uction were on the same plasmid and
d together. Whether these observations
genic *E. coli* isolated from animals are
rains isolated from man is unclear.
nic *E. coli* isolated from the Far East are
stant to multiple antibiotics and resis-
rred by R plasmids. Furthermore, genes
biotic resistance and enterotoxin produc-
ansferred together in vitro. We do not
smids are transferred with R plasmids in
inal tract of man, or whether the use of
ncreasing the prevalence of enterotoxi-
Our experience suggests, however, that the
of antibiotics may increase the distribu-
c *E. coli* by selecting for plasmid-contain-
ch carry genes for both enterotoxin pro-
tibi resistance.

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PERCUTANEOUS LIVER BIOPSY AND CHRONIC LIVER DISEASE IN HÆMOPHILIACS

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Summary Systematic screening of forty-seven hæmophiliacs in Sheffield revealed abnormal liver-function tests in thirty-six (77%), with a tendency for these abnormalities to persist. To assess the importance of these abnormalities, percutaneous liver biopsy was carried out on eight symptom-free patients under factor-VIII cover. A wide spectrum of chronic liver disease was demonstrated, including chronic aggressive hepatitis and cirrhosis. The liver pathology bore no relation to clinical history or to biochemical findings. Hepatitis-B-virus markers were common, but evidence suggests that this is not the only factor contributing to the development of liver disease. The high incidence of chronic liver disease seems to be a recent development and is probably related to factor-concentrate replacement therapy.

Introduction

CLOTTING-FACTOR concentrates have been increasingly used for patients with hæmophilia and have undoubtedly improved their overall care and management. However, reports indicate that abnormal liver-function tests are common in regularly treated hæmophiliacs.[1,2] These tests do not provide accurate guidance on the nature or severity of the underlying liver disorder. In an attempt to elucidate the importance of these abnormalities we carried out percutaneous liver biopsies in eight hæmophiliac patients in whom abnormal liver-function tests had persisted for at least 6 months.

Patients

We assessed the frequency of liver disease in patients under our care by testing liver function in forty-seven patients with hæmophilia. All patients had received factor-VIII replacement therapy on at least one occasion during the preceding 12 months. Apart from this selection was random. Persistent abnormalities (>6 months) of liver function were noted in twenty-five patients and eight of these were selected for liver biopsy. The selected patients were well known to us and were considered to be intelligent and responsible. The degree of biochemical abnormality was not a factor in selection. All were symptom-free at biopsy. The full nature of the procedure was explained to each patient and all eight gave their written consent.

Liver-biopsy Procedure

Before the biopsy, each patient's 1-stage prothrombin-time and platelet-count were checked and the presence of factor-VIII inhibitors excluded. A calculated dose of factor-VIII concentrate, sufficient to increase the factor-VIII concentration to 1·0 unit/ml, was given immediately before the biopsy. Factor-VIII plasma concentrations were monitored twice daily and further

factor-VIII concentrate was given to ma
concentration above 0·5 units/ml for t
biopsy was carried out by means of a F
cient tissue was obtained from each patie
tion. All biopsies were performed withou
tients were discharged from hospital 72 h

Methods

Liver-function tests, which were used
ure on all hæmophiliacs, included serum-l
mic-oxaloacetic-transaminase (S.G.O.T.),
pyruvate-transaminase (S.G.P.T.). These
standard autoanalyser methods. HB
anti-HB$_c$ were determined at the Virus
Colindale, in coded samples from thir
seven patients. Twenty-six of these p
liver-function tests and seven had
HB$_s$ Ag and anti-HB$_s$ were determined
(R.I.A.) and anti-HB$_c$ by immunoelectro
specimens were examined under light ar
by conventional techniques. The histo
classified by standard criteria for the
hepatitis.[3] Orcein staining and immu
were used to detect HB$_s$ Ag in the biopsy

Results

Liver-function tests were normal
forty-seven patients studied. The
patients had mild, moderate, or sev
half of them had a history of a
(table I). All thirty-six had raised s.
one third of the patients had raised
tions, clinical jaundice was appare
70% had persistently abnormal liver
trarily defined as persisting for mor
this figure is likely to be an underest
the remaining 30% have been follow
6 months to date.

Markers of hepatitis B (HB$_s$ Ag,

TAB

Patient	Age	Factor VIII (units/ml)	Months since clinical hepatitis	Mor bioch at ma
1	45	0·10	26	
2	40	0·03	–	
3	26	<0·01	22	
4	23	<0·01	–	
5	34	<0·01	–	
6	31	<0·01	43	
7	51	0·07	11	
8	55	<0·01	10	

* Known duration.
† No. of patients with abnormal values at tim

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factor-VIII concentrate was given to maintain the factor-VIII concentration above 0·5 units/ml for the next 72 h. Liver biopsy was carried out by means of a Klatskin needle. Sufficient tissue was obtained from each patient on the first aspiration. All biopsies were performed without incident and the patients were discharged from hospital 72 h after the procedure.

Methods

Liver-function tests, which were used as a screening procedure on all hæmophiliacs, included serum-bilirubin, serum-glutamic-oxaloacetic-transaminase (S.G.O.T.), and serum-glutamic-pyruvate-transaminase (S.G.P.T.). These were carried out by standard autoanalyser methods. $HB_s Ag$, anti-HB_s, and anti-HB_c were determined at the Virus Reference Laboratory, Colindale, in coded samples from thirty-three of the forty-seven patients. Twenty-six of these patients had abnormal liver-function tests and seven had normal biochemistry. $HB_s Ag$ and anti-HB_s were determined by radioimmunoassay (R.I.A.) and anti-HB_c by immunoelectrophoresis. Liver-biopsy specimens were examined under light and electron microscopy by conventional techniques. The histological features were classified by standard criteria for the diagnosis of chronic hepatitis.[3] Orcein staining and immunoperoxidase methods were used to detect $HB_s Ag$ in the biopsy tissue.

TABLE I—DATA ON 36 HAEMOPHILIAC PATIENTS WITH ABNORMAL LIVER-FUNCTION TESTS

Clinical and biochemical features	No.	%
Factor-VIII concentration:*		
<0·01 units/ml (severe)	24	67
0·01–0·05 units/ml (moderate)	1	3
>0·05 units/ml (mild)	11	30
History of clinical hepatitis	18	50
Abnormal liver-function tests:		
Increased S.G.P.T. (>45 units)	36	100
Increased S.G.O.T. (>45 units)	30	84
Increased bilirubin (>17 μmol/l)	12	33
Persistently abnormal liver-function tests (>6 mo)	25	70
Hepatitis B†:		
$HB_s Ag$	2	7
Anti-HB_s	14‡	54
Anti-HB_c	18	69
No H.B.V. markers	5	19

* Severity of hæmophilia.
† Hepatitis-B markers examined in only 26/36 patients.
‡ An additional 6 sera gave low counts on R.I.A., possibly reflecting passive antibody rather than immunity.
$HB_s Ag$= hepatitis-B-surface antigen.
Anti-HB_s =hepatitis-B-surface antibody.
Anti-HB_c =hepatitis-B-core antibody.

Results

Liver-function tests were normal in only eleven of the forty-seven patients studied. The thirty-six remaining patients had mild, moderate, or severe hæmophilia and half of them had a history of a hepatitis-like illness (table I). All thirty-six had raised S.G.P.T., and although one third of the patients had raised bilirubin concentrations, clinical jaundice was apparent in only one case. 70% had persistently abnormal liver-function tests, arbitrarily defined as persisting for more than 6 months, but this figure is likely to be an underestimate since many of the remaining 30% have been followed up for less than 6 months to date.

Markers of hepatitis B ($HB_s Ag$, anti-HB_s, and anti-HB_c) were studied in twenty-six of the thirty-six patients with abnormal liver-function tests. At the time of testing, only two patients were found to be $HB_s Ag$-positive and in both cases this state has persisted for more than 6 months. Anti-HB_c was the most commonly detected marker (69%) and five patients had no detectable markers of hepatitis B. Sera from seven patients with normal liver-function tests were also examined for hepatitis B. $HB_s Ag$ was absent, but anti-HB_s was found in three and anti-HB_c in five. Only two had no markers of hepatitis B.

Table II shows the biochemical, serological, and histological data on the eight patients selected for liver

TABLE II—DATA ON 8 PATIENTS UNDERGOING LIVER BIOPSY

Patient	Age	Factor VIII (units/ml)	Months since clinical hepatitis	Months of biochemical abnormalities*	Liver-function tests†			Hepatitis-B markers			Liver histology
					Bilirubin (<17 μmol/l)	S.G.O.T. (<45)	S.G.P.T. (<45)	$HB_s Ag$	Anti-HB_s	Anti-HB_c	
1	45	0·10	26	24	12	67	100	–	–	–	Micronodular cirrhosis
2	40	0·03	–	15	22	108	224	–	–	+	Chronic persistent hepatitis and granulomas
3	26	<0·01	22	20	14	117	215	–	+	+	Chronic aggressive hepatitis
4	23	<0·01	–	9	17	338	334	–	+	–	Chronic persistent hepatitis
5	34	<0·01	–	18	29	82	190	–	–	–	Chronic lobular hepatitis and granulomas
6	31	<0·01	43	9	7	50	67	–	+	+	Chronic persistent hepatitis
7	51	0·07	11	10	20	125	135	–	+	+	Micronodular cirrhosis
8	55	<0·01	10	10	34	320	245	+	–	+	Chronic aggressive hepatitis

* Known duration.
† No. of patients with abnormal values at time of liver biopsy.

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biopsy. All eight liver-biopsy specimens showed either hepatitis or cirrhosis. $HB_s Ag$ was not detected in the biopsy tissue and no specific features were identified on electron microscopy. Unexplained granulomas were present in two patients.

Discussion

77% of our treated hæmophiliacs had abnormal liver-function tests and a history of a hepatitis-like illness was elicited in 50%. This contrasts with earlier reports on the frequency of liver-function test abnormalities in treated hæmophiliacs of 11·7% in 1970[4] and 3·8% in 1974.[5] In 1977 Levine et al.[2] reported abnormal liver chemistry in 68% of treated hæmophiliacs. This increase in abnormal liver-function tests seems to be associated with the introduction of clotting-factor concentrates in the treatment of hæmophilia. Previously, simple joint bleeds were treated by cryoprecipitate. Since each bag of cryoprecipitate is derived from a single blood-donation, the risk of exposure to hepatitis viruses is quite small. The introduction of factor-VIII concentrates considerably increased this risk, since each vial may contain material from as many as 2500 pooled donations. Like others,[1 2] we found that these abnormalities tend to persist.

We confirmed earlier observations[1 6] that percutaneous liver biopsy can be carried out safely in hæmophiliacs, given adequate factor-VIII cover and appropriate laboratory control. As with any non-hæmophiliac patient, there is a risk of hæmorrhage with this procedure but our experience supports the statement of Lesesne et al.[6] that "the potential risks of complications from liver biopsy in hemophiliacs are outweighed by the therapeutically important histologic information gained from the biopsy." We also found a wide spectrum of chronic liver disease, including benign self-limiting chronic hepatitis, potentially treatable chronic aggressive hepatitis, and established cirrhosis. All our patients were symptom-free at biopsy and it was impossible to differentiate between the different forms of liver disease on the grounds of biochemical abnormalities. Since the patients undergoing biopsy had been arbitrarily selected it is reasonable to conclude that a large proportion of hæmophiliacs receiving treatment with factor VIII have important chronic liver disease.

Although liver biopsies have been performed before in hæmophiliacs[1 6–8] this is the first report from the U.K. While the prevalence of hepatitis B is much lower in the U.K. than in other parts of the world, the incidence of liver disease in the hæmophilic population and the frequency of hepatitis-B markers are comparable. We used simple liver-function tests as a screening test and this may have underestimated the frequency of liver abnormalities, since five of the seven patients with normal liver chemistry had anti-HB_c in the serum, which is thought to reflect continuing virus activity. Any hope that the frequency of liver disease may fall as a result of more sophisticated blood-tests for $HB_s Ag$ may be unduly optimistic. Blood containing $HB_s Ag$, diluted to such an extent that the antigen is no longer detectable by R.I.A., may nevertheless induce $HB_s Ag$ +ve hepatitis in laboratory animals.[9] Cases of type-B post-transfusion hepatitis have been traced to donor blood lacking both $HB_s Ag$, and anti-HB (although anti-HB_c was present),[10] while Spero et al.[1] reported persistent biochemical abnormalities and $HB_s Ag$ infection in hæmophiliacs treated only with concentrates negative for $HB_s Ag$ by R.I.A.

In addition, non-A non-B hepatitis may well be an important factor and observations in four of our eight patients support this possibility. Patients 5 (chronic lobular hepatitis) and 1 (micronodular cirrhosis) have no serum markers of hepatitis B. Patient 7 (micronodular cirrhosis) had a well documented bout of acute $HB_s Ag$-positive hepatitis and $HB_s Ag$ had cleared from his serum within 3 months. Liver biopsy only 13 months after the acute hepatitis showed a quiescent well-established cirrhosis. We feel that the time interval and clinical pattern makes it unlikely that the cirrhosis was caused by the hepatitis-B infection, preferring to implicate some earlier non-hepatitis-B agent. Patient 3 (chronic aggressive hepatitis) had an episode of acute hepatitis 18 months before his liver biopsy. At the onset of hepatitis, his serum was negative for $HB_s Ag$ but positive for anti-HB_s and anti-HB_c. This suggests that he had probably acquired at least two separate hepatitis infections, although it is impossible to tell which was responsible for the liver lesion.

Granulomas were identified in two liver-biopsy specimens, an observation not previously recorded in hæmophiliacs. In neither case was there clinical evidence of sarcoidosis or tuberculosis and specific pathogens were not identified in the biopsy specimen. Although hepatic granulomas may be seen in many diseases[11] they may be associated with factor-VIII therapy.

We conclude that histological liver disease is common in hæmophiliac patients. The nature and severity of these abnormalities can only be assessed by biopsy, which under suitable control can be carried out without undue risk. It is noteworthy that two patients with cirrhosis (1 and 7) were mildly affected hæmophiliacs requiring only occasional factor-VIII transfusion. Such patients may perhaps benefit from the newly developed synthetic vasopressin analogue 1-deamino-8-D-arginine vasopressin.[12]

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