BRITISH MEDICAL JOURNAL

LONDON SATURDAY OCTOBER 29 1960

CONGENITAL BILIARY ATRESIA

BY

Sir ROY CAMERON, LL.D., F.R.C.P., F.R.S.

Department of Morbid Anatomy, University College Hospital Medical School, London

AND

G. L. BUNTON, M.Chir., F.R.C.S.

Surgeon, University College Hospital, London

[WITH SPECIAL PLATE]

Congenital biliary atresia, though a relatively uncommon occurrence, still remains one of the most disappointing of neonatal surgical problems, relieved all too rarely by occasional success in treatment. Following John Thomson's original survey of 50 cases in 1891, it was not until 1916 that J. B. Holmes suggested treatment by surgical means after reviewing over 100 patients. Though the first recorded unsuccessful attempt was made by Giesel and Witze in 1895, it was not until 1927 that Ladd first performed a successful anastomosis, and up till 1952 he, and later Gross, quoted the extraordinary total of 146 babies with biliary atresia, among whom they obtained a cure in 12 cases. This does not include those children with inspissated bile in the ducts—a condition which must often spontaneously regress, even though laparotomy may be needed to diagnose it. We doubt if any series can match this either in numbers or cures; in fact, even in large children's centres in Britain such a case must rank as a rare occurrence.

Aird records having had ten cases, none of which were operable. Our own recent inquiries throughout medical centres in Great Britain, Europe, and America have revealed that not a single pathologist has come across a case in which sections of the liver before and after the relief of congenital atresia were available. Indeed, they all write quite emphatically that they had never encountered a subject thought to be suitable for operation. After the completion of this paper, however, we heard of three patients who had been successfully operated on at the Children's Hospital of Philadelphia and were followed up for five years. No post-operative material was available for study. We are indebted to Dr. Irving J. Wolman for this information.

Table Showing the Larger Published Series of Cases of Biliary Atresia

Authors	Origin of Report	No. of Cases	No. Corrected	Correctability Rate %
Donovan, 1937	New York	11	1	9
Amberg and Zuschlag, 1944	Mayo Clinic	30	4	13
Gray <i>et al.</i> , 1948	Washington Children's Hospital	18	9	50
Leyva and Madigan, 1948	Indiana	31	8	26
Moore, 1953	Boston	146	27	19
Ladd and Gross, 1953	New York	27	10	37
Redo, 1954				

The larger published series are given in the Table, which shows the percentage correctability rate (as opposed to cure) to vary from as low as 9% to 50%, the average being 34%.

Recently at University College Hospital we have had four such patients, of whom one was successfully treated surgically, and it seemed worth while recording them, not so much for this fact alone, but as a background to the changes in the liver which are associated with biliary atresia.

Case 1

A girl first seen when 6 weeks old, jaundice having been first noticed when she was 2 weeks old. At operation a small rudimentary gall-bladder containing no lumen was found. There was no trace of any other extrahepatic biliary apparatus. The liver was dark green, finely nodular, and hard. She lived for eleven months, having to be admitted on seven occasions for abdominal paracentesis.

At necropsy the liver showed characteristic biliary cirrhosis with dilatation of the bile-passages. No major ducts could be demonstrated. There were also characteristic oesophageal varices.

Case 2 (Mr. H. R. I. Wolfe's Patient)

A boy first seen when 4 weeks old. Jaundice became apparent at 12 hours, and gradually increased without remission. At operation the gall-bladder was found to be absent, being represented by a small strand of fibrous tissue. A stenosed cystic duct joined a rather larger stenosed hepatic duct, which was thought to be the left-hand one. The common bile-duct and right hepatic duct were absent. The fibrous cord was dissected out backwards into the portal fissure and a very small fusiform dilatation was found. On cutting across this a few drops of clear bile appeared, but only a fine lacrimal probe could be introduced into the lumen of this duct.

An anastomosis was attempted between this fine duct and the duodenum. The liver was greatly enlarged, dark green, and granular. Following this the jaundice was not relieved. This child also survived eleven months, but unfortunately no necropsy was performed.

Case 3

A boy first seen when 2 weeks old. He had been what was described as "slightly yellow" from birth, and on admission was quite obviously jaundiced. A liver biopsy was done, which showed gross biliary cirrhosis with stasis in the canaliculi. There was some evidence of scattered liver regeneration and there was much glycogen storage. At

laparotomy the gall-bladder was found to be represented by a thin fibrotic cord which had a tiny empty lumen. There was no trace of any bile-ducts. He collapsed and died within 24 hours of operation. At necropsy, section of the liver did not show any macroscopic dilatation of the bile-ducts or canaliculi. The beginnings of the two main hepatic ducts were visible, but had almost microscopic lumina.

Case 4

A boy who was first seen when 7 weeks old. He had been noticed to have pale stools and dark urine for a week, and following this progressively deepening jaundice. At operation the common bile-duct was found to be atretic to a point 0.5 cm. below the portal fissure, where the dilated end was clearly visible. This was anastomosed to the first part of the duodenum. The liver was enlarged, greenish, smooth, and hard, and a piece was taken for biopsy. A normal-coloured stool was passed on the second post-operative day, and he then made an uninterrupted recovery. The jaundice faded quickly, but there was a detectable trace when he was discharged two weeks later.

He has since remained well and has developed normally. He is now nearly 4 years old, and a short while ago a further biopsy was taken from the liver for comparison with the first specimen.

Pathology of Case 4

Before Operation for Relief of Obstruction

Fairly large sections (10 × 5 mm.) from pieces of liver removed at operation well fixed in 10% neutral formal saline. Stained with Ehrlich's acid haematoxylin and eosin, Van Gieson, Gomori's reticulum method, Best's carmine after absolute alcohol fixation, and the P.A.S. method.

Sections Stained with H. and E.

Capsule.—Mostly normal in width, cellularity, and type of fibrous tissue. Here and there small flat plaques of collagen protrude, free from cells. The lining mesothelial cells are flattened and scanty. No inflammatory-cell infiltration. Scattered broad, wedge-shaped bands of cellular connective tissue pass inwards to the centre of a lobule. Blood-vessels immediately beneath the capsule are congested. There is no fibrin or fibrous adhesions.

Portal Canals.—(1) The connective tissue is increased as islands (insular) and also growing outwards (inter-insular) to join up here and there with other tracts. Inter-lobular fibrosis, however, is not complete. A few lobules show very wide bands joining together the portal tracts. From all of the fine bands small groups of collagen fibres are growing into adjacent lobules. Fibroblasts are present in all stages of active proliferation, together with a few polymorphonuclear leucocytes, eosinophils, fairly numerous lymphocytes, an occasional plasma cell, and many monocytes, all of which are healthy and free from inclusions or deposits, though many monocytes contain bile pigment. In a few tracts there are small collections of lymphocytes, but these are without a truly lymphoid pattern or germinal centres. They are found in the largest portal tracts and are often close to the portal veins. There is no necrosis, but free bile pigment is present. There is no haemorrhage and no other pigment is obvious. (2) Bile-ducts: These are increased in all portal tracts. There are signs of proliferation of the small ducts in the form of tall cuboidal epithelium containing prominent nuclei with a large chromatin network. Obvious epithelial budding is also present. There is great variation in the size of the duct nuclei, some being several times normal size and ovoid. Many cells contain free bile pigment. A few mitoses found after a prolonged search. There is no degeneration or necrosis in the bile-duct epithelium. Large and medium-sized bile-ducts are normal. There is no obvious distension anywhere. The basement membrane is difficult to see in the section stained with H. and E. The lumen of the small ducts contains bile, occasionally blood, well-formed bile thrombi, occasional

lymphocytes which are single or clumped, and very rarely mucus (see P.A.S. staining). There is no obvious periductular fibrosis or cystic dilatation. Here and there small bile-ducts are growing inwards, invading lobules, but they do not go very far. Small bile-ducts are numerous within the bands that enclose some of the lobules. (3) Blood-vessels: The portal vein branches are normal and not thickened, nor are they increased. There is no thrombosis, haemorrhage, hyaline degeneration, or necrosis in the walls. They are not inflamed, though the new connective tissue encroaches on them. The capillaries are not obvious, the hepatic-artery branches are normal. A few distended lymphatics are present, but they have to be looked for. They contain no leucocytes in the lumen. The centrilobular and hepatic veins are not obviously thickened. They are congested, but not necrotic or haemorrhagic.

Liver Lobules.—These seem somewhat larger than normal, but are not nodular. They are usually well defined by bands either completely or partially surrounding them. There is some intralobular fibrous tissue here and there as narrow bands. The canals of Hering can be traced into the lobules, but not very far. There is no focal, zonal, or isolated cellular necrosis. There is no haemorrhage, but some patchy sinusoidal congestion. There are no bile lakes and no definite regeneration.

Liver Cells.—These are mostly swollen, pale, and vacuolated with glycogen. There is no necrosis, and mitoses are not found. There are no giant cells. Bile pigmentation is seen as a fine stippling in many cells, with some clumps of inspissated bile here and there throughout the lobule, but there is no special distribution of these. There is no fibrosis deep in the lobules; the liver cells near the bands are shrinking.

Kupffer Cells.—These are not increased and contain no mitoses. Bile pigment is seen in many, with occasional clumps of bile. There are no proliferating groups of cells.

Bile Canaliculi.—Canalicular stasis is patchy and prominent with many bile thrombi and microliths, and there are many inspissated amorphous bile masses. The canaliculi are dilated, but not infiltrated with cells. There is no epithelial lining for the canaliculi anywhere.

Sections Stained with Van Gieson

These show a moderate amount of fuchsinophilic collagen, but less than would have been supposed from the sections stained with H. and E. Fine collagen bands penetrate the lobules at the periphery. The capsule of the liver is shown to be broader than in the H.-and-E. sections.

Reticulum-stained Sections

The reticulin is much increased everywhere, the portal tract being especially affected. Bands spreading out around the lobules are largely reticulum, and there is also much in the bile-duct walls.

Glycogen (Best's Carmine)

About one-half to two-thirds of the liver cells give deep-red staining; others remain unstained. There is none in the bile-duct epithelium. Saliva test is positive.

P.A.S. Staining

There is no increase of P.A.S. material in the portal tracts.

Three Years After Operation

The specimen consists of a small piece of liver about 4 × 2 mm. fixed in 10% formal solution.

Sections Stained with H. and E.

Capsule.—Normal, with a few spindle-shaped plaques or buttons projecting here and there. It is acellular, with no fibrin or adhesions. There are no wedge-shaped scars dipping into lobules.

Portal Canals.—(1) Connective Tissue: Normal in amount and distribution. The pattern also is almost normal. Fibroblasts are present, mostly elongated and spindle-shaped, but a few are still plump. A few scattered lymphocytes and

some tiny clumps of lymphocytes, but very few monocytes and no polymorphs, eosinophils, or plasma cells. The cellularity is just a little in excess of normal. There is no bile effusion or haemorrhage and no pigmentation of any kind. (2) Bile-ducts: Mature, mostly small and not increased. They are of normal structure, with no bile stasis or inflammation. There is certainly no evidence of proliferation or dilatation, and they are not invading the lobules. Medium and large ducts are normal. The canals of Hering are difficult to find. (3) Blood-vessels: All normal.

Liver Lobules.—Ill-defined but normal.

Liver Cells.—A moderate amount of glycogen only. No mitoses are present. There are no giant cells, no bile pigmentation, and no fibrosis or necrosis.

Kupffer Cells.—Normal. No bile stasis.

Bile Canaliculi.—Difficult to find because they are empty. No thrombi.

Other Stains

Normal results were obtained with Van Gieson and reticulum staining.

Comment

This case has much of interest for the student of regeneration in general and of liver restoration in particular. It conforms to the usual picture which is found in babies with atresia of two months' duration. At this time the liver is usually enlarged, though it may shrink with further obstruction. It is deep green, seldom irregular, and firm. It cuts with resistance, and presents a fine white or grey uniform mottling corresponding to the abnormal portal tracts. Microscopical examination shows that the capsule is seldom abnormal. The portal tracts are enlarged from hyperplasia and distension of the bile-ducts and progressive fibrosis. Periportal connective tissue is broad and may embrace lobules as inter-insular and annular cirrhotic bands. Some of these penetrate the lobules to some extent, and are accompanied by proliferating bile-ducts and inflammatory cells. A few lobules are often segregated by the encroaching periportal bands; others are actively regenerating and resemble tiny adenomata.

The most constant feature is bile-duct hyperplasia and distension; when this is severe, hyperplastic ducts ring around and invade lobules. Transitions between liver-cell trabeculae and new bile-ducts are quite easily found, pseudo-ducts and solid buds of cuboidal-duct epithelium are numerous, but the medium-sized and large ducts often remain unchanged apart from distension. Bile-duct increase is not correlated with the length of obstruction, and infants dying within two to three months may show more ducts than those living for seven months or more. Occasionally small granulomata are present in the walls of large ducts that are caused by effusion of bile, but the bile lakes of extrahepatic obstruction in adults are rarely seen. Bile stasis is always prominent, both in the interlobular and perilobular bile-channels and in the intercellular canaliculi. The latter stand out very distinctively because of their distension with green inspissated bile, greenish-black thrombi, and laminated microliths. Close to these thrombi the liver cells are sometimes necrotic and bile-stained, but necrosis is seldom extensive. Binucleate liver cells are numerous and multinucleate giant cells are easy to find.

So far as we know, convincing evidence of recovery in human beings from the effects of fairly prolonged biliary obstruction has not previously been presented. A firm experimental basis has, of course, existed ever since the early work of Bell (1926) in America and that of

Cameron and Oakley (1932) in Great Britain with dogs and rats. In a recent investigation of spontaneous restoration of the ligated common bile-duct in rats Cameron and Prasad (1960) show that quite severe biliary cirrhosis may completely disappear by a process of liver-cell proliferation, dedifferentiation of hyperplastic intrahepatic bile-ducts, and the formation of many new liver lobules. Fibrous tissue is incorporated in the framework of regenerating lobules, though some of it actually vanishes. There is, however, a limit to the reversible stage of biliary cirrhosis beyond which the liver does not recover when relieved from the strain of extrahepatic duct obstruction.

Embryology

Most of the liver and its bile-vessels comes from the endoderm of the foregut as a diverticulum adjacent to the yolk-sac at the end of the fourth week of embryonic life (Cameron and Hou, 1961). Cells derived from the upper portion of this diverticulum rapidly penetrate the septum transversum as cords or cribriform sheets and become closely aligned to the vitelline veins. Some observers maintain that mesenchymal or mesothelial cells of the septum join with the endodermal component to produce new liver cells. Whether this is true or not, trabeculae of functioning liver cells are clearly recognizable at about the sixth week, and they grow vigorously during the second and third months. A lobular pattern is, however, slow in emerging. Bile canaliculi are present in large numbers by the sixth week, some time before the hepatic cells begin to secrete bile. At this time, too, the embryonic hepatic duct is sending out many branches, which join up with canaliculi attached to the liver cells. Quite a number of liver cells apparently are derived from these tiny branches.

Small bile-ducts differentiate towards the end of the second month by a kind of transformation process that involves the liver-cell trabeculae that lie near branches of the portal vein. In the 16-mm. foetus they are quite distinct, but every variety of transition between unchanged liver cells and differentiating bile-ducts may be made out. As a result of successive transformations a network of ductules emerges and spreads out round the portal vessels, from which they are separated by mesenchymal cells. In this way the portal tracts are produced, which contain new ducts of varying sizes linked to the liver-cell trabeculae and the minute canaliculi by the tiny ductules known as the canals of Hering. At 12 weeks the liver is excreting bile, haematopoiesis having begun in the sixth week and reaching its greatest activity during the fifth month. The extrahepatic bile-ducts are derived from the caudal portion of the primary diverticulum. As is usual with hollow tubes in the embryo, hyperplasia obliterates the lumen in the fourth month, but subsequent canalization restores this space by the fifth month. Anomalous bile ducts occasionally develop, leading straight into the gall-bladder, and two cases are recorded in which a duct passed from the left lobe of the liver behind the lesser omentum towards, but not communicating with, the duodenum.

Aetiology

Incomplete development of the above process can account for some cases of atresia, but does not satisfactorily explain all of them. In some instances an intrahepatic atresia occurs, sometimes associated with

extrahepatic atresia. In a few, large cyst-like spaces full of bile are found in the liver, which are probably an expression of maldevelopment of the intrahepatic biliary system. Anastomosis and drainage of these cysts has not been followed by relief of the obstruction, as it appears that they may not communicate with the rest of the biliary system and drain only a very small portion of liver substance.

Common main types of atresia. Possible permutations and combinations of these are, of course, very great.

Inflammation, congenital syphilis, and erythroblastosis have in the past been blamed without any solid foundation of proof. It seems strange that minor expressions of such maldevelopment are not more often seen in adult life causing trouble with biliary flow if an embryonic explanation is the only one, but inquiry has failed to elicit any evidence of this.

Moore feels that these anomalies arise from "gross embryonic aberration" rather than a simple arrest of development. Whitten and Adie (1952) review the histories of eight families in whom the defect appeared familial, and added the record of a further family. From one to as many as six children in one family are reported as being affected. The incidence of this abnormality appears to be about one in 25,000 to 30,000 children.

Diagnosis

Progressive jaundice in infancy may be due to several causes. Icterus neonatorum, though sometimes causing alarmingly deep colour, can usually be easily diagnosed, for the jaundice diminishes rapidly in the first 10 to 16 days, there is no liver enlargement, and the urine and stools are normal.

Erythroblastosis Foetalis

If the possibility of this occurring is unsuspected, confusion may inevitably arise, but investigation of the blood should rapidly clear up any difficulty. A positive Coombs test, the blood group of the mother, a marked anaemia with immature red cells in the baby, form a characteristic pattern. There is, however, the possibility that liver damage and blocking of the bile-ducts with inspissated bile from rapid breakdown of red cells may sometimes superimpose the picture of an obstructive jaundice on this condition, and occasionally surgical exploration and "flushing" of the bile-ducts is necessary before an atresia can definitely be ruled out. In the past many cases of inspissated bile have probably been confused with neonatal hepatitis.

Congenital Syphilis

This is fortunately becoming much rarer and is not often missed. The mother's history and a positive Wassermann reaction are usually sufficient evidence.

Neonatal Hepatitis

This group of conditions appears to be becoming more frequent and is often a great source of confusion. Its origin is obscure and its effect sometimes prolonged. The baby is said to be more ill with hepatitis and the stools are not completely without pigment. Liver-function tests are usually invoked to try and tell the difference, but frequently liver damage and obstruction are severe enough to make such tests inaccurate and confused. However, Lehmann *et al.* (1957) state that estimation of the pseudo-cholinesterase level always differentiates clearly between biliary obstruction and hepatitis, being normal in the former and low in the latter.

We think it is fair to say that surgery in treatment is not necessary in children with atresia who are seen early in life. As we have shown, liver damage is largely reversible, and a week or two spent in careful investigations and observations will not be time wasted. It is perhaps inevitable that, even so, a certain number of cases of hepatitis may be explored unnecessarily. In any case, exploration in the first week or two of life is hazardous once the first 48 hours are past, and liver damage may make it more so. A slightly older child seems better able to withstand what may be a prolonged operation.

Jaundice in biliary atresia is not necessarily progressive from birth. The first meconium is often normal. It is said to be so because it is formed at the fourth month and is usually green in colour. Following this the contents of the bowel lose their colour and the stools rapidly become putty-coloured. However, as jaundice progresses bile may be excreted in the intestinal juices, and this may frequently impart a tinge of colour to the stools, which varies a little from day to day and may be taken as an early sign of remission. The urine becomes dark before the clinical onset of jaundice, and remains so with a high bilirubin level. Strangely enough, the blood picture shows little change. The platelet count is normal, and bleeding and clotting times are not altered until late. It is usually not until the fourth to sixth months that liver damage is sufficient to impair prothrombin production with the onset of haemorrhage, even though vitamin K absorption is poor throughout, and it is wise to give it pre-operatively. In fact, it is a tragic occurrence that so many of these babies for whom nothing can be done survive for so long, sometimes for over a year, and these are said to be those in whom intrahepatic atresia is present. By this time they exhibit all the characteristics that might be expected—namely, a huge liver with large spleen, ascites, and oesophageal varices.

Treatment

In view of what has been said and written about blockage of imperfectly formed bile-ducts, we have tried as a pre-operative measure the effect of dehydrocholic acid with a fine tube so placed that it was hoped the tip lay in the duodenum. While it was obviously not successful in any of our cases, it does seem that there is a place for a trial of this sort, as it may serve to identify those babies who will improve without surgery. Ladd and Gross record two cases in which no obvious

bile-ducts were found at operation, but subsequently the jaundice cleared and the babies recovered. Otherwise the only course open is a minute examination of the anatomy from the portal fissure down to the duodenum through an adequate incision in the hope that a sufficiently dilated piece of hepatic or common duct may be discovered that can be anastomosed to the intestine. If the block is below the entry of the cystic duct and the end of the common duct is very small, the gall-bladder itself can be used, and this is of course technically much easier. If the ducts appear tiny and fibrous, it is worth trying to distend them by injecting through the gall-bladder, if it is present, either saline or methylene blue. Alternatively, diodone may be used and radiographs taken on the table. Such a procedure would obviously be that of choice in those cases with tiny ducts blocked with solid material which can be washed through.

Summary

Four cases of congenital atresia of the bile-ducts are described in which operation for relief of obstruction was attempted, with a successful outcome in one. Microscopical examination of the liver before and three years after operation in the successful case showed complete recovery, with regeneration of damaged liver regions, restoration of biliary flow, and removal of fibrous bands that had begun to grow around the liver lobules.

The clinical and pathological changes produced by congenital atresia are discussed in terms of embryology, and suggestions are put forward for a rational treatment of the condition.

We are grateful to Dr. G. R. Osborn, pathologist to the Derbyshire Royal Infirmary, for the photomicrographs.

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A recent British Standard (B.S. 3277:1960) has been prepared at the request of the Ministry of Health. It deals with tuber-bearing thigh corsets (bucket tops) made of blocked leather (sometimes called moulded leather), non-tuber-bearing thigh corsets of blocked leather, and cuff tops. It distinguishes clearly the essential differences between the three types of appliances and ensures a reasonable quality for the finished product. The standard deals primarily with materials and those details of construction that are independent of individual clinical requirements. Copies may be obtained from the British Standards Institution, Sales Branch, 2 Park Street, London W.1, price 3s., postage extra to non-subscribers.

FOUR HAEMOGLOBINS IN ONE INDIVIDUAL

A STUDY OF THE GENETIC INTERACTION OF Hb-G AND Hb-C

BY

A. B. RAPER, M.D., M.R.C.P.

Royal Infirmary, Bristol

D. B. GAMMACK, B.Sc., M.S.

E. R. HUEHNS,* M.B., B.S.

AND

E. M. SHOOTER, M.A., Ph.D.

Department of Biochemistry, University College, London

[WITH SPECIAL PLATE]

Hitherto the finding of a variant of adult haemoglobin together with the normal pigment in any individual has been taken to indicate the presence of a single abnormal gene, and this hypothesis has been confirmed (except for haemoglobin H) whenever adequate family studies have been made. In those instances where two abnormal, but different, genes for haemoglobin production have been inherited one of two results has been found: either the two variants appear without haemoglobin A (as in sickle-cell-haemoglobin-C disease), in which event the genes for the variants concerned can be regarded as two allelomorphs of a normal gene, which they entirely supplant; or the two variants appear together with haemoglobin A (as found, for example, in the simultaneous occurrence of haemoglobins A, D, and K) (Cabannes and Portier, 1959), when one locus no longer suffices. The view that the difference between normal and an abnormal haemoglobin is determined by a single gene has now been clarified by the work of Hunt and Ingram (1959), who have shown that certain of the abnormal haemoglobins differ from normal adult haemoglobin in the chemical structure of only one of the two types of polypeptide chain of which the haemoglobin molecule is composed. Thus the presence of a single abnormal gene is finally seen as a chemical difference in a single polypeptide chain. Recently Itano, Singer, and Robinson (1959) have suggested that in certain circumstances an abnormal haemoglobin may be formed which differs from normal adult haemoglobin not in just one but in both polypeptide chains, and, furthermore, that when this occurs the individual would be expected to possess four major adult haemoglobins. Their argument depends on a consideration of the composition and interaction of the haemoglobin variants.

Human adult haemoglobin (Hb-A) consists of two pairs of polypeptide chains, designated the α^A and β^A chains, with a haem group attached to each chain (Rhinesmith *et al.*, 1958; Perutz *et al.*, 1960), and its molecular formula may be written $\alpha_2^A\beta_2^A$. The abnormal haemoglobin S (Hb-S) differs from Hb-A in the amino-acid composition of the β -chains, one glutamic-acid residue being replaced by a valine residue, but not in the composition of the α -chains, and thus its formula may be written $\alpha_2^A\beta_2^S$ (Ingram, 1959). Other abnormal haemoglobins have the same β -chains as Hb-A, but differ in the α -chains. For example, Murayama and Ingram (1959) have shown that in haemoglobin I (Hb-I) the α -chains are abnormal, and

*Beit Memorial Fellow.

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FIG. 1.—Liver from first biopsy, showing wide interlobular bands composed of proliferating small bile-ducts, reticulin and collagen fibres, and clumps of lymphocytes and macrophages. Much bile stasis in the lobules. (H. and E. $\times 60$.)

FIG. 2.—Detail of Fig. 1 showing bile-duct hyperplasia, reticulin and collagen bands, and much bile stasis. (H. and E. $\times 120$.)

FIG. 3.—Liver from second biopsy, showing normal structure. (H. and E. $\times 60$.)

FIG. 4.—Detail of Fig. 3 showing normal portal tract and liver cells. (H. and E. $\times 120$.)