Serological and histological diagnosis of primary biliary cirrhosis

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SYNOPSIS A simple immunofluorescence test for antibody to a mitochondrial antigen present in many tissues is a reliable method of distinguishing most cases of primary biliary cirrhosis from jaundice due to extrahepatic biliary tract obstruction. Of 30 cases diagnosed as primary biliary cirrhosis, 26 had antimitochondrial antibody whereas none of 77 cases with jaundice due to extrahepatic bile duct obstruction showed this serological abnormality. The antibody was also found in the serum of three of 42 patients who had other forms of cirrhosis and in two of 266 patients with no evidence of liver disease.

Clinical, biochemical, and serological findings favour the view that primary biliary cirrhosis is a real entity which, in our present state of knowledge, cannot be defined clearly by any single method of investigation. In particular, the liver may show a variety of histological appearances which, interpreted without regard to the other features of the case, may lead to errors in diagnosis.

Primary biliary cirrhosis is a rare condition of unknown cause characterized clinically by pruritus, hepatomegaly, and jaundice of obstructive type in the absence of extrahepatic biliary tract obstruction and without pain or fever. Pathologically the best known features are a fine 'monolobular' cirrhosis of the liver, pericholangitis affecting the medium-sized bile ducts in the portal tracts, and disappearance of many of the small portal bile ducts; in the early stages true cirrhosis may not have developed, and in the late stages the appearances of the liver may be indistinguishable from those of portal cirrhosis (Sherlock, 1963).

In 1965, Walker, Doniach, Roitt, and Sherlock reported that antimitochondrial antibody was demonstrable by means of an indirect immunofluorescence technique in the serum of all of 32 patients with primary biliary cirrhosis. The antibody was not found in patients with extrahepatic biliary tract obstruction. These findings support the concept that primary biliary cirrhosis as described above is a nosological entity, and they may shed some light on the causation of this obscure condition; they also provide the basis for a simple test to differentiate primary biliary cirrhosis from other forms of obstructive jaundice, and so may prevent undesirable surgical exploration of the extrahepatic biliary

passages. We have repeated the studies of Walker *et al.* (1965) and in this paper report partial confirmation of their findings. In addition we draw attention to the frequency of misleading reports on liver biopsy material in primary biliary cirrhosis and emphasize some of the difficulties in making an accurate histological diagnosis.

CASES STUDIED

Fluorescent antibody tests were carried out on the serum of 204 patients with jaundice due to a variety of causes, on five patients with primary biliary cirrhosis who did not have jaundice, and on 266 patients without jaundice or other evidence of liver disease.

PRIMARY BILIARY CIRRHOSIS (30 CASES) In view of the difficulty in defining rigid diagnostic criteria, the main clinical and biochemical features of the 30 cases are given in Tables I and II. In 24 cases the diagnosis of primary biliary cirrhosis had been made before serological testing. The diagnosis of three patients thought to have portal cirrhosis (cases 2, 24, and 25) and of three patients thought to have lupoid hepatitis (cases 1, 9, and 26) was later changed after serological testing and review of the clinical and biochemical findings.

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TABLE I

CLINICAL DETAILS OF PATIENTS WITH PRIMARY BILIARY CIRRHOSIS OR MITOCHONDRIAL ANTIBODY

Case No.	Age	Sex	Duration	Pruritus	Xanthoma	Titre of Antibody Complement-fixation Test	Titre of Antibody Immunofluorescence	Evidence against Extrahepatic Biliary Obstruction
<i>Primary Biliary Cirrhosis</i>								
1	54	F	5 yr.	—	—	2,048	2,048	Needle biopsy
2	67	F	3 yr.	+	—	2,048	2,048	Necropsy
3	54	F	10 yr.	—	—	256	2,048	Laparotomy, cholangiogram
4	67	F	3 yr.	—	—	256	128	Necropsy
5	64	F	1 yr.	+	—	256	1,024	Needle biopsy
6	43	F	4 yr.	+	+	128	128	Laparotomy
7	37	F	3 yr.	+	Xanthelasma	512	512	Laparotomy
8	45	F	1 yr.	+	—	256	128	Needle biopsy
9	60	F	6 yr.	+	—	—	1,024	Needle biopsy
10	51	M	10 yr.	+	+	128	128	Laparotomy
11	43	F	6 mth.	+	Xanthelasma	2,048	> 8,192	Needle biopsy
12	55	F	6 mth.	+	+	1,024	128	—
13	59	F	3 yr.	+	Xanthelasma	> 4,096	512	Needle biopsy
14	67	F	9 mth.	—	+	1,000	2,048	Laparotomy
15	49	F	7 yr.	+	—	1,000	128	Needle biopsy
16	42	F	2 yr.	+	+	1,000	> 8,192	Needle biopsy
17	72	F	3 yr.	—	—	256	512	Laparotomy
18	38	F	1 yr.	+	—	512	512	Cholangiogram
19	56	F	1 yr.	+	+	512	512	Laparotomy
20	44	F	1½ yr.	+	—	1,000	512	Laparotomy, cholangiogram
21	50	F	2 yr.	+	—	2,048	1,024	Cholangiogram
22	41	F	9 yr.	+	+	500	2,048	Laparotomy, cholangiogram
23	50	F	11 yr.	+	+	500	128	Necropsy
24	46	F	5 yr.	+	—	4,096	128	Needle biopsy
25	51	F	3 yr.	—	—	—	1,024	Needle biopsy
26	54	F	5 yr.	—	—	—	256	Laparotomy
27	51	F	2 yr.	+	Xanthelasma	< 8	Neg.	Cholangiogram
28	65	F	2 yr.	—	—	16	Neg.	Laparotomy, cholangiogram
29	58	F	6 yr.	+	—	< 8	Neg.	Laparotomy
30	56	F	2 yr.	—	—	< 8	Neg.	—
<i>Portal and Postnecrotic Cirrhosis</i>								
31	48	F	3 yr.	—	—	64	128	Necropsy
32	56	F	8 yr.	—	—	2,048	2,048	Laparotomy
33	39	F	15 yr.	—	—	> 4,096	512	—
<i>Controls</i>								
34	67	M	—	—	—	500	32	—
35	48	F	—	—	—	128	512	—

PORTAL AND POSTNECROTIC CIRRHOSIS (42 CASES) Histological evidence of portal or postnecrotic cirrhosis was obtained in 29 cases; the remaining 13 subjects had (1) a clinical history of chronic liver disease with recurrent jaundice of at least one year's duration; (2) portal hypertension, ascites, or episodes of hepatic coma; (3) abnormal serum turbidity and flocculation tests; (4) serum alkaline phosphatase below 35 King-Armstrong (K.A.) units/100 ml.; (5) serum transaminase activities consistent with cirrhosis as described by Wróblewski (1958).

EXTRAHEPATIC BILIARY TRACT OBSTRUCTION (72 CASES) AND SECONDARY BILIARY CIRRHOSIS (5 CASES) Fifty-eight patients had operative or necropsy evidence of extrahepatic biliary tract obstruction due either to stone or tumour. In five there was histo-

logical evidence of secondary biliary cirrhosis. The biliary tract was not explored in 19 who had clinical and biochemical evidence of obstructive jaundice; of these, 13 were known to have carcinoma, and the remaining six had gallstones or a non-functioning gallbladder demonstrated radiologically.

INFECTIVE HEPATITIS (25 CASES) All had typical features of the disease, including a prodromal illness and an acute attack of jaundice lasting not more than six weeks. In 20 the serum glutamic pyruvate transaminase (S.G.P.T.) was at least 1,000 units/ml.; in the other five, who were all under 23 years old, the transaminase levels estimated late in the illness were not diagnostic.

DRUG-INDUCED JAUNDICE (10 CASES) This consisted of transient jaundice following administration of

TABLE II

BIOCHEMICAL FINDINGS IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS OR MITOCHONDRIAL ANTIBODY¹

Case No.	Serum Bilirubin (mg./100 ml.)	Serum Alkaline Phosphatase (K.A. units/100 ml.)	Thymol Turbidity (units)	SGOT (units/ml.)	SGPT (units/ml.)	Serum Cholesterol (mg./100 ml.)
<i>Primary Biliary Cirrhosis</i>						
1	3.8	120	18	125	78	233
2	5.4	62	11	117	67	410
3	8.1	62	15	86	41	684
4	13.4	96	9	70	40	170
5	3.0	69	12	38	46	130
6	7.6	92	4	140	60	1,190
7	20.0	220	8	260	220	1,435
8	2.8	80	5	30	60	250
9	1.6	85	9	26	25	355
10	5.7	70	4	196	112	540
11	50.0 ^a	100	14	180	202	936
12	2.5	115	17	138	65	1,694
13	1.7	43	10	98	50	370
14	0.9	86	10	49	14	323
15	5.2	70	6	60	58	285
16	0.7	76	2	36	42	360
17	15.6	80	4	104	74	480
18	0.7	97	4	115	186	505
19	24.0	154	6	200	250	1,650
20	1.1	108	11	80	150	415
21	2.8	50	9	72	234	300
22	26.0	208	12	98	180	675
23	22.8	90	25	215	155	1,650
24	6.2	90	10	103	70	473
25	0.8	27	12	132	56	240
26	3.1	46	13	196	82	264
27	11.2	100	14	168	248	630
28	13.6	140	23	104	220	885
29	16.0	136	3	41	250	1,250
30	1.6	105	26	46	22	347
<i>Portal and Postnecrotic Cirrhosis</i>						
31	32.8	20	13	496	402	157
32	2.2	30	5	28	12	195
33	2.3	35	8	124	45	165
<i>Controls</i>						
34	0.4	16	1	14	12	310
35	0.5	12	4	15	13	310

¹Maximum recorded values.^aIsolated finding.

drugs known to be hepatotoxic: para-aminosalicylic acid (4 cases), chlorpromazine (3), imipramine (1), phenindione (1), and alcohol (1). In eight, liver biopsy showed appropriate histological changes.

HAEMOLYTIC JAUNDICE (4 CASES) The patients had typical haematological and biochemical features.

MISCELLANEOUS FORMS OF JAUNDICE (21 CASES) These included recurrent intrahepatic cholestasis (1 case), primary shunt hyper-bilirubinaemia (1), Gilbert's disease (1), jaundice associated with pernicious anaemia (1), cirrhosis complicating ulcerative colitis (1), jaundice associated with muscular dystrophy (1), jaundice due to chronic venous congestion of the liver (4), lupoid hepatitis (4), and seven cases in which a definitive diagnosis has not yet been made.

PATIENTS WITHOUT JAUNDICE General hospital

patients included 183 patients with a variety of diseases routinely admitted to a general medical unit, and one selected patient (case 34) noted to give granular staining of thyroid epithelium (see below) in a test for thyroid microsomal antibody. In 20 patients with severe chronic thyroiditis the diagnosis was confirmed by a precipitin reaction with thyroglobulin. Nineteen patients with pernicious anaemia, 22 with rheumatoid arthritis, 18 with systemic lupus erythematosus, and three with idiopathic hyperlipaemia and xanthomatosis were also tested.

METHODS

IMMUNOFLUORESCENCE TECHNIQUE Blocks of renal cortex from freshly killed rats were frozen on to metal chucks with CO₂ snow, and 6 μ sections were cut in a cryostat. Serum to be tested was diluted 1 in 8 in normal saline buffered with veronal (pH 7.2) and applied to the unfixed

sections for 30 minutes at 20°C. After washing with buffered saline for 10 minutes fluorescein-conjugated antihuman globulin serum (Burroughs Wellcome and Company) was applied for 30 minutes and after a final 10-minute wash in buffered saline the sections were mounted in buffered glycerol and examined with a Gillett and Sibert conference microscope using blue light. To reduce non-specific fluorescent staining, the fluorescein-conjugated antihuman globulin serum had been previously absorbed with cell fragments (obtained by centrifugation at 35,000 *g* for one hour at 4°C.) of rat kidney homogenized in buffered saline. Sera which gave positive results when diluted 1 in 8 were titrated in fourfold dilutions in parallel with similar dilutions of normal control serum. All tests were read independently by two observers who were unaware of the nature of the sera being tested.

COMPLEMENT-FIXATION TESTS These were performed on all but three of the sera giving immunofluorescent staining of rat kidney epithelium. Guinea-pig complement preserved in Richardson's fluid, sensitized sheep red cells, standardized by the method of Osler, Strauss, and Mayer (1952) and complement-fixation buffer (Oxoid) were used. The antigen was prepared by homogenizing rat liver in 0.25 M-sucrose at 4°C. with a teflon pestle; after removing nuclei and cell debris by centrifugation at 1,500 *g* for 15 minutes, the crude antigen was made by suspending in complement-fixation buffer the deposit obtained by centrifugation at 35,000 *g* for 60 minutes at 4°C.

Tests were performed in perspex trays using 0.1 ml. of doubling dilutions of serum inactivated by heating at 56°C. for 30 minutes, 0.2 ml. of complement containing 5 HD₅₀, and 0.1 ml. of a suitable dilution of antigen (usually the button obtained from 1 g. of liver in 40 ml. of buffer). After one hour at 37°C. with frequent shaking, 0.1 ml. of 2% sensitized sheep cells was added and the trays incubated for a further hour at 37°C., then left overnight 4°C. at before reading. Anticomplementary activity of serum was assessed by testing serum dilutions in the presence of 3HD₅₀ of complement with 0.1 ml. of buffer instead of antigen; 50% haemolysis judged visually was taken as the end point.

RESULTS

Figure 1 shows positive and negative immunofluorescence tests on rat kidney. In the positive test with serum from a patient with primary biliary cirrhosis the cytoplasm of the renal tubular epithelial cells shows brilliant fluorescence and the glomeruli appear as dark areas in the section; with normal serum there is faint general staining of all the renal structures, sometimes with brighter staining of the luminal margins of the tubular cells and of casts within the tubules. These tests have been performed with sera diluted 1 in 8, since undiluted normal serum causes considerable immunofluorescent staining which is difficult to distinguish from a true posi-

FIG. 1. Frozen section of rat kidney stained by indirect immunofluorescence. (a) With primary biliary cirrhosis serum. There is brilliant granular fluorescence (white) of the cytoplasm of renal tubular epithelium. The two large dark spaces are unstained glomeruli. (b) With normal serum. There is diffuse faint non-specific staining of tubules and glomeruli (top right and bottom left).

FIG. 2a

FIG. 2b

FIG. 2c

FIG. 2. Frozen section of human thyroid stained by indirect immunofluorescence. (a) With primary biliary cirrhosis serum. There is distinctly granular staining of the epithelium lining the follicles. (b) With thyroiditis serum. The epithelial cytoplasm is uniformly stained. (c) With normal serum. The epithelium is virtually unstained apart from very weak nuclear fluorescence.

tive reaction. (In case 23, due to unusually strong non-specific staining at a dilution of 1 in 8, an obviously positive result was obtained only after diluting the serum 1 in 32.)

Sera reacting positively with rat kidney were also shown to give positive results with similarly prepared sections of rat liver, brain and adrenal, human gastric mucosa, adrenal cortex and medulla, liver, and thyroid. As noted by Walker *et al.* (1965), the staining of human gastric mucosa closely resembles that seen with the majority of pernicious anaemia sera, whereas the staining of thyroid epithelium has a much coarser granular appearance (Fig. 2) than that obtained with the thyroid-specific microsomal antibody found in thyroiditis serum.

Details of antibody titres in patients with positive immunofluorescence tests are shown in Table I and the results of all immunofluorescence tests are summarized in Table III. Antibody was demonstrated in 26 of 30 (87%) cases with primary biliary cirrhosis; in the positive cases titres obtained by immunofluorescence were not less than 1/128 and most of the titres were much higher. Antibody was found in three patients (7%) with portal or postnecrotic cirrhosis. Case 31 had postnecrotic cirrhosis demonstrated at necropsy three years after a clinically typical attack of infective hepatitis, case 32 had histological evidence of portal cirrhosis, and case 33, with hepatosplenomegaly, bled from oesophageal varices 15 years after an attack of jaundice. The sera of cases 34 and 35 gave a granular staining pattern of thyroid epithelium during tests for thyroid microsomal anti-

TABLE III
IMMUNOFLUORESCENCE WITH RAT KIDNEY IN JAUNDICED PATIENTS AND CONTROLS

	No. Tested	No. Positive
<i>Jaundiced Patients</i>		
Primary biliary cirrhosis	30	26 (87%)
Portal and postnecrotic cirrhosis	42	3 (7%)
Extrahepatic biliary tract obstruction	72	0
Secondary biliary cirrhosis	5	0
Infective hepatitis	25	0
Drug-induced jaundice	10	0
Haemolytic jaundice	4	0
Miscellaneous	21	0
<i>Patients without jaundice</i>		
General hospital patients	184	1 (0.5%)
Chronic thyroiditis	20	0
Pernicious anaemia	19	0
Rheumatoid arthritis	22	1 (<5%)
Systemic lupus erythematosus	18	0
Idiopathic hyperlipaemia and xanthomatosis	3	0

body, and were subsequently tested with rat kidney. Case 34 had osteoarthritis, chronic bronchitis, and an E.S.R. of 48 mm. in one hour, and case 35 had classical rheumatoid arthritis. Both have slightly raised serum cholesterol levels. These two specially selected cases have been included in the appropriate control groups in Table III, and give an exaggerated incidence of positive tests in general hospital patients (0.5%) and in cases of rheumatoid arthritis (5%). Positive tests were not found in the other jaundiced and control groups studied.

Figure 3 illustrates positive and negative complement-fixation reactions using the 35,000 g sediment

FIG. 3. Complement-fixation test with sera from primary biliary cirrhosis. The control rows contain serum, 3HD_{50} of complement and no antigen (test for serum anticomplementary activity). The test rows contain serum, 5HD_{50} of complement and antigen prepared from rat liver. Tests 21 and 22 are negative, and 23 and 24 are positive (titre 256).

of rat liver homogenate as antigen. Details of titres obtained are shown in Table I. Positive complement-fixation reactions were found in those patients whose serum gave immunofluorescent staining of rat kidney epithelium. The correlation coefficient (r) between $\log_2$ immunofluorescence and complement-fixation titres is 0.72 ($t = 8.04$, $p < 0.001$).

HISTOLOGICAL STUDIES

The following description is based on the appearance of paraffin sections stained by haematoxylin and eosin of the eight wedge biopsies of liver obtained at laparotomy (cases 3, 7, 10, 14, 17, 20, 22, and 28) and on the four necropsy cases (cases 2, 4, 17, and 23) which were available to us for study.

EVIDENCE OF CIRRHOSIS Well-developed monolobular cirrhosis with fibrous bands regularly extending round liver lobules to link adjacent portal tracts

was seen in one of the wedge biopsies and in two of the four necropsy cases, while monolobular cirrhosis with linking of only some of the portal tracts was seen in three wedge biopsies. In three biopsies there were fibrous bands extending out from the portal tracts (portal tract fibrosis) but continuity between adjacent tracts was not established. The cirrhosis was of distinctly multilobular type in three cases, all of three years' duration or longer.

CHOLESTASIS Cholestasis, as shown by bile thrombi and/or intracellular granules of bile pigment, was present in only five wedge biopsies but was a consistent feature in the necropsy material.

CELLULAR INFILTRATION OF PORTAL TRACTS This was present in every case, and consisted mainly of lymphocytes with a variable number of plasma cells. The infiltrate was diffuse, frequently with nodules of densely aggregated cells. The infiltrating cells,

together with fibroblasts, frequently invaded the limiting plate of liver cells round the portal tracts giving an eroded appearance. A few eosinophil polymorphs were present in the portal infiltrate in most cases, and in a third of the cases small numbers of neutrophil polymorphs were noted.

SMALL INTRAHEPATIC BILE DUCTS Those less than $50\ \mu$ in diameter were markedly reduced in number in 10 of our 12 cases (Fig. 4). In four of these there was focal proliferation of tubular structures simulating bile duct proliferation and composed of flat eosinophilic cells lying near the hepatic lobules and possibly derived therefrom. Marked proliferation of bile ducts was observed in case 23, which was of 11 years' duration (Fig. 5).

MEDIUM SIZED INTRAHEPATIC BILE DUCTS Those between 50 and $100\ \mu$ in diameter showed pericholangitis in six of the seven wedge biopsies. This was characterized by accumulation of lymphocytes and plasma cells around these structures and by infiltration of the bile duct epithelium by lympho-

cytes, with swelling, eosinophilia, and heaping-up of the epithelium and absence of small branches (Figs. 6 and 7). This feature was identified in only one of the four necropsy cases possibly due to difficulties imposed by post-mortem autolysis.

LIVER CELL DEGENERATION Liver cell degeneration of feathery type was common, especially in areas showing erosion of the limiting plate. Areas of focal liver cell necrosis within the lobules, and usually surrounded by polymorphs, were seen in six of the wedge biopsies. The frequency of this change in the necropsy cases is uncertain because of post-mortem autolysis. Mild fatty change was seen in half the total cases.

GRANULOMATA Localized aggregates of endothelioid cells resembling sarcoid follicles were found in the portal tracts and in the hepatic parenchyma in two of the wedge biopsies (Figs. 8 and 9). In two other wedge biopsies less clearly demarcated clusters of endothelioid cells as described by Greville Williams

FIG. 4. Portal tract in primary biliary cirrhosis, showing fibrosis, focal and diffuse lymphocytic and plasma cell infiltration, erosion of the limiting plate of liver cells, and absence of bile ducts. Near the liver lobules are a few small tubular structures, probably derived from liver cells (haematoxylin and eosin $\times 135$).

FIG. 5

FIG. 5. *Proliferation of small bile ducts in fibrous tissue in primary biliary cirrhosis of long duration (haematoxylin and eosin $\times 135$).*

FIG. 6

FIG. 6. *Medium sized bile duct in primary biliary cirrhosis. There is massive lymphocytic and plasma cell infiltration of the portal tract and these cells have invaded the bile duct. There is epithelial degeneration in the left lower part of the duct. Note the absence of small branches (haematoxylin and eosin $\times 150$).*

FIG. 7. *Medium sized bile duct in primary biliary cirrhosis. The heaped-up epithelium is infiltrated by lymphocytes and plasma cells as is the periductal tissue. There is an ill-defined cluster of endothelioid cells at the top right hand corner (Masson $\times 370$).*

FIG. 7

FIG. 8

FIG. 9

FIG. 10

FIG. 8. *Primary biliary cirrhosis. Sarcoid-like follicle composed of endothelioid and small giant cells in a portal tract (haematoxylin and eosin $\times 205$).*

FIG. 9. *Primary biliary cirrhosis. Sarcoid-like follicle within liver lobule (haematoxylin and eosin $\times 205$).*

FIG. 10. *Primary biliary cirrhosis. Necrotic focus with Langhans giant cell and endothelioid cells, resembling tuberculous follicle (haematoxylin and eosin $\times 205$).*

(1965) were seen in the portal tracts (see Fig. 7). In one necropsy case necrotic follicles with Langhans type giant cells were noted (Fig. 10); no tubercle bacilli were demonstrated in sections from this liver, which was unfortunately the only organ available for examination.

DISCUSSION

Our finding that 87% of patients diagnosed as cases of primary biliary cirrhosis and a small proportion of patients with other types of cirrhosis have circulating antibody which is readily demonstrable by granular immunofluorescent staining of rat kidney epithelium largely confirms the observations of Walker *et al.* (1965).

Complement fixation between primary biliary cirrhosis sera and tissue extracts has previously been described (Gajdusek, 1958; Deicher, Holman, and Kunkel, 1960; Walker *et al.*, 1965) and our demonstration of a significant correlation between complement-fixation titres with rat liver extract and immunofluorescent staining of rat kidney suggests that both methods may broadly reflect the same antigen-antibody systems. The occasional finding of disparity between titres by the two methods may be a consequence of the indefinite end point observed with titrations, especially by the immunofluorescence technique, or may indicate some variation in the specificity of the antibody in individual patients. Beyond confirming absence of organ and species specificity, we have nothing to add concerning the nature of the antigen concerned in these reactions.

Following the studies of Ahrens, Payne, Kunkel, Eisenmenger, and Blondheim (1950) primary biliary cirrhosis has been established as a clinical entity though it is difficult to define it precisely. Probably the lack of a precise clinical definition accounts for our finding of antibody in only 87% of cases of primary biliary cirrhosis in contrast to the 100% reported by Walker *et al.* (1965). Our sero-negative cases are indistinguishable from those with positive antibody tests on the clinical, biochemical (Tables I and II), and histological evidence available to us. It remains to be seen whether the sero-negative cases are fundamentally different from those with antibody, or are suffering from essentially the same disease in which antibody formation is merely a common epiphenomenon.

The finding of antibody in occasional cases of portal or postnecrotic cirrhosis may indicate that these cases are atypical variants of primary biliary cirrhosis (*e.g.*, case 33 which has a relatively high serum alkaline phosphatase of 35 K.A. units/100 ml.) or may be taken as further evidence against the fundamental importance of antibody formation in the pathogenesis of primary biliary cirrhosis.

Experimental findings favour the latter view since liver damage by carbon tetrachloride causes anti-mitochondrial antibody formation in rats, but not the development of biliary cirrhosis (Weir, 1963); and in diseases in which autoimmunity is believed to have a pathogenic role, *e.g.*, experimental allergic encephalomyelitis or thyroiditis, sensitized lymphocytes (delayed hypersensitivity) and not circulating antibody are thought to be the harmful agents (Paterson, 1960; Felix-Davies and Waksman, 1961).

Whether or not antibody is of importance in the pathogenesis of primary biliary cirrhosis, it is clear that the immunological test is an important new parameter in the study of human liver disease. It strongly supports the view that, by and large, primary biliary cirrhosis as described in clinical and biochemical terms is a real entity. Furthermore it provides a useful confirmatory test for distinguishing the majority of cases from conditions, such as extrahepatic biliary tract obstruction in which surgical exploration is called for, and from drug-induced cholestatic jaundice.

The biochemical features observed in the present subjects conform to the pattern usually associated with primary biliary cirrhosis (Ahrens *et al.*, 1950; Sherlock, 1959). This includes disproportionately high levels of serum alkaline phosphatase relative to the frequently mild degree of jaundice. The very high bilirubin values recorded for cases 4 and 11 were sporadic or terminal phenomena, whereas the alkaline phosphatase was never very much lower than the highest observed levels documented in Table II. Thymol turbidity was usually abnormal in established cases, though not infrequently normal on first presentation. In general, values for this test were higher than those encountered in extrahepatic obstruction, though it was uncommon to find such dramatic elevations as are seen in a high proportion of cases with portal cirrhosis. On the other hand, transaminase activities were usually higher than those met with in portal cirrhosis with comparable bilirubinaemia. Unlike the pattern most often encountered in non-malignant extrahepatic obstruction, activity of serum glutamic oxalacetic transaminase (SGOT) was frequently higher than that of SGPT in the same specimen. In many cases there was a slight or moderate elevation in serum globulin, but compared with typical cases of portal cirrhosis, the albumin was only slightly depressed. The serum cholesterol level was raised in 25 cases and, where measured, serum phospholipids were also elevated. These abnormalities need not be associated with dermal xanthomata and should always be sought in patients with long-standing jaundice since they are most useful indices for distinguishing primary biliary from other forms of cirrhosis.

The pathological changes in the liver in primary biliary cirrhosis seem to be less widely known than the clinical features. In the cases described in this paper several incorrect histological diagnoses were initially made which misled the clinicians; these included portal cirrhosis, cryptogenic cirrhosis, continuing hepatitis, lupoid hepatitis, and cholangitis. These errors undoubtedly arise from unfamiliarity with this rather uncommon disease, from attempted diagnosis on biopsies too small to reveal the characteristic changes in the medium-sized and small bile ducts, from variation in liver histology depending on the duration of the disease, and from the conflicting accounts of the disease in the literature. We can confirm Sherlock's statements (1963) that needle biopsy is useful mainly for the exclusion of extrahepatic biliary tract obstruction in which there is marked proliferation of the intrahepatic bile ducts (Fig. 11), and that instead of the well-known monolobular cirrhosis there may be merely portal tract fibrosis or frank portal cirrhosis in patients with primary biliary cirrhosis. The statement by Cameron and Hou (1962) that bile thrombi are invariably present is apparently based on necropsy material; as emphasized by Popper, Rubin, and Schaffner (1962) they may be absent, particularly in the early stage of the disease. Undoubtedly the most characteristic histological lesion of primary biliary cirrhosis is the reduction in number of the small intrahepatic bile ducts, but focal proliferation of structures resembling very small bile ducts is not uncommon, and in the late stages, when portal cirrhosis is established, bile duct proliferation may be widespread. The other findings favouring a positive diagnosis of primary biliary cirrhosis is the pericholangitis of medium-sized intrahepatic bile ducts described by Greville Williams (1965), though this lesion is often difficult to find and is sometimes absent. The presence of granulomata resembling sarcoid follicles has previously been noted by Rubin (1963), and Scheuer (1966) has drawn our attention to his finding of these lesions in about one third of cases of primary biliary cirrhosis.

The infiltration of portal tracts by lymphocytes and plasma cells, the proliferation of fibrous connective tissue, and the alteration of epithelial structures found in the liver in primary biliary cirrhosis may be regarded as analogous to the lesions of autoimmune thyroiditis and gastritis. Localized granuloma formation is not, however, a feature of the lesions associated with organ-specific autoimmunity. Granulomata composed of endothelioid cells are sometimes found in the lesions of ulcerative colitis (Ackerman, 1959) and of systemic lupus erythematosus (Teilum, 1946) and on morphological grounds there might be

FIG. 11. Secondary biliary cirrhosis showing florid proliferation of small bile ducts (haematoxylin and eosin $\times 135$).

reason to suspect that primary biliary cirrhosis has a peculiarity of immunological reactivity in common with these diseases.

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