread one involving other members of the reticulo-endothelial system is shown by the observations on bone marrow smears and on biopsied lymph glands. Bone marrow smears have shown large numbers of atypical lymph nodes (Young and Osgood, 1935; Freeman, 1936). Lymph gland biopsies have demonstrated a variable picture with hyperplasia of the lymphoid and reticulo-endothelial elements. (Downey and Stasney, 1935). The presence of focal necroses in the liver suggests that infectious mononucleosis is an infection, possibly a virus. This is in keeping with the suggestion of Barber (1941).

Hepatomegaly as opposed to splenomegaly is rather uncommon. Bernstein (1940) found only 12 per cent of palpable livers in his series of 65 cases. The instances of enlarged liver have usually been in association with jaundice, but instances without icterus have been described (Farley, 1937). The increase in size of the liver is probably due to the large numbers of mononuclear cells present in the sinusoids. The hepatomegaly of leukaemia may have a similar causation.

Jaundice is a more frequent concomitant. The relation is fully discussed by De Vries (1938). The most common explanation put forward is an obstructive jaundice due to enlargement of lymph glands in the porta hepatis (Martin, 1941; Priest, 1942). Other workers believe that there is an acute hepatitis (Howard, 1942; Kilham and Steigman, 1942; Morris, Robbins & Richter, 1944). Davis and his coworkers (1945) obtained a liver
biopsy at operation and describe distended sinusoids containing leucocytes, the picture is described as that of a mild hepatitis. The term hepatitis usually implies inflammation of the entire liver and liver cell necrosis is an essential contributory feature. The only hepatic cell lesion in infectious mononucleosis is the minute focal necroses. The lesion, like that of leukaemia or kala-azar, is not a true hepatitis. The extent of the disturbance is not sufficient for the production of jaundice. Jaundice in infectious mononucleosis is almost certainly due to pressure on bile ducts by enlarged glands.

Summary.

Aspiration liver biopsy findings in a case of infectious mononucleosis are described. The causation of hepatomegaly and jaundice in this condition are discussed.
CHAPTER 22.

ASPIRATION LIVER BIOPSY IN THE DIAGNOSIS OF GAUCHER'S DISEASE.

This rare disease of cellular metabolism was first described by Gaucher in 1882. Since then descriptions of approximately 170 cases have appeared in the literature. The condition consists essentially of the accumulation of the cerebroside keratin in the cells of the reticulo-endothelial system. The present case was diagnosed by aspiration liver biopsy, although the diagnosis had been suspected after sternal marrow examination.

Case Report. The patient was a Czechoslovak Jewish woman of 32. For 10 years she had noticed that she bruised easily with minimal trauma. Three years ago she had had bleeding from the gums for about a week. Two years ago there had been menorrhagia associated with 2-3 of the menstrual periods, and for the past two years she had noticed that she was becoming very easily tired. Previous to this illness she had enjoyed a healthy life. Family history was negative. In November 1944 she was examined at the Czechoslovak Red Cross Dispensary and an anaemia was discovered. The erythrocytes were 3.13 million per cu.mm., haemoglobin was 60 per cent. The
leucocytes were 3,400 per cu.mm. Liver extract and iron were administered without any effect on the anaemia and she was referred to the Czechoslovak Unit at Hammersmith Hospital for further study. On 27th February 1945 the erythrocytes were 4.13 million per cu.mm., haemoglobin was 70 per cent (11.5 per 100 ml.), the leucocyte count was 6,300 per cu.mm. with 68.6 per cent of polymorphs, 4.8 per cent of proleucocytes, 10 per cent of monocytes and 22.4 per cent of lymphocytes. The blood film showed some anisocytosis and polychromasia of the erythrocytes.

In March 1945 the erythrocytes were 3.6 million, the haemoglobin was 61 per cent. The peripheral blood film now showed some primitive cells, there being an occasional myelocyte and myeloblast, and there was one erythroblast to every 250 leucocytes. The peripheral blood always showed abundance of platelets; the platelet count was 275,000 per cu.mm. The provisional diagnosis was an aleukaemic leukaemia or a leuco-erythroblastic anaemia.

On 13th May 1945 the haematological findings were similar to those reported in March, except that immature cells could no longer be demonstrated in the peripheral blood. Adequate iron medication had produced no improvement in the anaemia. The haematological findings are tabulated (Table 38):
TABLE 38

THE HEMATOLOGICAL FINDINGS IN THE CASE OF GAUCHER'S DISEASE DESCRIBED.

<table>
<thead>
<tr>
<th>Date</th>
<th>Erythrocytes</th>
<th>Hb. % (Eadén)</th>
<th>W.B.C. per c. mm.</th>
<th>Polymorphs</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Prothrombocytes</th>
<th>Platelets</th>
<th>Bleeding time (Duke's minutes)</th>
<th>Blood film</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.11.44</td>
<td>3.13</td>
<td>60</td>
<td>3,400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anisocytosis &amp; polychromasia of red cells</td>
</tr>
<tr>
<td>27.2.45</td>
<td>4.13</td>
<td>70</td>
<td>6,200</td>
<td>68.6</td>
<td>22.4</td>
<td>1.0</td>
<td>4.8</td>
<td></td>
<td></td>
<td>Occasional myeloblast myelocyte and late erythroblast</td>
</tr>
<tr>
<td>10.3.45</td>
<td>3.6</td>
<td>61</td>
<td>4,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.5.45</td>
<td>3.7</td>
<td>72</td>
<td>6,000</td>
<td>49</td>
<td>46</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.6.45</td>
<td>3.8</td>
<td>80</td>
<td>5,000</td>
<td>70</td>
<td>27</td>
<td>3</td>
<td>0</td>
<td>275, 5½</td>
<td></td>
<td>No primitive cells seen</td>
</tr>
</tbody>
</table>

She was admitted to hospital for further investigation.

The patient was of normal colour. There was no pigmentation of the legs or elsewhere. There were no pingueculae. On the right hip two well demarcated circular bruises approximately 3 cms. in diameter were observed. The tourniquet test was positive.
FIG. 131.
Gaucher's disease. Smears of sternal marrow.
Presence of large atypical cells with hyperchromatic nuclei.
Stained Leishman. X 335.

FIG. 132.
Sternal marrow smears. A group of four Gaucher cells. Note the ribbliary cytoplasm and eccentric hyperchromatic nucleus.
Stained Leishman. X 700.
FIG. 133.

Gaucher's disease. Liver sections show areas between the columns of liver cells filled with large pale cells. The liver cells themselves appear normal. Stained Best's Carmine. X 190.

FIG. 134.

Liver. A group of Gaucher cells. With this stain the cytoplasm appears clear. The nuclei are small and dark. Stained Best's Carmine. X 370.
Liver. A group of Gaucher cells. The longitudinal fibrils in the cytoplasm and the eccentric nucleus with open chromatin network and nucleolus are well demonstrated.

Stained Masson's trichrome and light green. X 800.
The spleen was palpable 6 cm. below the left costal margin. It was firm in consistence. The liver was palpable 4 cms. below the right costal margin.

Radiological examination of the skull and long bones failed to show any abnormality.

Sternal marrow puncture revealed an active normo-blastic bone marrow; there was an abundance of megakaryocytes. Large pale cells were seen rather reminiscent of "Gaucher cells"; however, the sternal puncture had been difficult and it was thought that these might be osteoclasts from the adjoining cancellous bone (Figs. 131 and 132).

Aspiration liver biopsy was now undertaken. The specimen obtained was small and of an unusual appearance. It was grayish-brown and rather glistening. The contour was smooth instead of the usual lobulated appearance.

Microscopy showed areas between the columns of liver cells filled with large pale cells (Figs. 133 and 134). These cells were 20-60 μ in diameter. They were oval, round or polygonal in shape. The protoplasm had a fibrillary structure usually running from one pole of the cell to the other. The reticular fibres were very delicate. Each cell contained one or two nuclei placed eccentrically. The nuclei contained a fairly open chromatin network and usually possessed
a nucleolus. Mallory's aniline blue stain has been recommended to demonstrate the characteristic reticular structure of the Gaucher cells (Risel, 1909; Epstein, 1924). This method did not prove very satisfactory with the alcohol-fixed material; a much more cosmetic picture was seen with Masson's trichrome and light green stain. The cytoplasm of the Gaucher cells took up the green dye and the fibrils in the protoplasm were well demonstrated. (Fig. 135). A thionine protoplasmic stain gave a beautiful demonstration of the fibrils but unfortunately faded rapidly and was only of transitory value. The lipoid in the cells failed to rotate the plane of polarised light. The cells showed no particular arrangement within the liver lobules. One portal tract was seen; it was quite normal and not infiltrated with Gaucher cells. This is contrary to the view of Pick (1924) that the cells may arise particularly from the histiocytes of Glisson's capsule. In this case an origin from the Kupffer cells seemed much more likely. Normal Kupffer cells could not be recognised in the liver section.

Biochemical findings were as follows:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum bilirubin</td>
<td>0.5 mg. /100ml.</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>5.2 units /100ml.</td>
</tr>
<tr>
<td>Phosphates (as P)</td>
<td>3.4 mg. /100ml.</td>
</tr>
<tr>
<td>Calcium</td>
<td>10.7</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>173</td>
</tr>
<tr>
<td>Plasma</td>
<td>194</td>
</tr>
<tr>
<td>Whole blood</td>
<td>177</td>
</tr>
<tr>
<td>Total serum proteins</td>
<td>7.5 g.</td>
</tr>
<tr>
<td>Albumen</td>
<td>4.2</td>
</tr>
<tr>
<td>Globulin</td>
<td>3.3</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>1.2</td>
</tr>
<tr>
<td>Plasma lipoid nitrogen</td>
<td>2.4 mg.</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1.36</td>
</tr>
<tr>
<td>Serum colloidal gold test (Maclagan)</td>
<td>00000</td>
</tr>
</tbody>
</table>
Discussion.

This case failed to show many of the characteristic manifestations of Gaucher's disease. Graham & Blakelock (1927) first mentioned skin pigmentation; this could not be demonstrated in this case. Brill (1901) reports that pigmeauleae are almost constantly found, but these were also absent in this case.

Bony changes were not demonstrated radiologically. Other authors report absence of X-ray changes (Rupanner, 1940; Petit & Schleicher, 1943).

The anaemia was quite non-specific. There was no leukopenia. The finding of immature leucocytes and nucleated red cells in the peripheral blood is in keeping with the cases described by Graham & Blakelock, Melamed & Chester, 1938, and Erf, 1938. Mandelbaum, Berger and Lederer (1942) suggest that this may be due to crowding of the bone marrow by the Gaucher cells. The bleeding tendency in Gaucher's disease is usually associated with a diminished platelet count and an increased bleeding time. (Welt, Rosenthal & Oppenheimer, 1929; Sherlock & Learmonth, 1942). In this instance the bleeding tendency was manifested in the history of bleeding gums and menorrhagia and the presence of bruises caused by insignificant traumata. The platelet count, however, was
normal and the sternal marrow showed an abundance of megakaryocytes. The bleeding time was only at the upper limit of normal and the tourniquet test not markedly abnormal. In this case true purpura has never been observed. It is difficult, however, to explain the bruising. Bloem, Groen & Postma (1936) suggested that, as the lipoid kerasin contains nitrogen, cases of Gaucher's disease might show an increase in the lipoid nitrogen concentration of the plasma. In three of their five cases such an increase was noted. Kerasin does not contain phosphorus; consequently in all their cases the lipoid phosphorus was normal. In the present case both the lipoid nitrogen and lipoid phosphorus were normal and gave no diagnostic information. As in most other cases reported in the literature, other biochemical investigations gave essentially normal results.

In view of all these negative and contradictory findings, too much stress could not be placed on the finding of large pale cells in the sternal marrow. The liver biopsy findings were of great value in clinching the diagnosis. Sternal marrow puncture has often been used to make diagnosis of Gaucher's disease. Emanuel (1941) states that about a dozen cases have been diagnosed by this method. Splenic puncture, although a hazardous procedure, has also been utilised
in this way (Chalmers, 1940; Rupanner, 1940). Casal (1943) mentioned one case diagnosed by puncture biopsy of the liver; our case seems to be only the second diagnosed by this procedure. Obviously liver biopsy has a large part to play in the diagnosis and elucidation not only of Gaucher's disease, but of the whole group collectively described as lipodystrophies.

Summary.

The use of aspiration biopsy in the diagnosis of a case of Gaucher's disease is described.
CHAPTER 23.

THE USE OF ASPIRATION LIVER BIOPSY IN
THE STUDY OF LEUCOERYTHROBLASTOSIS.

The term leucoerythroblastic anaemia is used to describe an anaemia characterised by the presence in the peripheral blood of immature red cells and a few immature white cells of the myeloid series (Vaughan 1934). The condition is obviously one of multiple aetiology. The case to be described obeyed the criteria for diagnosis set down by Vaughan. The part that aspiration hepatic biopsy played in its elucidation will be discussed.

Case Report. A 65 year old housewife was first admitted to Hammersmith Hospital on 27th October, 1943. Six years previously she had been a patient in another hospital with haematemesis. At that time she was said to have vomited about a pailful of blood and blood transfusion was necessary. The spleen was noticed to be enlarged. Recovery was complete and she remained well until the beginning of 1943. She then noticed that the abdomen was becoming enlarged and the swelling was progressive during the succeeding nine months. Six months previous to admission she began to have hot sweats during the day-time, and three months ago she had experienced fainting
attacks. The faints now occur 2-3 times a week, they are preceded by drowsiness and the patient often sat on the floor to avoid falling down. During the past year she had become increasingly breathless on exertion; there was no history of nocturnal dyspnoea or ankle swelling. The patient had lost about 2 stones in weight.

Past health and family history were uneventful. Examination revealed a sallow thin old woman. There was no lymphadenopathy. The nails were brittle. There was a moderate degree of hypertension (B.P. 170/95), but cardiac enlargement could not be detected clinically. A soft systolic cardiac murmur was heard to the left of the sternum. Examination of the abdomen revealed a huge mass filling up the left side and extending anteriorly towards the right iliac fossa. The mass was clearly spleen (Fig. 136). The liver was not palpable.

Urine analysis showed no abnormalities. The haematological findings are tabulated (Table 39).
TABLE 39
THE HAEMATOLOGICAL FINDINGS IN A CASE OF LEUCOERYTHROBLASTOSIS

<table>
<thead>
<tr>
<th>Date</th>
<th>R.B.C. (millions per c.mm.)</th>
<th>Hb. per cent</th>
<th>Colour index</th>
<th>Nucleated RBC/100WBC</th>
<th>W.B.C. per c.mm.</th>
<th>Differential leucocyte count</th>
<th>Platelets</th>
<th>Pro-</th>
<th>Metrizo-</th>
<th>Erythro-</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.10.43</td>
<td>5.0</td>
<td>62</td>
<td>0.62</td>
<td>1</td>
<td>30,000</td>
<td>6,900 300 21900 0 0</td>
<td>900</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.11.43</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>33,000</td>
<td>7,260 1650 22110 990 0</td>
<td>990</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9.11.43</td>
<td>5.1</td>
<td>61</td>
<td>0.6</td>
<td>0</td>
<td>47,000</td>
<td>10,610 3290 30080 0 0</td>
<td>2820</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>17.11.43</td>
<td>4.5</td>
<td>63</td>
<td>0.7</td>
<td>1</td>
<td>34,000</td>
<td>7,480 1360 22440 340 0</td>
<td>1020</td>
<td>1360</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24.11.43</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>37,000</td>
<td>8,510 1850 24050 0 740</td>
<td>1110</td>
<td>740</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25.11.43</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>34,000</td>
<td>8,160 1360 22780 680 0</td>
<td>1020</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24.12.44</td>
<td>4.3</td>
<td>66</td>
<td>0.77</td>
<td>3</td>
<td>17,000</td>
<td>4,420 680 7650 170 0</td>
<td>2890</td>
<td>1190</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5.12.44</td>
<td>3.7</td>
<td>70</td>
<td>0.95</td>
<td>3</td>
<td>42,000</td>
<td>9,660 2100 18900 840 0</td>
<td>7560</td>
<td>2940</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

5.11.45 Platelets 454,000 per c.mm.
9.11.45 Reticulocytes 2.2%
Absolute values 1.11.43

Erythrocytes 5,100,000 per c.mm.
Haemoglobin 70% (Haden) = 10.92 g/100 ml.
Packed cell volume 42%.
Mean corpuscular volume 82.35 c.μ.
" " Haemoglobin 21.4 g/dl
" " " concentration 26.07%.

Stained blood films. In all the films the red blood cells showed anisocytosis, poikilocytosis and polychromasia. The red cells occasionally assumed oval and elongated shapes. The primitive red cells seen were normoblasts and late erythroblasts.

Smears of Sternal Marrow, 9.11.43.
Per cent

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblasts</td>
<td>1.0</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>2.2</td>
</tr>
<tr>
<td>Myelocytes</td>
<td>3.6</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>5.1</td>
</tr>
<tr>
<td>Eosinophil metamyelocytes</td>
<td>0.1</td>
</tr>
<tr>
<td>Proerythroblasts</td>
<td>15.0</td>
</tr>
<tr>
<td>Polymorphs</td>
<td>61.2</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2.0</td>
</tr>
<tr>
<td>Large lymphocytes</td>
<td>2.2</td>
</tr>
<tr>
<td>Small &quot;</td>
<td>2.9</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.5</td>
</tr>
<tr>
<td>Haemocytoblasts</td>
<td>0.1</td>
</tr>
<tr>
<td>Early erythroblasts</td>
<td>0.1</td>
</tr>
<tr>
<td>Intermediate &quot;</td>
<td>0.5</td>
</tr>
<tr>
<td>Late &quot;</td>
<td>0.6</td>
</tr>
<tr>
<td>Normoblasts</td>
<td>0.5</td>
</tr>
<tr>
<td>Abnormal haemoglobinised  nucleated red blood cells</td>
<td>0.2</td>
</tr>
<tr>
<td>Unclassified myeloid cells</td>
<td>0.2</td>
</tr>
<tr>
<td>Degenerate cells</td>
<td>0.1</td>
</tr>
<tr>
<td>Megakaryocytes</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Leucoerythroblastic ratio = 48.6 : 1
The marrow shows a greatly increased leucoerythroblastic ratio, with predominance of the neutrophil series. This is represented mainly by increase in the mature polymorphs, many of which show abnormal features, such as nuclei with five to eight lobes and coarse slightly basophil granules in the cytoplasm. Cells of the red cell series are considerably reduced in numbers, and abnormal forms are seen rather resembling the megaloblast of Ehrlich. These cells are larger than a normoblast, are well haemoglobinised and have large immature nuclei with coarsely reticulated chromatin.

Blood Wassermann and Kahn reactions were negative. Fractional test meal showed a histamine fast achlorhydria. Combined acidity was within normal limits. Blood was not detected in any specimen. Faeces contained no occult blood.

Radiological Findings.

1. Bones. Humeri shows bone of normal density and trabeculation. Pelvis: no bony abnormality detected; an enormous soft tissue mass is filling upper portion of pelvis and left side of abdomen. Hips and upper femora show bone of normal density with normal medullary cavity and trabeculation. Lower femora and upper tibia show bone of normal density with well defined trabeculation. The medullary canals were normal. No vascular calcification seen.
Leucoerythroblastosis. Liver architecture and liver cells normal. Increased cellular content of sinusoids and portal tracts. Giant cells can be seen even at this magnification.

Stained Best's Carmine. x 90.
**FIG. 138.**
Leucoerythroblasticosis. Giant cells, late erythroblasts, normoblasts and polymorphs are present in the hepatic sinusoids. Stained Best's Carmine. X 300.

**FIG. 139.**
Leucoerythroblasticosis. Giant cells, resembling the megakaryocytes of the bone marrow, in the hepatic sinusoids. Stained Leishman. X 500.
2. **Chest.** The left ventricle is enlarged and the aorta is unfolded. The hilar shadows are heavy and the retrocardiac space is obscured by a soft tissue mass, possibly glands. The oesophagus is deviated posteriorly in this region.

3. **Barium meal.** No intrinsic gastric lesion but the stomach is displaced to the right and anteriorly by a large soft tissue mass arising from the left hypochondrium.

**Aspiration liver biopsy (19.11.45)** was uneventful and a satisfactory sample of liver tissue was obtained for section. Histological findings are illustrated (fig.137). The liver architecture and liver cells are essentially normal. The liver cells contain their normal complement of glycogen. The hepatic sinusoids are distended and have a richly cellular content (fig.138). Many of the cells are multinucleated giant cells morphologically identical with the megakaryocytes of the bone marrow (fig.139). There are also many reticulum cells and there is some evidence of transformation of these cells into the giant cells. Other cells present are polymorphs in abundance and an occasional normoblast and late erythroblast. Apart from the sinusoids, aggregations of similar cellular content were found related to the portal tracts. The impression was myeloid metaplasia in the liver with numerous megakaryocytes.
Progress. The patient was treated with ferrous sulphate, gr. 3, thrice daily. The medication produced little change in the blood picture. On 29.11.43 she was discharged home feeling reasonably well. Soon after leaving hospital the patient contracted a chill which was followed by pneumonia. She was treated at home: recovery was slow. The breathlessness on exertion became more marked and ankle swelling was noticed in the evening. She felt very weak and was losing much weight. On 22.8.44 she was readmitted to hospital. Physical examination revealed very similar findings to those recorded previously. The patient was thinner. The huge spleen seemed even larger than before and now the liver could be palpated 9 cm. below the right costal margin (fig.136). Peripheral lymph nodes were not enlarged.

Investigations. Haematological findings are tabulated.

Radiological findings.

1. Bones.

Right humerus and shoulder. Bone of average density and the trabeculation appears normal. The medullary cavity is well defined and is, if anything, slightly larger than the average.

Right femur. Bone of average density and trabeculation also appears normal. The medullary cavity is
quite well defined and is, if anything, slightly larger than the average. Within the medullary cavity trabeculation is poor, being slightly blurred but no gross abnormality is seen.

2. Chest. The upper mediastinal shadow is broader than the average. The posterior mediastinum is clear. The appearances suggest slight enlargement of hilar glands.

Further progress. The patient improved with rest and ferrous sulphate 9gr. daily. She was discharged home on 9th September, 1944. It was later reported that during January 1945 she had died at home. A post mortem examination was not performed.

Discussion.

The relation of leucoerythroblastosis to bone changes.

Ever since 1908 when Donhauser published an account of a case of splenomegaly with sclerosis of the bone marrow and with histological evidence of myeloid metaplasia with many giant cells in the spleen, many reports of similar cases have appeared in the literature. The relation of the bone changes to the peripheral blood picture is still in doubt. The type of bone lesion associated with the leucoerythroblastosis is very variable. Secondary carcinoma, myelomatosis, Cooley's anaemia and myelosclerosis are among the related bone lesions, and the haematological findings bear no relation to the extent of the bone involvement (Vaughan,
1936). Tudhope (1937), for instance, describes a case in which the osteosclerosis involved the upper part of one femur and elsewhere there was only gelatinous bone marrow. In our case definite bone changes could not be demonstrated radiologically. This may indicate localised bone disease in a part not exposed to radiographs. It may indicate a bone lesion of such a nature that changes in bone density are not produced; fibrous changes may be present in the marrow; the radiological changes in such cases are often minimal (Chapman, 1933). On the other hand, the possibility exists that bone changes may not be present. It has been postulated that the clinical and pathological picture of leucoerythroblastic anaemia can occur in the absence of bone changes (McMichael, 1935; McMichael and McNee, 1936). These latter authors report three such cases and describe five additional cases collected from the literature. In our case, in the absence of a post mortem examination, it is impossible to state whether the case falls into the "idiopathic" group or whether bone lesions were present, unrecognised by radiographs.

The relation of leucoerythroblasts to leukaemia.

The presence in the peripheral blood of a small number of immature red cells is a well known accompaniment of myeloid leukaemia. This led to the group of cases now designated "leucoerythroblastic anaemia" to be included in the "atypical leukaemias". They were variously termed "leukanaemia" (Leube & Arnett, 1902) and
"myeloid splenic anaemia" (Vaquez & Aubertin, 1904). Other authors were impressed by the conspicuous giant cell (megakaryocytic) proliferation in the bone marrow, liver, spleen and other organs and suggested the term "megakaryocytic myelosis" (Hewer, 1937; Bamforth and Kendall, 1939). The present case is not strictly comparable to a leukaemia; the almost constant presence of young red cells in the peripheral blood with such mild anaemia is unusual in leukaemia (Vaughan & Harrison, 1939); and although in our case, as in that of Downey et al. (1930), there was a suggestion of the formation of megakaryocytes from the local reticulum in the liver, the changes included myeloid elements. It is very unusual for more than one cell type to be involved in a leukaemic process. The duration of life is usually longer than that enjoyed by even a case of chronic myeloid leukaemia; our case lived for 7½ years after discovery of the splenomegaly. However, it seems unwise to separate the leukoerythroblastic group entirely from the leukaemias. A gradation may exist from one type to the other. In our case the height of the leucocyte count in the peripheral blood and the undue preponderance of leukoid elements in the sternal marrow suggested that the myeloid elements predominated in the process and could represent one phase in the progression towards leukaemia. The two cases of Mettier & Rusk (1937) presented leukaemoid blood pictures. Lindboom (1938) believes that the condition
lies midway between the reticuloses and leukaemia. Bamford & Kendall present a case with a frankly leukae-
moid blood picture; the bone marrow, lymph glands, liver and spleen contained large numbers of primitive
cells, mainly myeloblasts and megakaryocytes. The bone marrow was very widely involved and there were
subperitoneal deposits in the vertebrae producing a paraplegia. The cells possessed invasive properties
and were invading peripheral nerves and the fibres of voluntary muscle. These authors stress the malignant
nature of their case and postulate a further relation between megakaryocytic myelosis and chlorosis.

The Diagnosis of Leucoerythroblastosis.

A careful clinical history and examination, together with examination of smears of sternal marrow
and repeated study of the peripheral blood, are essential. Although splenic puncture has been used
as a diagnostic aid (Emile Weil et al., 1936), liver puncture does not seem to have been employed for this
purpose. The method has obvious potentialities. The puncture in the liver of myeloid metaplasia with large
numbers of megakaryocytes is almost specific. It does not resemble the picture seen in a true leukaemia. The
method is likely to fail only in the few cases, such as that of Hewer, where the liver escapes involvement.
Where liver change is present, the picture is diffuse and sampling errors are unlikely.
The treatment of leucoerythroblastosis.

Accurate diagnosis from leukaemia is essential because the treatment of the two conditions is diametrically opposite. The usual haematinics are valueless. Bomford & Rhoads (1941) include the condition in the refractory anaemias. X-ray therapy is dangerous and splenectomy is often fatal. No treatment seems of any avail. Iron may be given as a placebo.

Summary.

A case of leucoerythroblastosis was submitted to aspiration liver biopsy. The characteristic picture of myeloid metaplasia and giant cell proliferation was demonstrated.

The relations of leucoerythroblastosis to bone changes and to leukaemia are discussed.
CHAPTER 24

ASPIRATION LIVER BIOPSY IN THE ELUCIDATION OF "REFRACTORY" ANAEMIAS.

Although the use of sternal marrow punctures has enabled great strides to be made in the classification and study of the anaemias, it is suggested that in obscure cases, especially when the myelogram is equivocal, aspiration liver biopsy may have a part to play in reaching a correct diagnosis. This contention is illustrated by the following case.

Case Report. A 54 year old grocer was admitted to hospital on 16.11.43., giving a 9 months' history of pains in the legs, exhaustion, breathlessness and pallor. 2 months previously a blood examination had been carried out, and following this, he was given iron and intramuscular liver extract. This had caused no improvement. There had been no weight loss. He had not been in contact with any possible haemotoxic agents. The patient was a well-developed man. The skin had a yellowish sallow tinge. There was no enlargement of lymph nodes. The tongue was pale and smooth. The fundus oculi showed bilateral new and old haemorrhages. One of the haemorrhages showed a white centre. In the abdomen the liver was not palpable and the spleen could just be felt on deep aspiration under the left costal margin.
Examination of the central nervous system revealed no abnormalities. Urine showed constant excess of urobilin. Stained blood films showed constant anisocytosis and anisochromasia of the erythrocytes. There was occasional poly-chromasia and poikilocytosis. The nucleated red cells seen were normoblasts with very occasionally a late erythroblast.

**Absolute values 17.11.43.**

Mean corpuscular volume 107.5 c.u.

" " haemoglobin 37.06

" " concentration 34.48%

Sternal punctures were performed on 17.11.43. and 15.2.44. Both showed relative cellularity. There was no evidence of megaloblastic reaction and the predominant cells present were small with deeply staining nucleus, and scanty basophilic cytoplasm. The majority appeared to be erythroblasts but in many instances the cells resembled lymphocytes. The granulocytes were present in rather diminished numbers.
### TABLE 40. THE HAEMATOLOGICAL FINDINGS IN THE CASE DESCRIBED

<table>
<thead>
<tr>
<th>Date</th>
<th>RBC per c.mm.</th>
<th>Hb. %</th>
<th>C.I.</th>
<th>Nucleated RBC/100 WBC</th>
<th>Reticulocytes %</th>
<th>WBC per c.mm.</th>
<th>Polymorphs</th>
<th>Lymphocytes</th>
<th>Mononuclears</th>
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</thead>
<tbody>
<tr>
<td>16.11.43</td>
<td>2</td>
<td>38</td>
<td>0.95</td>
<td>5</td>
<td>0.8</td>
<td>3000</td>
<td>1530</td>
<td>1410</td>
<td>60</td>
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<tr>
<td>17.11.43</td>
<td>1.6</td>
<td>38</td>
<td>1.18</td>
<td>2</td>
<td>1.2</td>
<td>3000</td>
<td>1410</td>
<td>1590</td>
<td>30</td>
</tr>
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### FIG. 140.

![Graph showing hematological findings](image-url)
Fractional test meal showed a histamine fast achlorhydria. Barium meal showed no organic lesion of the gastrointestinal tract. Occult blood was absent from the stool on 3 occasions.

Progress. Apyrexial apart from blood transfusions. No response to ferrous sulphate gr.3 t.d.s., anaehaemin 12 cc. intramuscularly, plexan 8cc. intramuscularly, or ascorbic acid 500 mg. daily. On 26.11.43 given a packed red cell transfusion (1 pint of blood of haemoglobin concentration 120% (Haden) ). Haemoglobin rose from 37% to 47.5% Haemoglobin fell off and on 9.12.43 the patient was given a further packed cell transfusion, and the haemoglobin rose to 50%. The patient was given proteolysed liver extract by mouth (2 oz. daily) and was discharged on 22.12.43, having maintained the 50% haemoglobin level for 3 weeks. Reticulocytes in the peripheral blood, however, never rose above 1.2%.

During the next 6 weeks, despite the administration of the proteolysed liver, the haemoglobin level fell to 41% and on 4.2.44 the patient was readmitted to hospital for transfusion. A pint of packed red cells was given and the haemoglobin remained at 42%. The liver was now noticed to be enlarged and was felt 4 c.m. below the costal margin in the nipple line. The spleen could now be readily felt 3 cm. below the left costal margin. It was decided to perform an aspiration liver biopsy.
Reticulosis. Conspicuous accumulation of nucleated cells in the sinusoids and portal tracts. Liver architecture is essentially normal.

Stained Best's Carmine. X 120.
FIG. 142.
Reticulosis. Portal tract showing round cell infiltration and some bile duct proliferation. Stained Best's Carmine. X 265.

FIG. 143.
Reticulosis. The lobule centre showing the nucleated cellular increase in the sinusoids. Many of the cells are primitive in type and give the impression of being formed from the sinusoidal epithelium. Stained Best's Carmine. X 265.
Histiocytic medullary reticulosis. "Butterfly" eruption on nose and cheeks.
FIG. 145.
Histiocytic medullary reticulosis. Confluent well demarcated rash on trunk.

FIG. 146.
Histiocytic medullary reticulosis. Maculo-papular demarcated rash on legs.
Aspiration Liver Biopsy (7.2.45) was uneventful. A satisfactory specimen was obtained. Sections showed an unusual picture. The chief change was a conspicuous accumulation of nucleated cells in the sinusoids and, to a less extent, in the portal tracts. (Figs. 141 and 142) These cells were primitive in type and gave the impression of being formed from the sinusoidal endothelium. There was no evidence of phagocytic activity. The cells were very poorly differentiated. (Fig. 143)

Iron-stained sections showed a well-marked diffuse siderosis of the liver cells and Kupffer cells.

Further Progress. The patient became febrile on 6.2.44 and a steady fever of 103 - 104.5° persisted until his death on 23.2.44. Blood culture was bacteriologically sterile. The haematological changes are best seen in Table 40 but the progressive leucopenia should be noted. On 19.2.44 a macular papular rash was noticed on the trunk and limbs, excluding the face, hands and feet. The rash was raised, purplish-red in colour, and in circular well-demarcated areas becoming confluent on the neck and chest. By 22.2.44 the rash was very marked and was becoming everywhere confluent. A slight "butterfly eruption" was noted on the sides of the nose and on the cheeks (Figs. 144, 145 & 146) The conjunctivae were now icteric and the serum bilirubin level had risen from 0.5 mg. per 100 ml. on 7.2.44. to 8.8 mg. per 100 ml. on 23.2.44. There were no fundal
haemorrhages, but a bleeding tendency could now be
demonstrated. Capillary resistance test strongly positive.
Bleeding time (Duke's) 3 minutes. Bleeding time (Ivy) 30 minutes. Coagulation time (Lee & White) 60 minutes.
Prothrombin time 41 secs. Prothrombin index 61%.
Platelets per c.mm. 68,000. The patient died on 23.2.44.
The provisional diagnosis was refractory anaemia due to a reticulosis, probably of histiocytic medullary type.
Necropsy was confined to an abdominal incision. The essential findings will be enumerated:

Naked eye:

1. The body showed no evidence of wasting. Height 5ft. 10ins. Weight 10st. 2 lb. The rash and the jaundice previously described were still present.

2. The liver weighed 1800 g. It was pink in colour, with a suggestion of fatty change. The tissue gave a deep prussian blue reaction for iron.

3. The spleen weighed 280 g. It was soft and had rather a red-currant jelly appearance. There was diffuse siderosis.

4. A few vertebral lymph nodes showed enlargement. Lymph nodes elsewhere were not conspicuous.

5. The vertebral bodies were full of dark red jelly-like marrow. The lower end of the sternum contained similar marrow.

Microscopically:

1. Sections of liver showed no essential difference from those obtained by liver biopsy.
2. The spleen showed a normal reticulin structure. It was very cellular and was particularly rich in erythrocytes and reticulum cells. Erythrophagocytosis by reticulum cells was readily demonstrated. Iron stains showed excess iron, both free and in the histiocytes.

3. The vertebral lymph nodes were seen to be haemolymp nodes. The sinuses of the glands were filled with sinus reticulum or 'Littoral' cells. Erythrophagocytosis was clearly demonstrated. One focus of possible erythropoiesis was seen.

4. The vertebral bone marrow showed a diffuse cellularity. The picture was of apparently rich erythropoiesis. The cells seen were mainly erythroblasts with a few basophil normoblasts.

DISCUSSION.

It is not proposed to give a full discussion of the various types of "refractory anaemia" and reticulosis, but rather to analyse the clinical and pathological picture presented by the above case and to outline the part aspiration liver biopsy played in its elucidation.

The first clinical impression of the patient was that of Addisonian pernicious anaemia. This impression was largely based on examination of the peripheral blood. The high mean corpuscular volume was particularly suggestive. However, this diagnosis had already been reached elsewhere and correct treatment
instituted with no beneficial effects. It was essential, therefore, to analyse the cause of the macrocytosis more closely by study of the sternal bone marrow. The marrow did not present the classical megaloblastic picture of Addisonian pernicious anaemia. It was relatively acellular and of the red cell series basophilic erythroblasts and normoblasts predominated with a diminution in the members of the white cell series and a reversal of the leucoerythrogenetic ratio. At this time reports were appearing in the literature of "refractory anaemia": this condition is defined as "Anaemia which failed to yield to any treatment except blood transfusion." Cases in which secondary to other diseases, e.g. cancer, tuberculosis, nephritis, etc. are excluded (Bomford and Rhoads, 1941). The group have been further clarified by Davidson and his co-workers (1943). The present case, which failed to respond to iron, ascorbic acid, yeast or various liver preparations, was considered to fall into the group of refractory anaemias. These anaemias have been further classified according to the sternal marrow findings, and the case described seemed similar to the group 3 of Bomford and Rhoads and of Davidson et al. The group may be designated "refractory anaemia with immature cellular marrow or chronic granulocytopenia" (Bomford and Rhoads, 1941). The case, however, did not fall entirely into this group, although there was evidence of difficulty of white cell forma-
tion. The leucopenia in the peripheral blood was not very marked and the anaemia was rather more severe than that usually described in these cases. Davis and Davidson (1944) have described benefit from proteolysed liver extract by mouth in the refractory cases with megaloblastic marrow, and, although the sternal marrow was not typically megaloblastic, it was thought worth while to try this remedy. It was unsuccessful. The patient was sustained by blood transfusions, and it was on admission to hospital for transfusion that the increasing size of liver and spleen was noted and the patient submitted to aspiration liver biopsy.

The liver biopsy findings at once made the type of refractory anaemia described above seem unlikely. The picture of hyperplasia of the reticulo-endothelial system in the liver seemed to fit into the group of conditions known as reticulooses (Letterer, 1924; Pullinger, 1932). This is using the term in the widest sense described by Scott and Robb Smith, 1936. These authors define reticulooses as a condition with progressive hyperplasia throughout the haemopoetic and lymphatic tissues. The histological type of the proliferating cells varies, but they owe interrelation-ship to the common mesenchymal origin of these cells. The group includes conditions as widely different as Hodgkin's disease, leukaemia and Gaucher's disease. It was therefore necessary to place our case in its
correct category. The case approximated most nearly to the histiocytic medullary reticuloses (Scott and Robb-Smith, 1939). The clinical course with the rapid demise, asthenia, fever, splenomegaly, hepatomegaly and, terminally, jaundice and haemorrhagic eruption was compatible. The feature missing was lymphadenopathy. The peripheral blood findings were also consistent with this diagnosis. At autopsy erythrophagocytosis was demonstrated in spleen, lymph nodes and bone marrow, and there was diffuse haemosiderosis. The failure to demonstrate erythrophagocytosis by the Kupffer cells of the liver has also been reported in the case described by Beaver and Johnson (1934). Aspiration liver biopsy provides a method, in addition to sternal puncture and lymph gland biopsy, of studying the morphology of the reticulo-endothelial system. It, therefore, has a place in the study of obscure anaemias. Moreover, it seems likely that the method may be used to separate many cases of refractory anaemia from the wider group of reticuloses.

Summary.

A 54 year old man presented with a macrocytic anaemia refractory to treatment with known haematinics. The sternal marrow showed preponderance of erythroblasts and diminution of the leucopoietic elements. Eventually sections of liver obtained by aspiration liver biopsy enabled the case to be included in the group of
reticuloses. The eventual course and autopsy showed that the case most closely approximated to histiocytic medullary reticulosis. The use of liver biopsy in the study of obscure anaemia is discussed.
CHAPTER 25.

ASPIRATION LIVER BIOPSY IN THE DIAGNOSIS OF

HAEMOLYTIC ANAEMIA.

Aspiration liver biopsy was used to assist in the diagnosis of three cases of haemolytic anaemia. Two were instances of the "acquired" type, the third, of "congenital" type, occurred in a young man who had complicating features in his past medical history. A description of this latter case will be used to illustrate the applicability of the method.

Case Report. A 26 year old Army officer was admitted to hospital on 22.1.45. He complained of feeling generally run down and tired. There were no other specific complaints. At the age of 9, while at boarding school, he had an attack of jaundice; there had not been other cases in the school. At 19 there was a further attack of jaundice; at that time he felt only moderately ill. The urine was darker than usual. The spleen was found to be slightly enlarged. At the age of 22 (July 1941), one week after landing in India, there was a slight attack of jaundice. There was diarrhoea, but his appetite remained good. There was no nausea. The stools and urine were dark. At the age of 23 (June 1942) he fainted on a boat in the Red Sea and was found to be pyrexial; there was no jaundice. The provisional diagnosis was malaria.
In May 1943 the patient suffered a severe attack of infective hepatitis. He was extremely jaundiced, there was anorexia and nausea at the onset, the temperature was 101°F, the urine was dark and the stools pale. The symptoms soon subsided but the patient was retained in hospital seven months because of a moderate persistent icterus. He felt well. In June 1944 the patient suffered from benign tertian malaria confirmed by blood smears. The spleen was enlarged, He was given a blood transfusion and iron and liver medication, but remained anaemic. He was taking suppressive mepacrine. The family history was of little moment. His father was said to have suffered from gall-stones in India and his sister had recently been in hospital for investigation of her gall-bladder.

Examination showed a sallow thin man. He was aipyrexial. The spleen was readily palpable 4 cm. below the left costal margin. The liver was not felt. Physical examination was otherwise negative. Urine showed variable quantities of urobilin; sometimes there was only a trace, sometimes excess.

Haematology: Erythrocytes 4.6 million per cu.mm.
Haemoglobin (Haden) 92 per cent. Colour index 1.0.
Leucocytes 7,000 per cu.mm. with a normal differential count. The M.C.V. was 84.22 cu.μ, M.C.H. 33.29
M.C.H.C. 39.52 per cent and the M.C.D. 6.2. μ (Halometer)
The M.C.A.T. was 2.79 μ and the diameter thickness
ratio 1.33. Reticulocytes varied between 8 and 4 per cent. A stained blood film showed slight anisocytosis, anisochromasia and polychromasia of the erythrocytes. Some of the red cells were small and stained darkly. They were thought possibly to be spherocytes. No malarial parasites were seen even after provocative subcutaneous injection of 5 minims of 1:1000 adrenaline hydrochloride followed by blood smears 10, 20, 30 and 60 minutes later. Red cell fragility: The curve was normal. The median corpuscular fragility was 0.414 g. per cent sodium chloride (Fig. 147) A sternal marrow puncture had been performed elsewhere and smears had been reported as normal.

**FIG. 147.**

![Red Cell Fragility Curves](image)
Haemolytic anaemia. Essentially normal liver structure.

Stained Best's Carmine. X 115.
**Fig. 149.**
Haemolytic anaemia. Haemosiderosis of liver cells.
Stained Prussian blue. X 370.

**Fig. 150.**
Haemolytic anaemia. Spleen. The sinusoids are packed with erythrocytes so that the splenic trabecular structure is difficult to differentiate.
Stained H.E. X 95.
Biochemistry. Serum bilirubin 2.9 mg. per 100 ml.
V.D.B. reaction indirect.
Serum alkaline phosphatase 6.0 units/100 ml.
" cholesterol 131 mg./100ml.
" albumen 4.9 g./100ml.
" globulin 1.5 g./100 ml.
A/G ratio 3.3.
Colloidal gold test (MacIlagan) 0
Hippuric acid synthesis normal.
Galactose tolerance normal.

Liver biopsy was performed. The sections showed no hepatatis, fibrosis or disorganisation of the liver structure (Fig.148) Sections stained by the Prussian blue method for iron revealed heavy haemosiderosis of the hepatic cells (Fig.149) The Kupffer cells showed no haemazoin pigment.

Treatment: Splenectomy was performed (15.2.45) At the same time the gall-bladder was found to contain several small hard pigment stones, and was also removed.
The spleen weighed 1260 g. The organ was deep purple-red in colour. The capsule was smooth and slightly wrinkled; it was not thickened. The spleen cut easily and the cur surface poured dark blood. The malpighian corpuscles were not easily differentiated.
Sections of spleen showed the characteristic picture of acholuric jaundice. The sinusoids were packed with erythrocytes so that the typical splenic structure was difficult to differentiate. The malpighian bodies were not conspicuous. Some of the arterioles showed hyaline change of their walls. (Fig.150)

Progress: The patient made an uninterrupted recovery
and was discharged to a convalescent home 4 weeks after operation. The effect of the splenectomy on the serum bilirubin, the erythrocytes and haemoglobin, the reticulocytes and platelets is seen in Fig. 151. Urobilin disappeared from the urine. The fragility of the erythrocytes in saline remained virtually unchanged.

**FIG. 151.**

**Discussion.** The multiplicity of known aetiologial factors in this case made diagnosis difficult. The points in favour of a diagnosis of excessive blood
destruction were:-

(1) The bilirubinaemia with an indirect Van Den Bergh reaction.

(2) The urobilinuria.

(3) The reticulocytosis in the presence of a falling haemoglobin and red cell count (Fig. 151) That this was probably a congenital acholuric jaundice was suggested by:-

(i) The history of repeated attacks of jaundice, starting early in life and during which the patient was more icteric than sick, and in which the stools were often noted to be dark in colour.

(ii) The haematological findings which were very suggestive of spherocytosis.

Against this diagnosis were:-

(i) The negative family history. However, as the relatives lived at a distance, it was not possible to investigate all of them thoroughly. Latent cases such as those described by Vaughan (1936) might have been encountered.

(ii) The normal fragility of the erythrocytes. However, 10 per cent of cases of congenital acholuric jaundice are said to have normal red cell fragility (Gänslen, 1922; Gänslen et al., 1925)

However, as spherocytosis and excessive fragility are believed to be related factors, it is surprising that, although fragility was normal, spheroc-
cytosis could be demonstrated. The possibility of a chronic malaria had also to be considered. This is notorious for the frequency with which it mimics other conditions, especially those associated with excessive haemolysis. However, in this instance the presence of such marked evidence of increased red cell breakdown, with no pyrexia and without being able to demonstrate the parasite in the blood even after provocative injection of adrenaline or in the sternal marrow, was against such a diagnosis.

The relation of the findings to the preceding severe attack of infective hepatitis must also be considered. There was a possibility that the acute illness might have passed into a chronic hepatic cirrhosis; however, the blood picture was not at all similar to that observed in chronic liver disease (see Wintrobe, 1936) and it seemed more likely that the acute hepatitis had upset the balance between red cell regeneration and red cell destruction, and so precipitated a haemolytic crisis. This had necessitated the patient being 7 months in hospital. The malarial attack may have acted in a similar fashion.

With all the side issues involved, it seemed essential to use all available methods to clinch the diagnosis of acholuric jaundice before submitting the patient to the major operation of splenectomy. Liver biopsy at once put a cirrhosis or hepatitis out of court. Moreover, the absence of the characteristic
malarial pigment from the reticuloendothelial cells of the liver was strong negative evidence for chronic malaria. As these features were found in the presence of increased iron content of the liver suggesting increased red cell destruction, the diagnosis of a primary (congenital) haemolytic jaundice seemed likely.

The spleen, when removed, showed the characteristic features of acholuric jaundice. Malarial parasites, pigment or fibrosis were not demonstrated. The benefits of the splenectomy have been maintained to date (August 1945).

SUMMARY.

A case of congenital acholuric jaundice in a young officer was complicated by a past history of infectious hepatitis and malaria. Aspiration liver biopsy showed a normal liver structure, the only abnormality being haemosiderosis of the hepatic cells. This added confirmation to the diagnosis of acholuric jaundice. Splenectomy was performed with benefit.
CHAPTER 26.

CASES IN WHICH ASPIRATION LIVER BIOPSY FINDINGS WERE WRONGLY INTERPRETED.

In the preceding group of cases the study of the case was greatly facilitated by examination of aspiration liver biopsy material. This section on the diagnostic application of the method will be concluded by descriptions of two cases in which a definite diagnosis was not made even after very full investigation. In one the correct answer was given by laparotomy; in the other the diagnosis was only reached at autopsy.

Case I. A 29 year old officer was admitted to hospital on 24th January, 1944. He gave a long history of ill-health. In September 1941, while in Iraq, he suffered from epigastric pain occurring two hours after meals. A barium meal was performed and a diagnosis of gastric ulcer was made. He was treated along the usual lines and six months later a further barium series showed that the ulcer had healed. In September 1942 there was a recurrence of similar epigastric pain, but on this occasion a barium meal showed no abnormality. He was treated as for a peptic ulcer with subsidence of symptoms. In May 1943, while in India, the patient suffered severe diarrhoea with blood and mucus in the stools. Entamoeba histolytica was demonstrated in the faeces and a course of 10 injections of emetine
hydrochloride was given. There was a marked febrile reaction to the injections. In June 1943 for the first time it was noted that his liver was enlarged. At that time the patient suffered from stitch-like pains in the right and left side and a general ache in the epigastrium unrelated to the consumption of food. Since June 1943 the pains were still occurring intermittently. The patient noticed himself increasingly weak and easily fatigued. There had been weight loss. One year before admission he weighed 13 stone; now his weight was only 10 stone 5 lbs. Very occasionally he had febrile attacks, lasting only for a few hours. The temperature in these attacks was noticed to be 101°. On specific enquiry he would admit to no urinary symptoms. There was no frequency of micturition or haematuria, and he did not rise at night to pass water.

His habits were moderate and he took very little spirits...

In the past he had had his appendix removed at the age of 19. While in India he had suffered from dengue and sandfly fever. He had never been jaundiced.

Examination showed a pale man. He looked ill. His cheeks were sunken and he was underweight. There was no enlargement of peripheral lymph nodes.

A small pink circular elevated nodule about
1 cm. in diameter was noticed on the left chest in the 5th. intercostal space anteriorly. This seemed to be situated in the skin itself. It had been present 3 years. In the abdomen a large mass could be palpated in the right upper quadrant. This mass had a firm lower margin, and towards the right was markedly nodular on the surface. The mass was thought to be the liver. The spleen could just be tipped. General physical examination was otherwise negative.

Urine examination on 3 occasions showed no abnormalities. Bile, urobilin and albumen were absent. Examination of the centrifuged deposit showed only an occasional leucocyte.

Biochemical findings.

Serum bilirubin: less than 0.5 mg. per 100 ml.

" phosphatase: 3 units per 100 ml.

Fasting blood sugar: 90 units per 100 ml.

Liver function tests.

Bromsulphthalein excretion test (5mg/kilo body weight)

No retention of dye at the end of 30 minutes

Intravenous galactose tolerance test: normal.

Oral hippuric acid synthesis test: normal.

The stools were of normal colour and consistence. The Fat content was 15.5 g/100 cc. dried faeces and the fat was well split.

Occult blood was absent on 3 occasions.

No entamoebae were detected in the warm fresh stool.
Haematology. Erythrocytes 5.3 millions per c.mm.

Haemoglobin (Haden) 95 per cent., colour index 0.90.

Leucocytes 9,000 per c.mm., of which 1710 were Lymphocytes, 270 monocytes and 7020 neutrophil polymorphs.

Chest X-ray showed no abnormality. Both domes of the diaphragm moved well and evenly.

Biopsy of the nodule on the left chest wall was undertaken. Sections showed the lesion to be a well differentiated fibroma, probably a neurofibroma.

Liver biopsy was now performed. Only a small fragment was obtained. When penetrated by the trocar the liver substance felt very soft - like butter. No blood was aspirated with the biopsy. Sections of the liver proved extremely difficult to interpret on the small fragment available. The lobular pattern was lost and normal liver tissue was not seen in any part of the section. The individual cells seen were swollen and granular and appeared distended with lipid. They contained little glycogen but glycogenic nuclear degeneration was well marked. Between these cells was a growing tissue in which new reticulin fibres were being formed (figs. 152 & 153). No definite diagnosis was made.

Progress. While in hospital the patient complained of no new symptoms. On the night of the bromsulphthalein test the patient had a rigor and his temperature rose to 102°.

As a definite diagnosis could not be formulated
Figure 152. Case 1.
Normal liver tissue not seen. The individual cells are swollen and appear distended with lipid.
Best's carmine stain. X 120.

Figure 153. Case 1.
The individual cells are swollen and granular, and contain a hyperchromatic nucleus. They apparently contain lipid. Between the cells is a growing tissue in which new reticulin fibres are being formed.
Best's carmine stain. X 235.
the patient was strongly advised to submit to laparotomy. This recommendation was refused and for about three months there was a general improvement, the patient feeling less weak and gaining half a stone in weight. However, this remission was only temporary, and in November 1944 the patient underwent an abdominal operation. A large mass was found occupying the right upper abdomen and this was removed with difficulty. The mass was "as large as a soccer ball" and proved on section to be a very anaplastic hypernephroma. The remains of the right kidney were compressed over the lower pole of the tumour. There was no evidence of intra-abdominal metastasis. The patient recovered well from the operation, but evidence of generalised spread of the tumour was not long in appearing. He soon presented features of osseous, cutaneous and peritoneal metastases and has since died.

Comment.

It is difficult to know how a correct diagnosis could have been arrived at sooner. The patient had previously consulted two experts in tropical medicine, one of whom made a provisional diagnosis of hepatic cirrhosis, the other of hepatic lymphadenoma. There had never been any suggestion that the abdominal tumour might not be the liver, nor were there any symptoms drawing attention to the urinary tract. On the present admission the mass certainly appeared to be related to
the liver. The so-called liver biopsy was undoubtedly a puncture biopsy of the massive hypernephroma, which was invading the right kidney. The difficulty in interpreting the sections obtained arose from lack of knowledge of the correct site from which they were obtained, the small size of the material available for study and the unusual nature of the histology. When at operation a correct diagnosis was eventually reached, further reference was made to the liver biopsy sections. Although it was now evident that the lesion was a hypernephroma, the sections were atypical even of this pleomorphic tumour. Originally the presence of cells packed with lipoid and glycogen had suggested one of the rarer storage disorders of metabolism; it was now apparent that the cells contained only the content of lipoid usually described in hypernephroma and which was one of the points put forward for an adrenal origin of the tumour.

Summary.

A young officer presented with a large right upper abdominal mass thought to be the liver. This mass was submitted to puncture biopsy. The resultant histological picture proved impossible to diagnose. Later, at operation, the lesion was found to be a massive hypernephroma. It was puncture of this tumour and not the liver which had led to such a bizarre
histological picture being obtained.

**Case II.** A 51 year old lorry driver was admitted to hospital on 23rd August, 1943. In July 1941 he had contracted primary syphilis and during the succeeding year he had been treated by injections and had received a total of 12.0 g. stabilarsen and 2.8 g. bismuth. The last injection was on 15th July 1942.

In September, 1941, the treatment had been complicated by stomatitis and in December 1941, and August 1942, there were episodes of "threatened jaundice." These latter events were characterised by malaise, anorexia, occasional vomiting and generalised joint pains.

There had been no clinically recognisable jaundice, but the urine had contained an excess of urobilin.

He then remained well until 3 weeks before admission to hospital. The present illness commenced with a gastrointestinal disturbance. There was a heavy feeling in the epigastrium, occasional nausea, belching of gas and vomiting. The next day his urine was noticed to be darker than usual and a fortnight before admission he became jaundiced. The jaundice had progressed. Anorexia had been marked and during the 3 weeks he had lost an uncertain amount of weight.

The patient had been a fairly heavy consumer of alcohol. He used to take an average of 6 pints of beer nightly and some additonal spirits. In the past 2½ years he
had taken very little alcohol. Past health had been good. He did not usually suffer from indigestion. Family history was of interest in that his mother and one brother were both reported to have succumbed to cancer of the liver.

Examination showed a well-developed man of good nutrition. He was deeply icteric and dilated blood vessels were seen on the nose. The liver was palpable 4 cm. below the umbilicus in the right flank. The margin was smooth and firm. The spleen could not be felt. Physical examination was otherwise negative. The urine always contained much bile pigment and no urobilin. Microscopic examination of the centrifuged deposit showed an occasional coarsely granular cast.

Investigations: The biochemical findings are tabulated. (Table 41.)

**Table 41. The Biochemical Findings in Case 2.**

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</tbody>
</table>


**Figure 154. Case 2.**

On the right relatively normal liver tissue showing the features of obstructive jaundice. On the left a focus of malignant neoplasia.

Stained Best's carmine. X 145.

---

**Figure 155. Case 2.**

A higher power view of the anaplastic area. Many hyperchromatic cells are seen sometimes grouped into anaplastic bile ducts. There is an extensive stromal reaction and focal infiltration with lymphocytes.

Stained Best's carmine. X 300.
Figure 156. Case 2.
The liver at autopsy. Hepatic cirrhosis with very extensive bile duct proliferation.
Stained H.& E. X 28.

Figure 157. Case 2.
At the top frankly malignant tissue is invading the portal tracts. Below the liver shows the features of obstructive jaundice.
Stained H.& E. X 370.
thrombi). The remaining area appeared to be a focus of malignant neoplasia (on left in Fig. 154 and more highly magnified in Fig. 155) There were many large hyperchromatic cells of uncertain type. Some of these cells were grouped together into anaplastic bile ducts. There was an extensive surrounding stromal reaction, there being much newly formed reticulin which in parts was heavily infiltrated with lymphocytes. A further liver biopsy was performed on 23.9;43 and the histological picture was essentially similar to that seen in the earlier biopsy. The large malignant cells, however, were not noted. In most cases the small multiplying ducts appeared to be malignant. The interpretation of the biopsies was bile duct carcinoma with extensive stromal reaction.

Progress. The condition of the patient steadily worsened. Anorexia was complete. After any food the patient felt very distended and complained of a dull aching pain in the centre of the epigastrium. There were occasional episodes of diarrhoea. The jaundice progressively deepened. Vascular telangiectasia was noted on the face and the palmar eminences were flushed. The peritoneal cavity filled with ascitic fluid and on two occasions paracentesis was necessary. The liver enlarged and the surface became firm and hard knobs could be palpated on the surface. Death supervened on the 23rd September, 1943.
Necropsy. The essential findings are enumerated.

Naked Eye.

1. The peritoneal sac contained 1500 cc. of bile-stained fluid.

2. The pancreas, both body and head, was invaded with streaks of yellowish malignant tissue. The common bile duct was completely occluded by the pressure of the growth.

3. The liver weighed 2500 g. It was dark green in colour and on the surface were knobs of white malignant tissue. The cut surface showed the liver substance to be separated into nodules by white bands.

Microscopically

1. The pancreatic tumour was of undifferentiated glandular type. The origin was thought to be from the pancreatic ducts.

2. The liver as a whole showed the features of portal cirrhosis but there were areas of frank malignancy which were invading the dense connective bands of the hepatic cirrhosis. (Figs 156 & 157)

Discussion.

The provisional diagnosis on admission was hepatic cirrhosis. The two incidents during the period of arsenotherapy were believed to be subicteric arsenotherapy hepatitis and these had been followed by hepatic cirrhosis, at first latent, but later becoming active. (Bloomfield, 1938). The original blood biochemistry supported this hypothesis. However, the continued
absence of stereobilin from the faeces and of urobilin from the urine suggested rather an obstructive jaundice. This theorising took place before the liver biopsy findings were available. The histological picture of the liver at once suggested malignancy. The scattered groups of malignant cells simulating bile ducts, the overactivity of relatively normal bile ducts, the excessive surrounding stromal reaction and the picture of bile retention in the liver tissue made a diagnosis of bile duct carcinoma probable. Moreover, this was believed to have been superimposed on the marked bile duct proliferation encountered in cirrhosis. This suggestion was considered in spite of the knowledge that the carcinoma associated with hepatic cirrhosis is commonly a hepatoma (Counsellor and McIndoe, 1926; Wilbur, Wood and Willett, 1944).

As the disease progressed, the unremitting jaundice, the cachexia and the development of a hard irregular liver made the diagnosis of malignant disease of the liver certain. Necropsy confirmed the hepatic cirrhosis. The pancreatic carcinoma was not suspected during life. This lesion had produced an obstructive jaundice. Common bile duct occlusion acts as a stimulus to the minute bile ducts through the liver and there is usually a surrounding fibrous stromal reaction (Loeffler, 1927; Fiessinger, Albot and Dieryck, 1931; Cameron and Oakley, 1932). When this stimulus is superimposed on the bile duct proliferation already existing in cirrhosis, the remarkable picture observed in the
case described is easily understood. This is the picture of the second liver biopsy. The bands of fibrous tissue and proliferating bile ducts were being invaded by metastatic malignant cells from the pancreatic carcinoma. This accounts for the histology shown by the first hepatic biopsy.

Summary:

A 51 year old man presented with a history of three weeks jaundice. Two years previously he had been treated for syphilis by arsenic and bismuth injections, and had twice suffered "subclinical hepatitis." Liver biopsy showed the features of obstructive jaundice and also a band of fibrous tissue containing a great number of proliferating bile ducts, some of which appeared malignant. A diagnosis was made of hepatic cirrhosis (?) post hepatitis) with superimposed bile duct carcinoma. At necropsy a primary pancreatic carcinoma was discovered which had produced common bile duct occlusion. A hepatic cirrhosis of "portal" type was also present and malignant cells from the primary growth were invading the bands of fibrous tissue. The cause of the bile duct proliferation is discussed.
CHAPTER 21

SOME PHARMACOLOGICAL APPLICATIONS OF
ASPIRATION LIVER BIOPSY.

INTRODUCTION.

This is an age of therapy. Newer and better methods of treatment appear almost daily; often before any procedure is firmly established a more recent method has displaced it. For example, in the space of 10 years the treatment of urinary tract infections has been altered three times. First Mandelic acid therapy was used; then this was replaced by sulphonamides, and now penicillin seems to be displacing them both. Moreover, the development of these newer methods is inextricably linked with "big business", and their production is largely the concern of a small ring of drug houses. It is therefore extremely important for disinterested workers to use as many ways as possible to check the value of these newer agents. The original investigations must necessarily be on the experimental animal, and only later will their human therapeutic value be known. The difficulties in applying the results obtained by animal observations to man is self-evident. Aspiration liver biopsy findings, taken in conjunction with clinical observations and the results of laboratory biochemical tests, can be used to check the therapeutic value of any method in human liver disease and to decide its
toxic action on the liver. This chapter will deal with some observations made on the possible harmful effect of mepacrine on the liver, and with the therapeutic use of choline in steatosis hepatitis with cirrhosis.

**Aspiration liver biopsy in the study of mepacrine toxicity.**

Malaria has proved the major disease of the war just ended. In West Africa, for instance, at certain seasons, a 100 per cent incidence of malignant tertian malaria in the troops is expected (Drew and Reid, 1945). Quinine is not an entirely satisfactory suppressive. The expense and enemy control of sites of production have been deterrents to its general use. The evolution of the yellow acridine dye mepacrine soon led to its general use for troops in malarial regions. Complaints of the toxicity of the substance were soon forthcoming, largely from the Middle East Forces. The main symptoms were nausea, vomiting and insomnia. Moreover, as the drug causes yellow staining of the skin, there was some confusion with jaundice, and it was suggested that mepacrine was hepatotoxic. This was supported by observations on the experimental animal. It was discovered that large doses caused necrosis of the liver (De Mello and De Azevedo, 1932; Martin et al. 1939; Scudi et al., 1944). Clark et al. (1937)
showed that in dogs the drug produced impairment of the bromsulphthalein and bilirubin excretion tests. There was one report of mepacrine producing liver necrosis in man (Field et al., 1937). It therefore seemed important to investigate more fully the effects on the human liver of taking mepacrine for long periods.

**Material.**

In 1943 large numbers of men were returning to Britain from West Africa, having been on suppressive mepacrine for long periods. 102 of these men were investigated at Milbank Hospital (Drew and Reid, 1945). At the request of the Army authorities a number of volunteers were referred to Hammersmith Hospital for aspiration liver biopsy. Ten cases were selected having had a total of 11 - 36 g. mepacrine over a period of 6 - 17 months. In all the cases in which it was estimated there was a significant amount of mepacrine in the blood.

**Results.**

The clinical and laboratory findings are tabulated. (Table 39).
Of the 10 cases, 7 showed some clinical or biochemical abnormality. In 2, the liver was palpable; in 3, there was splenomegaly. 6 had an impaired hippuric acid synthesis test, i.e. less than 80 per cent excretion by the oral method. The remaining 3 cases, although they had taken full courses of mepacrine, presented none of these features.

Hepatic histology.

The findings are summarised in table 40.
Hepatic cells and lobular pattern. Normal in all the cases.

Glycogen content of the liver cells is normal.

Küpffer cells. One case only shows a very slight increase in the number of these cells.

Iron. 5 of the 10 cases show a trace of iron in the liver cells or Küpffer cells.

Haemozoin in the Küpffer cells. 9 of the 10 cases show increases varying from traces to moderate amounts.

Fat. 5 of the 10 cases show a minimal degree of fatty change in the liver cells.
Discussion.

In rats, Scudi et al. (1944) have reported severe liver necrosis due to mepacrine. Other workers describe a loose appearance and vacuolation of the cells in the centre of the lobule, mild parenchymatous degeneration and portal tract lymphocytic infiltrations; the Kupffer cells contain brown pigment like haemosiderin (Martin et al., 1939). It may be categorically stated that the liver sections in our cases did not show any of these features. The livers as a whole show no departure from normal with the exception of some haemozoin pigment in the reticulo-endothelial cells and some slight excess of iron in 50 per cent of cases. As shown in table 39, none of the ten men had suffered from malaria; some had had as many as ten attacks; the haemozoin is therefore not unexpected. The iron may have a like cause, or may be associated with the mild degree of anaemia present in most of the cases. Apart from these observations, the liver structure has been within normal limits.

The hepatic pathology described in animals was associated with doses of mepacrine far in excess of that used in human therapy. Martin et al. (1939) gave 0.2 g./kilo. body weight to their dogs. Scudi et al. (1944) found that in rats the quantity producing liver necrosis was 50 per cent of the lethal dose 50 (0.225 mg./kilo. body weight). The fallibility of
transferring observations made with massive dosage in experimental animals to human disease is obvious.

Drew and Reid (1945) have demonstrated the beneficial effects of suppressive mepacrine in preventing deaths from severe forms of malaria, in preventing complications such as blackwater fever, and in eliminating disability from chronic malaria. The observation that the drug has no demonstrable hepatotoxic action is therefore of some importance in facilitating the continuance of routine dosage. The unreliability of the hippuric acid synthesis test as a measure of hepatic function, as already discussed in this thesis (Chapter 9), is further emphasised.

The therapeutic efficacy of choline.

The last 20 years have produced a large literature concerning the dietetic production and treatment of liver lesions. In 1924 Allan, Bowie, Macleod and Robinson reported that depancreatized dogs, kept alive with insulin, eventually showed extensive fatty change in the liver. This fatty infiltration was later seen to progress to cirrhosis (Chaikoff, Connor and Biskind, 1938). Best, Hershey and Huntsman, (1932) found that choline would effectively remove the fat from such livers. Connor (1938 & 1939) has emphasised the importance of prolonged fatty infiltration in the development of cirrhosis in diabetes and chronic alcoholism; the fatty change is believed to be precirrhotic. It was
suggested that the findings of Allan et al. might be applicable to human liver disease.

The next group of observations concerned the dietetic production of hepatic lesions. György and Goldblatt (1939) found that the livers of rats kept on a diet deficient in vitamin B₂ showed parenchymatous and fatty degeneration, focal and massive necrosis, hyperaemia and haemorrhages, and sometimes perilobular fibrosis. Rich and Hamilton (1940) produced a diffuse portal cirrhosis in rabbits kept on a yeast deficient diet. Himsworth and Glynn (1944) encountered similar lesions in rats kept on a protein deficient diet. In animals, this dietetic cirrhosis could be prevented by the addition of choline (Lillie, Daft and Sebrell, 1941; Daft, Sebrell and Lillie, 1941). Experiments were next performed to ascertain whether choline would cure a dietetic cirrhosis. Obviously, complete structural resolution is not to be expected, but Lowry et al. (1941) found that choline or casein could arrest the cirrhotic process and improve the general condition of the animal, fatty change regressed, the liver cells improved in appearance, and the liver size diminished. The mechanism of the lipotropic action of choline is believed to be related to its labile methyl groups; these groups are probably necessary for the prevention of fatty infiltration of the liver (du Vigneaud, 1941). Methionine may act similarly.
The fallacy of transferring the results of animal experiments to human hepatic disease has already been mentioned. However, in 1941, Patek and Post reported impressive results obtained by treating cirrhosis of the liver with a nutritious diet and supplements rich in the vitamin B. complex. This suggested applications of the results of the animal observations to man might yield useful methods of treating hepatic diseases. The clinical reports of the use of choline have not been numerous. Brown and Muether (1942) gave cirrhotics 1 gm. choline daily for up to 2 years, and a number of patients responded well. Russakoff and Blumberg (1944) treated 9 cases of decompensated portal cirrhosis with a high caloric, high protein, high carbohydrate, low fat diet and the usual vitamins with choline; seven improved. Other reports have been mainly unfavourable. Yates (1943) had little success using this treatment in 15 cases of cirrhosis. Richardson and Suffern (1945) treated 16 cases of infective hepatitis with supplementary choline with no significant benefit. The related substance methionine has achieved no better results (Wilson, Pollock and Harris, 1945; Higgins, O'Brien, Peters, Stewart and Witts, 1945).

Aspiration liver biopsy has been used as a means of checking results of therapy by Iversen and Kvarup (1940). Five cases of alcoholic fatty liver were given vitamin B.1. Serial biopsies demonstrated
improvement, although not all the sections showed complete disappearance of the fat. We add to this our results in a case of cirrhosis, showing fatty change in the liver, treated with a high protein, low fat diet and vitamin supplements, and also given choline hydrochloride.

Case Report. This case has been described previously (Chapter 14, case 28).

A 33 year old ex-service man suffering from post-hepatitis cirrhosis of the liver. He took very little alcohol. Vascular "spiders" were prominent (figs. 87 & 88). The liver edge was tender and was palpable just below the right costal margin. The spleen was enlarged half way to the umbilicus. Aspiration biopsy sections (3.4.45) show a typical cirrhosis (fig.158). The cells at the periphery of the lobule are infiltrated with fat (fig.159). The fat takes the form of droplets of varying size. The connective tissue strands are fairly cellular and contain proliferating bile ducts. The liver cells contain a normal complement of glycogen, but towards the periphery of the lobule are of varying sizes.

Treatment. This seemed a suitable case to treat with a high protein, low fat diet and choline. On the 4th April,1945, the patient was placed on a diet of 325 g. carbohydrate, 116 g. protein (60% first class) and 28 g. fat. He was also given supplementary
Before treatment with choline, baker's yeast and high protein low fat diet.

Figure 158. Best's Carmine stain. X 115.

Figure 159. Osmic acid. X 130.
After treatment with choline, baker's yeast and high protein low fat diet.

Figure 160. Best's Carmine stain. X 115.

Figure 161. Osmic acid. X 130.
baker's yeast, 1 oz. daily. On the 21st. April choline hydrochloride was added to the dietary regime. 6 g. were given daily in 4 doses. The substance was suitably flavoured and was not unpleasant to take.

**Results of treatment.**

1. Symptomatic. No change. The patient still complained of general malaise, left abdominal discomfort, headaches and occasional epistaxis.

2. Clinical signs. While under treatment fresh vascular spiders appeared on the wrists. There was no change in the size of the liver on spleen or in the tenderness of these regions.

3. Hepatic histology. The treatment has produced no change in the histological picture (figs. 160 & 161). The fatty change is as pronounced as that noticed in the first biopsy sections.

4. Biochemical changes.

**TABLE 44.**

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</table>

Hepatic biopsies on 3.4.45 and 11.5.45.

Treatment 4.4.45 - 15.5.45. High protein, low fat diet with 1 oz. yeast daily.

21.4.45 - 15.5.45. Choline hydrochloride 6 g. daily.
The biochemical findings in the case are tabulated above. The only improvement is in the result of the bromsulphthalein test. Serum bilirubin and serum proteins show no significant change.

Discussion.

It is always dangerous to be dogmatic on the results of one experiment, however clear-cut the answer. Our case of cirrhosis following acute hepatitis may fall into the "toxic" cirrhosis group (Mallory, 1911), rather than the usual alcoholic portal (Laennec's) type. In the literature it is the nutritional-alcoholic type of lesion that has responded to the dietetic treatment plus choline. It is the trophopathic rather than the toxipathic type of cirrhosis (Himsworth and Glynn, 1944) which would be expected to benefit. McHenry and Patterson (1944) believe that the action of choline in preventing cirrhosis is different when methods other than dietetic are used for the production of fatty livers. It remains, however, that choline exhibited in full dosage to our patient with fatty liver failed to show any lipotropic action.

Some of the recent work reporting negative results with choline and methionine in hepatitis have been criticised on the grounds that inadequate doses of the substances were employed (Barclay, Kenney and Cooke, 1945). The dose curative in rats is 70 mg./kilo body weight (Best and Huntsman, 1935); the quantity used in
our patient should therefore have been adequate. Of the 7 cases of Russakoff and Blumberg which improved with choline 5 did so within 30 days. The period of treatment in our case should therefore have been sufficient.

Steatosis hepatitis has been infrequent in our series of cases of liver disease. The majority of cases of cirrhosis, whether post-hepatitis or of "Laennec's classical type, show little or no fat in the liver. This applies also to acute hepatitis. For this reason it has not been possible to extend the series of choline trials. In the absence of fat in the liver it is not surprising that choline and methionine have proved of little value in the treatment of acute hepatitis.

Summary.

The applications of aspiration liver biopsy to the study of drug toxicity and therapeutic efficacy are discussed.

The effect of mepacrine on the liver is described. No histological changes attributable to the drug were encountered.

The evolution of dietary methods for the treatment of liver diseases is described. A case of post-hepatitis cirrhosis with fatty change in the liver was treated with a high protein, low fat diet and supplementary baker's yeast and choline hydrochloride.
There was no significant change in the clinical or biochemical findings. The hepatic histological picture remained constant.
Chapter 28.

GENERAL CONCLUSIONS.

If used solely as a diagnostic aid, aspiration liver biopsy, used in conjunction with clinical and biochemical observations, will hold a place in general medicine. Not only can primary liver disorders be investigated, but conditions involving the liver as part of a generalised disease are also susceptible to study. The method can be used not only for the attempted elucidation of the jaundices but also in the study of reticulo-endothelial diseases such as kala azar, malaria, infectious mononucleosis, sarcoidosis and the leukaemias. Errors of metabolism such as amyloidosis, glycogen disease, haemochromatosis and the lipodystrophies can often be diagnosed by aspiration liver biopsy. In any obscure undiagnosed illness, if there is the slightest suspicion of liver involvement, the diagnostic value of an aspiration hepatic biopsy should be seriously considered.

The technique can be used to advance our knowledge of the histology and pathogenesis of conditions involving the liver. Hepatic sections in diseases not usually fatal become available. The simplicity of the procedure enables serial biopsies to be performed during the course of the illness, so demonstrating the progress of the lesion. Moreover, the sections have an advantage over those obtained at autopsy in that fixation is immed-
iate and the changes due to postmortem autolysis need not be considered. From time to time newer laboratory methods are devised to facilitate the study of liver diseases. Before coming into general use the tests must be tried on a large series both of "controls" and of liver diseases of certain diagnosis. Hitherto autopsy, or occasionally operation, has been the only sure method of controlling clinical diagnosis. Aspiration hepatic biopsy can be used to add weight to the clinical opinion. Moreover, one way of assessing the value of a liver function test lies in the comparison of the appearance of the liver in section with the test results. Similarly, the worth of the newer therapeutic agents used in liver diseases can be studied by reference to their effects on the histology of the liver.

This thesis has attempted to demonstrate the application of the aspiration liver biopsy technique to all these ends. The future will hold both a widening of these lines of investigation and also advances in other directions. A biochemical analysis of the small liver biopsy fragments is possible and can be utilised in studies on hepatic physiology. The liver material may be useful in providing an antigen for certain diagnostic serum tests for hepatic diseases. Bacterial culture of the hepatic sample may yield important information. These and other investigations are developments for the future.
Acknowledgments.

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