

2) Purpose of Proposed Investigation

The Haemophilia Surveillance Programme in the UK (1) has shown that the group of patients with the highest risk of contracting factor VIII or IX associated hepatitis are those transfused with freeze dried concentrate for the first time. Most mild haemophiliacs (factor VIIIc, 2%) normally require few transfusions, mainly of cryoprecipitate only. Since the risk of progression to chronic liver disease following acute non-A, non-B, hepatitis after transfusion is 20-40% (2), it is important that an accurate estimate is made of the incidence of symptomatic and asymptomatic hepatitis after first transfusions of concentrate in this group. This is particularly relevant when assessing the risk of chronic sequelae after transfusion of factor VIII in patients with mild coagulation defects for whom this treatment is not usually essential, when compared with the benefits of the treatment for which the concentrate was administered. The Haemophilia Surveillance Programme has only assessed the risk of symptomatic hepatitis, and the incidence of symptomless hepatitis can only be obtained by means of a prospective survey.

Preliminary work at the Oxford Haemophilia Centre in 28 patients who were studied prospectively and were followed up for 9 months after a treatment episode requiring cover with factor VIII or IX concentrate, has shown that all 9 patients who had not previously received concentrate contracted non-A, non-B, hepatitis. Seven of these had each received between 1440 and 27,000 factor VIII units as one batch of National Health Service (NHS) factor VIII concentrate prepared from plasma obtained from UK volunteer blood donors (3). The attack rate in patients who had received past transfusions of concentrate was 35%. The pool size of each batch of factor VIII used varied between 1413 and 2506 plasma donations. Other information suggests that a similar attack rate occurs after first treatment with commercial factor concentrate manufactured in the U.S.A. (US commercial factor VIII). This study suggests that there is no difference between NHS and US commercial factor VIII with regard to the risk of contracting transfusion hepatitis.

The purpose of this investigation is to continue these observations to obtain accurate information regarding the relative risk of hepatitis associated with different brands of factor VIII and IX concentrate; to evaluate the risk of chronic sequelae; and to obtain specimens suitable for attempts to devise tests for non-A non-B, hepatitis markers.

Since there are as yet no confirmed laboratory tests available for non-A, non-B, hepatitis, the only sure way of assessing the risk of transfusion hepatitis associated with new brands of concentrate where attempts have been made to inactivate hepatitis viruses by heat, ultra-violet light and propionolactone or other methods, is by the use of chimpanzee inoculation experiments, or trials of each product compared with an untreated product in a group subjects where the susceptibility to hepatitis is known to be high (4). We have demonstrated such a group in the patients with mild coagulation defects already studied at Oxford.

Studies might include:-

- (a) Trials of factor VIII or IX concentrate where attempts have been made to inactivate hepatitis viruses by pasteurisation and other methods.
- (b) The value of immunoglobulin in the prevention of non-A, non-B, hepatitis due to concentrate. A preparation for intravenous use will be available in about six months time from the Blood Products Laboratory of the National Blood Transfusion Service (5).
- (c) The value of factor VIII prepared from plasma donations obtained from blood donors specially monitored for evidence of acute or chronic liver disease.

3) Background to the Project

This project was submitted to the DHSS Small Grants Committee in January 1981. The committee suggested that a multi-centre study should be undertaken to obtain a sufficient number of patients, but they lacked sufficient funds to support such a project. At the suggestion of the DHSS, we approached the Medical Research Council, but they declined to support it. Subsequently I learned that they had completely discontinued support for research into transfusion hepatitis and the MRC Post-transfusion hepatitis Working Party of which I was a member has since been wound up. The pilot project at Oxford was undertaken with the aid of a grant from the Haemophilia Society and local support from Oxford. However further support is needed to complete an attempt to devise

diagnostic tests for non-A, non-B, hepatitis markers. An application for support for this project has also been made to the Dowding Trust.

Since 1969, the Oxford Haemophilia Centre on behalf of the UK Haemophilia Centre Directors' has collected information about the incidence of jaundice after transfusion of factor VIII and IX concentrates (6). The mainstay of the treatment of bleeding episodes in haemophiliacs before 1974 was cryoprecipitate made from plasma obtained from UK volunteer blood donors where each bag is made from one or two donations. This was supplemented by freeze dried NHS factor VIII prepared from UK volunteer donors' plasma with a pool size of about 400 donations per batch.

In response to the increased demand for Factor VIII, US commercial factor VIII prepared from plasma obtained by plasmapheresis of paid donors was imported to supplement NHS supplies. This was associated with an increase in incidence of overt hepatitis in recipients of this material from 2.31% in 1973 to 5.2% in 1974 (7). Further studies (8) showed that there was an attack rate of 17% for symptomatic hepatitis in patients first treated with Hemofil, the first brand of factor VIII imported from the U.S.A. Two types of hepatitis were observed; hepatitis B and what has since been shown to be non-A, non-B, hepatitis with an incubation period of 6 - 70 days (mean 30.2 days).

Further epidemiological evidence was subsequently found for the existence of 2 serotypes of non-A, non-B hepatitis, one associated with transfusions of US commercial factor VIII, and the second with NHS and European commercial factor VIII (9).

In 1978 a 3 year retrospective survey was started to study the incidence of acute hepatitis in haemophiliacs, and the risk of chronic sequelae. This was with the aid of a research grant from the DHSS. This survey has confirmed the existence of 2 types of hepatitis associated with factor VIII and IX treatment. Over 300 cases have now been reported to the Haemophilia Hepatitis Working Party, associated with all brands of concentrate now in use. The cumulative attack rate is about 2.5% per year for symptomatic hepatitis. However, the highest incidence, particularly non-A, non-B hepatitis, was in patients receiving their first infusion of concentrate, usually US commercial factor VIII. The prevalence of hepatitis B has declined since 1975 and is probably due to the effect of the screening of plasma donations for hepatitis B surface antigen (HBsAg) by radioimmunoassay (RIA).

Complications The main complication of factor VIII and IX associated hepatitis is chronic hepatitis. Between 20 and 40% of haemophiliacs on long term factor VIII treatment have abnormal serum aminotransferase levels. There is as yet no precise information regarding the likely risk of serious chronic liver disease, but a recent collaborative study (10) where liver biopsies and post-mortem material was investigated, showed that while nearly all the livers examined had abnormal histological changes, about 15% had changes which may be indicative of serious complications in future. Some children with cirrhosis have received concentrate for 6-7 years. It seems likely that most of the cases of chronic hepatitis are associated with non-A, non-B hepatitis, which is known to produce a chronic carrier state like that of hepatitis B. Most haemophiliacs with chronic hepatitis are hepatitis B antibody positive, but some patients exposed to concentrate for the first time in the past 3 years are negative for all hepatitis B markers. Only one of 152 patients at Oxford on regular factor VIII therapy was a carrier of hepatitis B virus, but 53 of the patients surveyed had persistently abnormal alanine aminotransferase levels (ALT) (11) for at least one year.

It is possible, therefore, that a significant proportion of haemophiliacs on regular factor VIII therapy may develop serious chronic liver disease in 10 - 20 years' time. It is, therefore, important to assess the risk of contracting acute and chronic hepatitis after first exposure to concentrate and to assess the relative risk for different brands of concentrate by means of a prospective study such as we propose and to evaluate new products where the risk of transfusion hepatitis may be reduced.

76

#### 4) Plan of Investigation

The clinical investigation of patients will be carried out at the Oxford Haemophilia Centre, Churchill Hospital, Headington, Oxford under the direction of the Physician-in-Charge, Dr C.R. Rizza. Laboratory tests for hepatitis A, B, cytomegalovirus, E.B. virus and toxoplasmosis will be carried out by Dr J. Craske at the Regional Virus Laboratory, Public Health Laboratory, Withington Hospital, Manchester. A library of serum specimens from patients at Oxford and the patients already studied has been established at Manchester Public Health Laboratory

##### a) Patient Selection

Patients at the Oxford Haemophilia Centre who have received less than 2 transfusions of factor VIII or IX concentrate in the past year are considered for this study. Categories of patient who are included are:-

1) Haemophilia A patients, carriers of the haemophilia 'A' gene, Christmas Disease or Von Willebrands Disease patients who because of their mild coagulation defect do not usually require factor VIII or IX concentrate, but who are about to undergo a elective procedure which will require treatment with concentrate.

2) Patients with serum inhibitors to factor VIII or IX who are treated infrequently with freeze dried concentrate.

##### b) Procedure

Patients who attend the Oxford Haemophilia Centre during the course of the study are selected. The objects of the study are explained to them and their consent, or that of their parents if under 18 years of age obtained.

1) Prior to the start of factor VIII or IX treatment, they undergo clinical examination, and blood is taken for hepatitis serology, full blood count and liver tests before treatment is started. If the patient is seen as an emergency, then as many tests are performed as is compatible with the clinical situation.

2) Patients are followed for 12 months after their treatment episode. Liver function tests and hepatitis serology are carried out at weeks 1, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 42, 46, and 52 post transfusion. The patient is visited in his own home by a health visitor appointed for the purpose of this study. If a patient develops evidence of acute hepatitis, his liver function tests and hepatitis serology are followed fortnightly or as often as possible until his condition resolves or until 3 months after the onset, and then at monthly intervals for six months. Follow up after is then 3 - 6 monthly

##### c) Definition of Hepatitis

A patient is considered to be suffering from acute hepatitis if he develops symptoms and signs as defined in j, page 4.

Hepatitis is defined as acute icteric (raised serum bilirubin)  
acute anicteric  
and acute symptomless

This may be of 2 varieties; - hepatitis B or non-A, non-B. Other causes of acute hepatitis not associated with transfusions of factor VIII or IX are excluded by appropriate laboratory tests as defined above

##### d) Follow-up

Patients whose liver function tests remain abnormal for more than 6 months after the onset of their attack of acute hepatitis or become carriers of hepatitis B virus, will be considered for referral to Dr. Joan Trowell's clinic at the John Radcliffe Hospital, Oxford. Patients are then reviewed at 3 - 6 monthly intervals for clinical and laboratory evidence of abnormal liver function. Return to normal liver function is defined as 3 successive serum aminotransferase levels within normal limits.

##### e) Transfusion Records

Detailed transfusion records are kept for all patients followed up in the project, which will last for 2 years with a follow up up to 2½ years.

##### f) Control Group

This is selected from patients attending the Haemophilia Centre who receive cryoprecipitate, fresh frozen plasma, or DDAVP to cover their treatment episode.

##### g) Family contacts

Family contacts are investigated prospectively and

#### 4) Plan of Investigation

The clinical investigation of patients will be carried out at the Oxford Haemophilia Centre, Churchill Hospital, Headington, Oxford under the direction of the Physician-in-Charge, Dr C.R. Rizza. Laboratory tests for hepatitis A, B, cytomegalovirus, E.B. virus and toxoplasmosis will be carried out by Dr J. Craske at the Regional Virus Laboratory, Public Health Laboratory, Withington Hospital, Manchester. A library of serum specimens from patients at Oxford and the patients already studied has been established at Manchester Public Health Laboratory

##### a) Patient Selection

Patients at the Oxford Haemophilia Centre who have received less than 2 transfusions of factor VIII or IX concentrate in the past year are considered for this study. Categories of patient who are included are:-

1) Haemophilia A patients, carriers of the haemophilia 'A' gene, Christmas Disease or Von Willebrands Disease patients who because of their mild coagulation defect do not usually require factor VIII or IX concentrate, but who are about to undergo a elective procedure which will require treatment with concentrate.

2) Patients with serum inhibitors to factor VIII or IX who are treated infrequently with freeze dried concentrate.

##### b) Procedure

Patients who attend the Oxford Haemophilia Centre during the course of the study are selected. The objects of the study are explained to them and their consent, or that of their parents if under 18 years of age obtained.

1) Prior to the start of factor VIII or IX treatment, they undergo clinical examination, and blood is taken for hepatitis serology, full blood count and liver tests before treatment is started. If the patient is seen as an emergency, then as many tests are performed as is compatible with the clinical situation.

2) Patients are followed for 12 months after their treatment episode. Liver function tests and hepatitis serology are carried out at weeks 1, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 42, 46, and 52 post transfusion. The patient is visited in his own home by a health visitor appointed for the purpose of this study. If a patient develops evidence of acute hepatitis, his liver function tests and hepatitis serology are followed fortnightly or as often as possible until his condition resolves or until 3 months after the onset, and then at monthly intervals for six months. Follow up after is then 3 - 6 monthly

##### c) Definition of Hepatitis

A patient is considered to be suffering from acute hepatitis if he develops symptoms and signs as defined in j, page 4.

Hepatitis is defined as acute icteric (raised serum bilirubin)  
acute anicteric  
and acute symptomless

This may be of 2 varieties; - hepatitis B or non-A, non-B. Other causes of acute hepatitis not associated with transfusions of factor VIII or IX are excluded by appropriate laboratory tests as defined above

##### d) Follow-up

Patients whose liver function tests remain abnormal for more than 6 months after the onset of their attack of acute hepatitis or become carriers of hepatitis B virus, will be considered for referral to Dr. Joan Trowell's clinic at the John Radcliffe Hospital, Oxford. Patients are then reviewed at 3 - 6 monthly intervals for clinical and laboratory evidence of abnormal liver function. Return to normal liver function is defined as 3 successive serum aminotransferase levels within normal limits.

##### e) Transfusion Records

Detailed transfusion records are kept for all patients followed up in the project, which will last for 2 years with a follow up up to 2½ years.

##### f) Control Group

This is selected from patients attending the Haemophilia Centre who receive cryoprecipitate, fresh frozen plasma, or DDAVP to cover their treatment episode.

##### g) Family contacts

Family contacts are investigated prospectively and

hepatitis in household contacts of a patient in this survey is investigated in collaboration with the patient's general practitioner. Blood is taken for liver function tests and hepatitis serology from adult contacts at the start of each patient's treatment and at 3 and 6 months after the transfusion episode in the index case. Children are only investigated where clinically justifiable.

#### h) Sample size

So far 40 patients have enrolled in the study of whom 18 have contracted non-A, non-B hepatitis. We plan to continue the main project until 60 patients are enrolled and then to continue the follow up for a further 2 years. At the present rate of enrolment 60 patients should be included in the project by June 1983. We then plan to review the project and then to conduct a small trial of a new 'hepatitis reduced' brand of factor VIII if available; or to undertake a trial of immunoglobulin in the prevention of non-A, non-B, hepatitis using the new preparation for intravenous use. The exact design of these studies will depend on the attack rate of non-A, non-B hepatitis in the main study.

#### i) Results

At the end of the main study, the attack rates of acute hepatitis, and of chronic sequelae, will be assessed in relation to:-

- 1) The type of product transfused
- 2) The transfusion history of each patient
- 3) The disease category and severity of coagulation defect
- 4) The ratio of symptomless to overt hepatitis for both B and non-A, non-B hepatitis.

5) The age of the patients

6) The number units and types of factor VIII or IX transfused.

7) The type of procedure for which the factor VIII or IX was given.

#### j) Definition of Hepatitis (Clinical and Laboratory features)

A patient is considered to be suffering from hepatitis when 3 or more symptoms or signs compatible with a diagnosis of hepatitis are present as indicated on the sickness record form, together with evidence of abnormal liver function tests. These are considered abnormal when a value of at least twice the upper limit of normal serum aspartic (AST) or alanine (ALT) aminotransferase levels or both is present on two occasions within 3 weeks of the onset of symptoms. A serum bilirubin level is considered abnormal if a figure of at least twice the upper limit of normal is obtained on the same as the serum enzyme tests are raised.

Cases of hepatitis are defined as B or non-A, non-B. The evidence on the basis of over 50 cases of non-B hepatitis suggests that hepatitis A is rarely, if ever, involved in concentrate related transfusion hepatitis. Hepatitis B is considered to be present when a serum is positive for HBsAg by reverse passive haemagglutination (RPHA) or RIA within one month of the onset of symptoms. The same patient should previously have been HBsAg and hepatitis B antibody (anti-HB) negative. Non-A, non-B hepatitis is considered to be present when all serum specimens from a patient with acute hepatitis are negative for HBsAg. Hepatitis cytomegalovirus, E-B virus and toxoplasma infections must be excluded by appropriate laboratory tests.

Symptomless non-A, non-B hepatitis is defined as 2 successive aminotransferase levels of at least two and a half times the upper limit of normal, having had previously had serum enzyme levels within normal limits. For symptomless hepatitis B infections, either a positive test for HBsAg or seroconversion to anti-HBs positive by RIA or RPHA and hepatitis B core antibody (anti-HBc) positive by RIA is considered to be evidence of recent infection.

#### REFERENCES

- 1) Craske J. The epidemiology of factor VIII and IX associated hepatitis in the UK Proceedings of a Symposium held at the Royal College of Physicians and Surgeons Glasgow, September 1980 Edited by C.D. Forbes and G.D.O. Lowe (1982) pp 5 - 14 published by the MTP Press Ltd
- 2) Berman, M., Alter, H.J., Ishak, K.G., Purcell, R.H., et al (1979) Ann. Int. Med. 91, 1 - 6.
- 3) Craske, J., Fletcher Mary, Paver, W.K., Rizza, C.R., and Trowell, Joan M., Factor VIII and IX related hepatitis:- Preliminary results of a prospective survey of patients treated with NHS factor VIII concentrate. In preparation.

- 4) Personal Communication, Dr R. Gerety, Chief, Hepatitis Section Bureau of Biologics NIH Washington, U.S.A. (1982)
- 5) Personal Communication, Dr Richard Lane (1982)
- 6) Biggs, R., (1974) Brit. J. Haemat. 26, 313-29
- 7) First Annual Report on Hepatitis Surveillance (1979)
- 8) Craske, J., Kirk, P., Cohen B., and Elise M. Vandervelde (1978) J. Hyg, Camb. 80, 327-336.
- 9) Craske J., Spooner, R.J.D. and Elise M. Vandervelde (1978) Lancet (ii) 1051-2
- 10) Non-A, non-B Hepatitis in Haemophiliacs, in Viral Hepatitis, Proceedings of the Third Symposium on Viral Hepatitis, New York, 1981 Edited by W. Szmunes and H.J. Alter pp 634-5 published by the Franklin Press (1982)
- 11) UK Haemophilia Centre Directors' Hepatitis Working Party (1980) Unpublished Observations

5) Reasons for Support Requested

Financial support for the follow up of patients will be required at Oxford. The part-time health visitor is required to pay regular visits to patients taking part in the project after their discharge from hospital on a prospective basis in collaboration with their general practitioner. She takes clotted blood samples by venepuncture and will, therefore, require malpractice insurance to cover the procedure from the Royal College of Nursing (see expenses).

Purchase of RIA diagnostic kits

This is to provide for the extra cost to the Public Health Laboratory at Manchester of carrying out diagnostic tests for hepatitis A and B which will be as part of the project.

Travelling Expenses

To pay for car journeys made by the Health Visitor at Oxford while visiting patients in their own homes.

Senior Medical Laboratory Scientific Officer

This person (to be appointed) will work under the Direction of Dr R.S. Tedd Senior Lecturer and Consultant Virologist, Middlesex Hospital Medical School; London on attempts to devise immunoassays for the detection of antigen/antibody systems in serum related to non-A, non-B, hepatitis viruses. He will also require finance for the purchase of reagents etc.

6) Detailed Financial Support Requested

a) Technical/Other assistance

	<u>Qualifications</u>		First year	second year
Oxford Haemophilia Centre. Part-time Health Visitor (25hrs/week)	SRN, CMB Part I Health Visitors Cert. B.A.	NH N11 Grade point 02 on scale annual increment	20% £4565 913	20% £4766 954
Mrs Mary Fletcher aged 50yrs (Open Univ.)		2.3.83		

Middlesex Hospital Medical School; Dept of Virology; Senior MISO

To be appointed

(Scale paid dependent on experience)

£8290.1658 £2583.1717

b) Materials and consumables

Purchase of diagnostic kits for RIA tests for the diagnosis of hepatitis A and B (Manchester Public Health Laboratory)	£2500	£2500
Purchase of reagents for developmental work at the Dept. of Virology, Middlesex Hospital Medical School, London	£1000	£1000

c) Travelling Expenses

Travelling expenses for Health Visitor at Oxford (26.4 pence per mile at 700 miles per month)	£2250	£2250
---	-------	-------

£18605.32511 £19097.32511

20% to cover national insurance, employee's contribution etc.

TOTAL = £47715

7) Attempts to Develop Serological Tests for Non-A, non-B Hepatitis

Preliminary work on this aspect of the project was carried out in collaboration with Dr R.S. Tedder, Senior Lecturer in Virology, Middlesex Hospital Medical School, London earlier this year. The object of this study was to devise a method for the detection of antigen/antibody systems in serum by solid phase radioimmunoassay. Sera from 19 patients at Oxford on long term treatment with factor VIII or IX concentrate was chosen as likely to contain a convalescent antibody to non-A, non-B hepatitis viruses. Patients with normal liver function tests were chosen since they were more likely to contain antibody and to have recovered from their non-A, non-B infection.

The IgG fraction of each serum was prepared by twice precipitation with ammonium sulphate and labelled with I125 by the Iodogen method (1). All 19 radio labelled IgG fractions were used in solid phase RIA tests. Whole, unlabelled serum was used to coat the wells of microtitre plates in the first layer of solid phase sandwich. Sera from acute cases of non-A, non-B hepatitis which were likely to contain non-A, non-B antigen were used as the 'jam' or second layer of the solid phase RIA test. After incubation over night the plates were washed and any bound 'antigen' detected by reaction with the radiolabelled 'antibody'. 'Antigens' and 'antibody' containing sera were all reacted with each other and with sera from volunteer blood donors as negative controls, so that a large chessboard was obtained with each radio-labelled being used against whole serum from the same patient and against all the acute sera as the 'jam' in the sandwich. Negative control counts were obtained by taking the mean of 20 tests using the same serum as the solid phase first stage and detector serum - third stage (Bread of the Sandwich) and sera from blood donors as the second stage or 'jam' in the sandwich.

By this system a technique was worked out for the detection of antigen/antibody systems in serum and confirmation of specificity by showing specific inhibition of antigen in a competitive RIA test using serum containing antibody. One antigen/antibody system was detected but this proved not to be related to non-A, non-B hepatitis when tested using sera obtained from patients in the Oxford Prospective Study. One group at Organon Laboratories has recently described an antigen/antibody system which may be related to US commercial factor related non-A, non-B hepatitis (2). If this work is correct, then the optimum time for the detection of non-A, non-B related antibody is between 12 and 15 months after the onset of acute non-A, non-B hepatitis. The convalescent sera we were using were obtained earlier than this and we propose to make further attempts using specimens of serum from patients 12 - 15 months after their hepatitis in the prospective study.

REFERENCES

- 1) Radiolabelling using Iodogen in Practical Immunology by L. Hudson and F.C. Heath pp 241 - 2 published by Blackwell Scientific Publications Ltd, Oxford 2nd Ed 1980
- 2) Duermeier- Proceedings of the Second Symposium on Virus Hepatitis, sponsored by the International Association of Biological Standardisation and the World Health Organisation, Athens, November 17-20 1982