HIV HAEMOPHILIA LITIGATION - FIRST PRELIMINARY REPORT - DR H GUNSON

CONTENTS

HIV HAEMOPHILIA LITIGATION

Report by H.H. Gunson, MD, DSc, FRCP, FRCP(E), FRC Path.

INTRODUCTION

This report is presented in two main sections, the second of which is divided into two parts.

In Section I the organisation of the Blood Transfusion Service for England and Wales is discussed.

In Section II the work of the Regional Transfusion Centres is considered in two parts; viz:

Section IIa, the collection of plasma for the production of coagulation factor products at the Blood Products Laboratory.

Section IIb, the screening of blood donations to ensure maximum safety for recipients.

These subjects are addressed in chronological order from approximately 1970 to the present.

Where it has been possible, supporting data for statements in the text are included as appendices. References to certain scientific articles are listed. Other scientific articles when, in the view of the author, have greater relevance to the report have been appended.

SECTION I

1.0 MANAGEMENT OF THE NATIONAL BLOOD TRANSFUSION SERVICE (NBTS)

During the second World War a Blood Transfusion Service operated under the Emergency Medical Service and the permanent Service, constituted in July 1946, was based on the University towns of Newcastle, Leeds, Sheffield, Cambridge, Oxford, Bristol, Cardiff, Liverpool and Manchester with two Centres to cover the Greater London area and South-East England. The Service was financed by the Ministry of Health. The newly created Centres were directed by a Regional Blood Transfusion Officer who met monthly under the Chairmanship of Dr. Maycock who was the Director of the "Plasma Drying Plant", later called Blood Products Laboratory (BPL).

2.0 TRANSFER OF MANAGEMENT TO REGIONAL HOSPITAL BOARDS

2.1 In July 1948, the management of the Transfusion Centres was devolved to the Regional Health Boards created by the National Health Service Act. Initially, the Transfusion Centres were those cited previously. With the subsequent creation of the N.E. Thames and Wessex Regions there were 14 Regional Transfusion Centres in England and Wales. Thirteen Regions each had a Transfusion Centre, the exception being S.E. and S.W. Thames which were served by one Centre. The Welsh Office managed the RTC in Wales. There were two Central Laboratories, the Blood Products

Laboratory (BPL) and its associated Plasma Fractionation Laboratory in Oxford and the Blood Group Reference Laboratory. The Central Laboratories were directly financed by the Ministry of Health and subsequently DHSS and administered on its behalf by the Medical Research Council which, in the case of BPL, delegated day to day management to the Lister Institute of Preventive Medicine until 1978. Between 1978 and 1st December 1982, the North West Thames RHA managed BPL on behalf of the Secretary of State for Health and Social Security. The Central Blood Laboratories Authority (CBLA) was created on 1st December 1982.

2.2 The Regional Blood Transfusion Officers, who became the Regional Transfusion Directors (RTDs) in 1949, continued to meet regularly to review the work of the Service. However, the meetings, although attended by some Ministry and subsequently Department of Health Officers, were informal only. Attempts were made to put forward uniform policies for the NBTS.

2.3 However, this degree of co-operation constituted no more than a loose federation and RTDs could develop their own policies in accordance with agreements reached with their respective Regional Boards. Over the years divergent activities developed in RTCs, although certain core policies such as the medical selection of donors operated according to national guidelines.

3 MANAGEMENT BY REGIONAL HEALTH AUTHORITIES

3.1 Before the 1974 reorganisation of the NHS, a Working Party of the RTDs Committee prepared a report on the organisation of the NBTS. In this report, it was pointed out that there was a growing divergence in the organisation and administration of the NBTS, reflecting in part the varying fiscal policies of Regional Boards. Furthermore, there was no central body to co-ordinate policy for the NBTS in scientific, technical, administrative and financial fields. This lack of central direction, it was claimed, inhibited the achievement of a nationally uniform service of a desired standard of efficiency. Policies recommended to Regions may not necessarily be accepted, or, if accepted, were unlikely to be interpreted or implemented uniformly.

3.2 In the 1974 reorganisation of the NHS, the Government's White Paper left the responsibility for the provision of the blood transfusion services with the newly created RHAs. It was recognised by the DHSS that the NBTS differed from other sections of the NHS in the services it provided and a degree of central co-ordination was desirable.

4. MANAGEMENT BY REGIONAL HEALTH AUTHORITIES

4.1 During the consultation period prior to the 1974 reorganisation of the NHS, the RTDs discussed the implications for the NBTS on this reorganisation. A summary

of these discussions is recorded in the minutes of the RTD meeting on 16th December 1970, item 3, Appendix 1.

In summary:

4.11 DHSS might think that NBTS worked efficiently and smoothly under the present system. In reality there were 14 quasi-independent regional centres and 2 central laboratories trying to provide a uniformly efficient service of high standard without a central co-ordinating body apart from the RTD meeting, which had no formal authority.

4.12 NBTS was a complex service which had grown unevenly because of its fragmented administration and the different responses of the Regional Hospital Boards to the needs of the Service. Uneven capital provision was an example.

4.13 It was considered that a uniform financial policy should exist.

4.14 Various categories of staff were, in general, performing the same duties, but grading and allowances were not uniform.

4.15 RHBs had no obligation to carry out a centrally-agreed policy.

4.16 The unique position of the NBTS, with its dependence upon the continuing goodwill of voluntary donors should be taken into account.

4.2 The meeting formed a group, Drs. Bowley, Darnborough, Cleghorn, Stratton and Maycock to prepare a report setting out the reasons why a centrally controlled and administered service was necessary.

This report, a copy of which I have been unable to locate, was presented to the Chief Medical Officer, on 1st September 1971.

4.3 Item 3 of the minutes of the RTD meeting on 25th October 1972 records the response of the DHSS to their requests given by Mr. Gidden. (Appendix 2).
In summary:

4.31 With respect to management of the RTCs, Regional Health Authorities (RHAs) would function in the same manner as the Regional Hospital Boards.

4.32 It was envisaged, in the reorganisation that a much more thorough planning procedure would be adopted which would allow the DHSS to monitor plans of RHAs on a continuing basis. This should help to ensure that the important requirements of the BTS were not neglected.

4.33 The DHSS recognised that the BTS, although a vital component of the hospital service, was unlike any other component and that a degree of central co-ordination in its operation was highly desirable if not essential. This existed already through meetings of RTDs which were held on an informal basis and it was not proposed to change this meeting to a statutory advisory committee.

4.34 The DHSS was proposing to examine whether it would be practical to introduce some special administrative arrangements which would permit uniform practice to be achieved in the NBTS.

4.35 It was minuted that the Working Group which had drafted the augural report might assist the DHSS in (4.34) above.

4.4 In January 1973 a report was presented to the Standing Medical Advisory Committee (SMAC) advocating the establishment of a centrally administered NBTS (Appendix 3).

SMAC recommended that the DHSS set-up a small committee to look into this matter (i.e. the formation of a centralised service) with Dr. Maycock and the Directors. DHSS acted on this suggestion and constituted an ad hoc committee with terms of reference:

"to consider whether any change should be made in the present organisation of the blood transfusion services in England and Wales and to make recommendations."

4.5 The ad hoc Committee met in July and November 1973 and their report is appended (Appendix 4). They considered a further paper presented by the two RTD members of the Committee at the November meeting which detailed the options for the formation of a centralised service (Appendix 5).

The report of the ad hoc Committee (Appendix 4) was accepted by the DHSS and a Central Committee for the NBTS was established in 1975 with the following terms of reference:

"to keep under review the operation of the National Blood Transfusion Service, including the Blood Products Laboratory and the Blood Group Reference Laboratory in England and Wales and to advise the Department of Health and Social Security and the Welsh Office on the development of these services".

Because of the Committee's size (Appendix 4, paragraph 15) and very general terms of reference it was unable to provide the advice the Department needed. The last meeting was in 1978 and in 1980 it was formally disbanded.

5 FORMATION OF DIVISIONS

5.1 In order to improve communications within and between RTCs, in October 1986 the RTCs formed themselves into three Divisions.

These were:

Eastern: E. Anglia, N.E. Thames, N.W. Thames, SE/SW Thames

Western: Oxford, S. Western, Wales, Wessex, West Midlands

Northern: Mersey, Northern, N. Western, Trent, Yorkshire

Membership of the Divisions comprised all consultant medical staff working in the respective RTCs. The Divisional Chairman was elected from RTD members of the Divisions. It was envisaged that meetings would be held prior to the RTD meeting so that discussions could take place on agenda items in a wider forum than had occurred previously.

5.2 Regional Donor Organisers have met regularly since 1946 to discuss matters relating to donor recruitment and since central publicity funding still continued, they had representatives together with RTDs on a DHSS Central Publicity Committee. This Committee apportioned funds from a DHSS central publicity budget for donor recruitment. During the next few years other groups of RTC managerial staff met two or three times per year and minutes of their meetings were sent to the RTD Committee. These Committees

comprised Managers/Administrators, Nurses and Head Laboratory Scientists.

5.3 The RTD Committee also created a number of Working Parties to examine certain aspects of the work of the Service. A number of these were also attended by representatives of the Scottish NBTS. Attempts were made through the Working Parties to introduce some degree of standardisation and uniformity in the operations of the Service in the UK. These were some notable successes such as the introduction of machine readable labels, a uniform pack design in England and Wales for plasma for fractionation, guidelines for the medical selection of blood donors and guidelines for apheresis of blood donors.

6.0 ADVISORY COMMITTEE ON THE NBTS

6.1 Major developments in the BTS were occurring, in particular, the increasing requirements for adequate supplies of products derived from human plasma (e.g. Factor VIII and albumin) and the need to supply increasing quantities of plasma to BPL. RTDs agreed that there was a greater need for co-ordination of the development and activities of Regional Transfusion Centres and the Central Blood Laboratories in England and Wales, for better liaison with the Scottish National Blood Transfusion Service and the Northern Ireland Blood Transfusion Service.

6 Regional Transfusion Directors, through their Chairman Dr. G. Tovey, Consultant Adviser in Blood Transfusion, proposed to the DHSS that a new Advisory Committee on the NBTS be formed to replace the former Central Committee for the NBTS. The DHSS agreed to this proposal and considered that the membership should be restricted to those most closely concerned with the Blood Transfusion Service and Health Authorities.

6.3 The Chairman of this Committee was a Deputy Chief Medical Officer of the DHSS and the membership comprised the Chairmen of the three Divisions of the RTCs, the Consultant Adviser in Blood Transfusion, the Director of the Blood Products Laboratory, a Regional Administrator, Regional Medical Officer, Regional Treasurer and a Regional Nursing Officer.

Its terms of reference were:
"to advise the Department of Health and Social Security and the Welsh Office on the co-ordination of the development and work of the Regional Transfusion Centres and Central Blood Laboratories in England and Wales and the English and Welsh Blood Transfusion Service with those of Scotland and Northern Ireland."

6.4 This Committee met on fourteen occasions between 1st December 1980 and 8th February 1988. In the context of

this litigation the major contribution of the Committee were as follows:

6.41 determination of the volume of plasma required to provide national self-sufficiency of fractionated plasma products. A Working Party under the Chairmanship of the author was established in the second meeting of the Advisory Committee. The recommendations of this Working Party will be included in the Section of this report dealing with plasma for the supply of fractionated plasma products (Section IIa).

6.42 the establishment of a pro-rata supply of fractionated plasma products determined according to the quantity of plasma supplied by each RTC. The aim of this policy was to encourage RTCs to reach their targets with respect to plasma for fractionation. The Advisory Committee approved the policy and agreed that the Blood Products Laboratory should, with consultation of RTCs, determine the arrangements for pro-rata supply and agreed that special allocations of Factor VIII should be made for special users. DHSS paper AC(81)3, (Appendix 6) and minutes of second meeting of Advisory Committee (Appendix 7). (See also Section IIa, paragraph 4.1).

Management arrangements for the NBTS continued unchanged until 1988 which encompasses the period under examination.

SECTION II

WORK OF REGIONAL TRANSFUSION CENTRES

1.0 Introduction

All Regional Transfusion Centres have a core function of blood collection from volunteer, unpaid donors, testing of the donations, processing of donations into blood products and the issue of these products to the hospitals in the region they service. Several products can be obtained from a donation of blood which contains two principal components, cells and plasma. The cellular components can be separated by centrifugation from the plasma and also from each other. The two major cellular components are red cells and platelets. Plasma can be used for clinical treatment in an unchanged form but the majority of plasma separated from blood donations is used for the preparation of fractionated plasma products.

RTCs also developed plasma collection by apheresis techniques. Initially these were performed manually and were reserved for the collection of plasma containing specific antibodies (noteably anti-D for the production of anti-D immunoglobulin). During the 1980s, however, automated plasmapheresis became more cost effective and was considerably safer for the donor. This was employed to collect normal plasma in addition to immune plasma.

SECTION IIa

PLASMA SUPPLY FOR THE PROVISION OF COAGULATION FACTORS FOR THE TREATMENT OF PATIENTS WITH HAEMOPHILIA

2.0 Introduction

During the 1960's, freshly collected whole blood, or plasma separated from whole blood within a few hours of collection, were the products used to treat patients with haemophilia. Following the discovery that Factor VIII could be concentrated by freezing plasma this product gained popularity in the treatment of haemophilia. This product was manufactured at RTCs.

2.1 Cryoprecipitate

2.11 Pool and Shannon (1965) described a method of preparing a concentrate of Factor VIII based on the fact that when plasma is frozen and subjected to controlled thawing the protein precipitated was rich in Factor VIII. The material also contained 30-50% of the original fibrinogen.

2.12 Cryoprecipitate can be frozen and when stored at -30°C retains its Factor VIII activity for at least two years (Pool 1970). Freeze-dried cryoprecipitate could also be manufactured but I am not aware that this product was used in the UK.

2.13 Frozen cryoprecipitate was in use for the treatment of haemophilia patients in 1967 (Biggs and Spooner 1977) and in 1969 comprised 68% of the amount of Factor VIII used for the treatment of haemophilia in the UK. This was prepared by Regional Transfusion Centres and each pack, derived from a single blood donation, contained an average of 70 iu Factor VIII although there was considerable variation. Because of this variability in Factor VIII concentration there tended to be overdosage and depending upon the degree of severity of the disease and the blood volume of the patient, between 2 and 10 packs of cryoprecipitate were used for a treatment dose (Biggs, 1974).

2.14 The use of cryoprecipitate reached a maximum between 1970 and 1975, but has declined since 1977 and by 1984 less than 5M i.u. Factor VIII were being provided by cryoprecipitate out of a total of approximately 72 million units of Factor VIII used (Gunson 1986).

2.15 It is true that because of the smaller number of donors to which the patient is exposed that the risk of transmission of infection is lower with the administration of cryoprecipitate, providing

it is not used to treat a patient with severe haemophilia.

2.2 **Supplies of cryoprecipitate**

In October 1970, Officers from the DHSS met with Directors of the London Haemophilia Centres ostensibly to discuss organisation of Haemophilia Centres in London. However, during the meeting the Haemophilia Centre Directors had pointed out that the application of the modern concepts of treatment necessitated freely available supplies of cryoprecipitate in larger quantities than presently produced (Appendix 1, minutes of RTD meeting 16th December 1970, item 6). After discussion it was agreed that:

2.21 some means of controlling the use of cryoprecipitate (and platelet preparations) and a uniform policy regarding their issue against agreed indications seemed desirable.

2.22 RTDs would report on the number of donations used to prepare cryoprecipitates (and platelets) in the last three months of 1970.

2.23 the subject would be discussed further at the next meeting.

2.3 At the RTD meeting on 5th May 1971 a review of cryoprecipitate production at RTCs was discussed and a wide variation was found, from 0 to 2 donations per 1000 of the population to 11-12 donations per 1000 of the population. An overall policy was requested so that inequalities could be smoothed out. (Appendix 8, minutes of RTD meeting 5th May 1971 and accompanying table).

2.4 A further review of cryoprecipitate production was carried out one year later and the regional differences remained. It was concluded that the main reasons were probably, the preference on the part of haemophiliacs to be treated at certain medical units, specialisation at certain medical units, presence of concentration of haemophiliacs in certain places, e.g. Alton and the lack of facilities for preparing cryoprecipitate.

3.0 SUPPLIES OF PLASMA FOR THE PREPARATION OF FACTOR VIII CONCENTRATE

3.1 A meeting of the Haemophilia Centre Directors was held on 31st January 1974 and this was attended by four RTDs. At that meeting it was confirmed:

3.11 an estimate that plasma from approximately 400,000 donations was needed to provide adequate treatment of the haemophiliac population in the U.K. and that plasma from 275,000 of these

donations should be used to prepare anti-haemophilic globulin (Factor VIII) concentrate.

3.12 there was a preference for concentrate over cryoprecipitate.

(Appendix 9, minutes of the RTD meeting, 20th February 1974, item 6).

3.2 At the RTD meeting on 3rd July 1974 a review was carried out of the number of donations which were issued as red cell concentrates, a measure of the quantity of plasma available for the manufacture of fractionated plasma products. This varied from nil to 30 per cent. It was concluded that the immediate aim was to achieve 30 to 35 per cent overall and for this additional capital and revenue expenditure would be required. RTDs were asked by the Chairman of the RTD Committee to do everything they could within the limitations of their present budgets. (Appendix 10, minutes of RTD meeting, 3rd July 1974, item 2d).

3.3 On 24th December 1974, DHSS announced the allocation of £0.5M to provide a co-ordinated programme for the increased production of blood products with the primary aim of making the NHS self-sufficient in AHG concentrate in 2 to 3 years. (Appendix 11, letter from B.O.B. Gidden to Regional Administrators (DS 364/74)).

3.4 At a special meeting of RTDs held on 19th February 1975 Appendix 12, item 4 of the minutes), Regional Centres were given targets for the production of plasma from 275,000 blood donations. (Appendix 13, paper RTD (75/1). Regional Administrators were informed of the quantity of blood donations required from each RTC to achieve these targets. The targets were subsequently extended to 1977 (Appendix 14, RTC(75)26).

3.5 By May 1976 Dr. Maycock was able to report to RTDs that the intake of fresh plasma at BPL and PF laboratories was approximately on target and that it was likely that the amount of blood products needed for the treatment of haemophilia would exceed the estimates on which the current programme was based. It was noted that requests for cryoprecipitate were increasing in some parts of the country (Appendix 15, minutes of the RTD meeting, 26th May 1976, item 4).

3.6 The supply of plasma on target with respect to the DHSS schedule was confirmed at the next RTD meeting in July 1976, (Appendix 16, minutes of RTD meeting, item 6), but it was reported at this meeting that the ad hoc Expert Group on Haemophilia had suggested that the demand of Factor VIII in the U.K. would rise to 35 million iu per year over the next five years; a figure in excess of the present target for NBTS Factor VIII production.

In July 1976 the RTD Committee appointed a Working Party to consider the variables involved in cryoprecipitate production since this is the starting material for the production of Factor VIII production. It was hoped that some indication may be given of the manner in which plasma could be collected to maximise the yield of cryoprecipitate. The report of the Working Party was published in 1978 (Appendix 17). The result was slightly disappointing in that mean Factor VIII activities derived from group A plasmas and CPD anticoagulant appeared to give increased potency when freshly collected plasma was used to prepare cryoprecipitate. With the volume of plasma used, it was impractical to restrict this to group A donors.

However, CPD did become the anticoagulant of choice. It was not cost-effective to separate plasma from donations in less than 4 hours where the benefit in yield was seen in some experiments since there was not a sufficiently increased yield of Factor VIII to justify the extra expense of arranging for donations to be brought back from mobile blood collection sessions to RTCs within this time-scale.

3.8 The author in a paper entitled 'Trends in Blood Transfusion Practice in England and Wales' published in Health Trends in November 1986, reviewed the use of Factor VIII during the years 1975 and 1984 (Appendix 18). It can be seen that the forecast of the Expert Group on Haemophilia was

accurate since the 35 million units per year was achieved in 1980. (Fig. 3 of the paper). This coincided with a plateau in the production of NHS Factor VIII.

3.9 Plasma supply increased each year from 1975. Data for the table (Appendix 19) was calculated for the preparation of the paper in Health Trends, but not published at that time. The equivalent amount of plasma from 275,000 donations, the target set in 1974 by the Haemophilia Centre Directors, is approximately 50,000 Kg. It can be seen from Appendix 19 that this target was reached in 1976/7 and was in line with the target date set by DHSS (Appendix 11).

Since 1983 the quantity of plasma sent for fractionation has risen sharply and in 1989 this reached 433,000Kg. Several regions have invested in plasmapheresis centres and by 1989 16% of total plasma was obtained by this technique.

4.0 In 1980 the Advisory Committee on the NBTS was formed (see earlier part of statement, (Section I, paragraph 6). This Committee contributed towards the increase in plasma supply.

4.1 Agreement was reached with respect to pro-rata distribution of blood products to regions. (Appendix 20, reference AC(80)5).

This step allowed regions to receive more products if they provided more plasma and was an incentive to provide an increased plasma supply.

4.2 At the second meeting of the Advisory Committee in February 1981, a Working Party to consider plasma supplies for self-sufficiency in blood products was created under the chairmanship of the author and comprised the following membership:

Dr. Diana Walford (DHSS)

Dr. Angela Robinson (Consultant Haematologist, Leeds RTC)

Dr. G.H. Tovey (Consultant Adviser, DHSS)

Dr. J.K. Smith (Blood Products Laboratory)

Dr. T.J. Snape (Blood Products Laboratory) was recruited at a later date

The Working Party's terms of reference were "to advise on supplies of plasma for self-sufficiency in blood products in England and Wales."

This Working Party met on several occasions and three members visited Belgium and concluded, from the evidence which was available at the time, that approximately 500,000 Kg plasma should be sufficient to provide by 1985 the 100 million i.u. Factor VIII the quantity defined by the representatives of the Haemophilia Directors and the NBTS at the meeting held at the DHSS on 23rd April 1981. (Appendix 21).

4.3 The report of the Working Party to Advise on Plasma Supplies for self-sufficiency in blood products was presented to the Advisory Committee in September 1981. Recommendations were put forward for the introduction of plasmapheresis in order to supplement the quantity of plasma obtained from whole blood donations. (Appendices 22 and 23), reference AC(81)11 and AC(81)18. The costs of producing this plasma were estimated. The plan for increasing the plasma supply called for an increase of approximately three-fold in the plasma currently being sent to BPL. The report of the Working Party was accepted by the Advisory Committee and revised targets were prepared for RTCs.

These were communicated to Regional Administrators in letters from J.F. Shaw on 4th February 1981 and 18th December 1981. These targets were given for 1984/5, a time when it was expected that the new laboratory would be commissioned. In collaboration with the Director of the Blood Products Laboratory, annual targets were provided for RTCs since it would take some time to build up the plasma supplies to target level.

Several factors affected the collection of plasma during the next few years.

4.31 On the positive side, the introduction of new technology involving the use of additive

solutions for the preservation of red cells allowed an increase of 50% plasma to be separated from each donation of whole blood. This was commented in the Author's paper in Health Trends (Appendix 18).

4.32 The acceptance of component therapy by clinical staff and the improvement of mobility of red cells suspended in additive solution made red cell preparations more acceptable. As a result of increased use of this product up to 70% of whole blood donations could be subjected to plasma separation instead of the 51% estimated by the Working Party in their enquiries in 1981.

The effect of these two factors was to reduce the need for plasma obtained by apheresis.

4.33 By 1984 it was realised that the new Blood Products Laboratory would be delayed and the revised date for commissioning was January 1986. In the event the new plant was not opened until 1987.

4.4 During the latter part of 1983 comments by Regional Transfusion Directors gave cause for concern that the targets which had been agreed in the planned programme were in jeopardy due to the difficulties in obtaining necessary

funding. The author, on behalf of the Advisory Committee, carried out a survey of RTCs and the results of this survey are given in Appendix 26, (reference AC(84)4). It was agreed that 450,000 Kg plasma would not be required until 1986. A further paper dated November 1984 (Appendix 27), reference AC(84)8 on the subject of plasma supply was presented to the Advisory Committee.

In the event, although the quantity of plasma did not approximate to target levels until 1989, a sufficient stockpile of plasma was available for BPL to fractionate at full capacity once it had been commissioned.
(Appendix 19).

5.0 COMMENT

The RTCs have endeavoured since 1970 to provide sufficient plasma for the preparation of coagulation factors for the treatment of haemophilia patients. The first target given was 400,000 donations of plasma of which 275,000 were to be sent to BPL for the preparation of Factor VIII concentrate. Following an injection of £0.5M the RTCs responded and this target had been reached in early 1977. Unfortunately, by that time, the changing pattern of haemophilia treatment had set a higher target for the next five years which was in excess of NBTS Factor VIII production.

The supply of plasma has increased every year since 1975,

but has not until recently reached levels which equate with self-sufficiency of Factor VIII supplies.

During the 1980's, although there was a slower response than the targeted quantities, sufficient plasma was available for full production once the new BPL had been commissioned. Indeed, it is fortuitous that an increased quantity of plasma had not been collected before 1985 since a greater proportion of the plasma stockpiled would not have been tested for anti-HIV and could not have been used for the production of Factor VIII concentrate.

SECTION IIb

TRANSFUSION ASSOCIATED INFECTIOUS DISEASES IN RELATION TO THE BLOOD SUPPLY

1.0 Introduction

Potentially there are many infections which can result from the transfusion of blood and blood products. Few, however, cause major problems because of the level of immunity in the patients and because potential blood donors are selected from the healthy population and where appropriate screening tests are applied to blood and plasma donations.

2.0 Examples, not exhaustive, of infectious agents which may be transmitted by transfusion include:

2.1 **Hepatitis A virus** (HAV) - The transmission of Hepatitis A virus is uncommon since a carrier state does not exist.

Infection can occur if the donor has been bled in the early stages of incubating the disease when there is a transient viraemia. Patients must be susceptible and all units devoid of anti-HAV.

2.2 **Hepatitis B virus** (HBV) - It has been known that this virus could transmit hepatitis for many years. The disease was formerly called "Homologous serum jaundice" and subsequently serum hepatitis. The discovery in 1968 of an antigen (hepatitis B surface antigen) associated with the disease (Blumberg et al, 1968) and subsequent screening of blood donations for this antigen was an important step in the control of transfusion transmitted hepatitis B.

2.3 **Hepatitis non-A, non-B** (NANB) - The commonest cause of transfusion transmitted hepatitis following the reduction in transmission of HBV. This virus (or viruses) was not isolated until 1989 when hepatitis C, which appears to be at least one virus causing NANB hepatitis was found.

2.4 **Retroviruses**

2.41 **HTLV I/II** - This class of viruses is associated with adult T cell leukaemia and tropical spastic paraparesis and is endemic in South West Japan and the Caribbean. The viruses have been transmitted by blood transufusion but not by fractinated plasma products.

2.42 **HIV I and II** - Causative agents of AIDS. Identified in 1984 and 1986 respectively. HIV I transfusion transmission will be considered in detail later in the report.

2.5 **Herpes Viruses**

2.51 **Cytomegalovirus** (CMV) - This virus is cell associated but also found free in plasma and secretions. Infection can arise in immunocompromised patients.

2.52 **Epstein-Barr virus** (EBV) - Approximately 90% blood donors have neutralising anti-EBV. Post-transfusion EBV disease is not common and symptomatic infection occurs infrequently due to the frequency of antibodies in the population, although it has been reported in young persons.

2.6 **Serum parvovirus-like virus** (SPLV) or B19 virus - Can precipitate aplastic-like crises in chronic haemolytic anaemias and has recently caused foetal death. The virus can be transmitted by blood and Factor VIII concentrates.

2.7 **Treponema pallidum** - This organism, the causative agent of syphilis, is not a major problem since most blood is stored at low temperatures in which the bacterium survives poorly, although it may be important with respect to platelet

concentrates stored at 22°C for 5 days. The test is useful in a non-specific manner as an indicator for sexually-transmitted diseases.

2.8 **Brucella abortus** - This bacterium has a long survival in stored blood. Donors with a history of brucellosis are rejected permanently.

2.9 **Malaria** - The incidence of malaria has been increasing during the past decade and with the increasing travel of donors to areas where malaria is endemic this requires a careful history being taken to exclude donors at risk. There is no risk of transmitting malaria with fractionated blood products.

3.0 When considering the introduction of routine screening of blood donations several factors must be taken into consideration. Thus:

3.1 the prevalence and epidemiology of the infectious agent in the country.

3.2 the incidence of the carrier state.

3.3 morbidity, mortality and long term effects of the agent in the country.

3.4 the availability of suitable screening tests.

3.5 the immune status of the recipients.

3.6 survival of the infectious agent in blood and/or blood products.

3.7 Furthermore, when screening tests are routinely introduced they must be easy to perform, rapid, reliable, reproducible, of high sensitivity with few false positives and cost-effective.

3.8 Consideration of the above criteria would rule out routine screening for the great majority of infectious agents, but there are some instances that routine screening will increase the safety of the blood supply.

4.0 TESTS ON BLOOD DONATIONS FOR MARKERS OF INFECTIOUS DISEASES

4.1 Syphilis

4.12 A test for syphilis has been performed on blood donations from the inception of the NBTS. Treponema pallidum, the causative organism of syphilis survives poorly in stored blood and transfusion transmission in current transfusion practice is a low risk. However, screening for treponema have been continued since such tests can act as non-specific markers for other infections which are sexually transmitted.

4.2 Hepatitis

4.21 It has been known for many years that hepatitis, resulting in jaundice could be transmitted by the transfusion of blood and blood products. Initially, this was called homologous serum

jaundice, and subsequently serum hepatitis, to distinguish it from infectious hepatitis which rarely causes a transfusion-associated infection. Following identification of the causative viruses, infectious hepatitis is now referred to as hepatitis A and serum hepatitis as hepatitis B. After introduction of screening tests for hepatitis B in the early 1970s (vide infra) it became clear that other viruses could be transmitted by blood and these have been called non-A, non-B hepatitis viruses.

4.22 **Introduction of screening tests for Hepatitis B**

In 1965 an antigen was defined in the serum of an Australian aborigine by Blumberg et al, 1965 which was initially thought to relate to leukaemia. Subsequently this was shown to have a relationship with hepatitis B, (Blumberg et al, 1968) although the precise status with respect to hepatitis transmission was not understood for some time.

By March 1970 it was estimated that by screening blood donations for the Australian antigen (now called hepatitis B surface antigen or HBsAg), the incidence of hepatitis transmission by transfusion could be reduced by as much as 40 per cent (Appendix 28). The methods for detection

of the antigen were being developed but the quantity of specific antibody available was in short supply. It was agreed at the RTD meeting on 11th March 1970 (Appendix 28, minutes of that meeting, item 5) that if routine testing was to be introduced it should be on a national basis. As an interim measure it was noted that the safety of donors could be deduced from the results of investigating recipients of previous donations.

RTDs were asked to provide sera for the screening of HBsAg and Dr. Zuckerman at the London School of Hygiene had agreed to test specimens from transfusion centres. (Appendix 29, minutes of RTD meeting, 20th May 1970, item 2(b)).

4.23 On 20th July 1970 a meeting was held at DHSS to discuss the problems of hepatitis-associated antigen in relation to blood and associated matters (Appendix 30). It was reported at the meeting that there was a scarcity of antisera for testing and there was a lack of reference standards. Following discussion it was agreed that facilities should be made available for the testing of blood donations even though this could not yet be organised on a national scale. In the light of knowledge at that time, a donation from

a given donor should be excluded if either the antigen or the corresponding antibody had been detected. The donor should not be bled again.

4.24 Dr. Maycock, Director of the Blood Products Laboratory, informed the meeting that he was arranging for a panel of "safe" donors to supply plasma for the preparation of fibrinogen and, in time, such plasma would be used for the preparation of anti-haemophilic (i.e. Factor VIII) globulin and Christmas Factor (i.e. Factor IX) concentrate but this could not be achieved until screening was widespread.

4.25 From that time, Regional Transfusion Centres (RTCs) began the introduction of screening of blood donations for HBsAg and this was accepted as policy by the DHSS in July 1971 following the recommendation of an Advisory Group on testing for the presence of hepatitis-associated antigen during 1970. By December 1972, all blood donations were being screened for HBsAg. (Appendices 31 to 38, items from the minutes of the RTD meetings from 22nd July 1970 to 10th January 1973).

4.26 Initially, screening tests were performed using techniques known as immuno-diffusion and counter-

immuno electrophoresis which were not particularly sensitive. By 1975 these had been replaced by a haemagglutination technique with greater sensitivity. From 1979 the so-called third generation tests were introduced, namely radio-immune assay and enzyme immuno-absorbent assay (ELISA).

4.3 Non-A, Non-B Hepatitis

4.31 Transfusion-associated hepatitis continued despite the screening of blood donations for HBsAg. It has been estimated in the USA that 90 per cent of such infections are caused by the non-A, non-B (NANB) hepatitis viruses.

4.32 Transfusion-associated NANB hepatitis usually runs a milder course than hepatitis B and many show no symptoms of hepatitis. Although the disease is mild there is a tendency for chronic liver damage in up to 50 per cent of patients. This may vary from mild persistent abnormality of liver function and chronic hepatitis to cirrhosis (Koziol et al).

4.33 Until 1989 there was no specific test available for the detection of viral agents which cause NANB hepatitis. Independent studies have suggested that there is a relationship between

transfusion-associated NANB hepatitis and both raised levels of the liver enzyme alanine amino-transferase (ALT) and the presence of anti-HBc in blood donors. (Koziol et al, 1986 Aach et al 1981, Alter et al 1981).

4.34 The association with raised ALT levels was reported in the Transfusion Transmitted Virus (TTV) study in the USA. (Aach et al, 1981), when it was shown that approximately 40 per cent of the cases of NANB hepatitis associated with transfusion, the donors had an ALT level greater than 45 units per litre. It was estimated that with transfusion of individual units of blood the incidence of NANB hepatitis may decrease by as much as 31 per cent if blood with an ALT value of less than 45 iu per litre was administered.

4.35 The presence of anti-HBc in blood donors may act as a non-specific marker for NANB hepatitis has been suspected for some time (Chataing B, 1983). Koziol et al (1986) found that 11.9 per cent of recipients developed NANB hepatitis when at least one donor was positive for anti-HBc compared with 4.2 per cent of patients receiving blood negative for anti-HBc. However these authors point out that both ALT and anti-HBc are surrogate tests for NANB hepatitis and the use of either to

screen blood donations will still lead to the continuance of 60-70% of transfusion-associated NANB hepatitis.

4.36 Allowing for the fact that the data from the above studies was derived from transfusions performed in the USA during the 1970s and that the incidence of sporadic NANB hepatitis varies from country to country (25 per cent of all sporadic hepatitis in the USA and 4.3 per cent in the UK, (Gitwick, 1984)) the results of the above studies may neither reflect the current situation nor apply in all countries.

4.37 The introduction of non-specific screening for NANB hepatitis has been widely debated (Hornbrook et al 1982, Lenes et al 1983; Khan 1982). Until recently only the Federal Republic of Germany and Italy have routinely screened donations for ALT. During 1986 the American Association of Blood Banks announced its intention to screen donations for both ALT and anti-HBc and similar action was to be taken by the American Red Cross.

Non-specific screening for NANB hepatitis has not been instituted in the UK. In the context of fractionated plasma products the presence of this infectious agent in Factor VIII concentrates,

would not have been eliminated by the use of the non-specific tests referred to above and would have lead to the unnecessary rejection of many donors who were non-infective. Alter (1984) reported that 60 per cent of abnormal levels of ALT were due to identifiable causes other than NANB hepatitis of which the commonest were obesity and excessive alcohol consumption. Two further studies identified similar causes. (Friedman et al 1987; GHillan et al, 1988).

4.38 The Department of Health commissioned in 1988 an investigation of ALT and anti-HBc tests on a sample of the current UK donor population in order to study such donors and to try and establish the presence or absence of disease. The study confirmed the results of previous studies in that the majority of persons with raised ALT values had an increased alcohol intake or were obese. The value of ALT and anti-HBc tests as non-specific markers for transfusion-associated NANB hepatitis can only be evaluated in a prospective study in the country concerned.

However, during the course of this investigation an antigen was produced which appeared to be associated with NANB hepatitis (called hepatitis C) and tests for anti-HCV had been

developed. The importance of the use of this test in relation to blood donations is currently being evaluated.

5.0 HIV AND THE SAFETY OF THE BLOOD SUPPLY

5.1 Information given to blood donors to encourage self-exclusion

5.11 Following the initial reports of the development of AIDS it was not immediately obvious that this illness could be transmitted by the transfusion of blood and blood products. In January 1983 in a review article in Science, (Appendix 39), Maix reported that an infant who received several infusions of blood and blood products had developed AIDS (reported by Ammann et al, 1983) and that one of the donors had subsequently developed the disease. The Communicable Diseases Centre in Atlanta (CDC) also reported the diagnosis in four haemophiliacs. This lead to the hypothesis that AIDS was caused by an infectious agent, possibly a virus, that could be transmitted by blood products.

5.12 The Regional Transfusion Directors discussed the situation with respect to transmission of AIDS by blood transfusion at their meeting on 18th May 1983. It was agreed that an information leaflet

on AIDS should be prepared for distribution to donors (Appendix 40, item 10). At this meeting the contents of a letter from the author to the Chairman of the RTD Committee (Appendix 41) was discussed and the options 1 and 2 were rejected since it was considered that the usual questions to donors on the health matters would be successful in rejecting such persons.

5.13 The wording for the AIDS leaflet was agreed and was issued on 1st September 1983, Appendix 42 gives the text of the leaflet. The exclusion categories were those which were suggested by the FDA in 1983. The leaflet was made widely available to blood donors either by sending the leaflet to donors with the call-up card, by handing the leaflet to donors or by making it available on blood collection sessions. The Chairman of the RTD Committee reviewed the situation after three months following the publication of the leaflet and the result of this survey is given in Appendix 43. Contacts were made by several RTCs with local GAY Societies to enlist assistance.

5.14 The North London RTC introduced a questionnaire for donors attending their permanent blood collection centre in London in July 1984. After

a successful trial at this centre, the questionnaire was used in a second permanent centre in April 1985 and on the mobile blood collection teams from June/July 1985 (Appendix 44). The reason for the use of this questionnaire was because it was suspected that the North London RTC was liable to have more donors at risk than RTCs in other regions (as has been proved, subsequently, with the introduction of anti-HIV tests on blood donations). The results of the trial use of this questionnaire and the tests carried out on donors who declared themselves at risk or not at risk were published in the BMJ, 9th March 1985 (Appendix 45).

5.15 As knowledge of AIDS increased a second leaflet was issued in January 1985. The text of the leaflet is given in Appendix 46. The issue of the leaflet was accompanied by Health Circular HC(85)3 requesting that this new advice for blood donors was distributed individually to every donor with the call-up notification (Appendix 47).

5.16 The issue of further leaflets to donors has occurred at the time of introduction of anti-HIV tests on their donations (para. 5.314) and in September 1986, February 1988 and April 1990.

The risk activities for self-exclusion of donors have been modified and up-dated as more information has become available on the epidemiology of the disease.

5.2 Surrogate tests for AIDS

5.21 A Central Committee for Research and Development in Blood Transfusion was created under the aegis of the CBLA in June 1983 with the following terms of reference:

"to advise the CBLA on research and development in blood transfusion and related fields."

5.22 This Committee formed a Working Group on AIDS whose first meeting was held on 14th October 1983. At this meeting the use of surrogate tests, particularly anti-HBc was discussed. Preliminary tests had shown that the incidence of anti-HBc positives in the Bristol RTC was 0.75 per cent (number of donors tested, 10,000) and 2.6 per cent in 2500 North London donors. (Appendix 48, minutes of Working Group on AIDS in relation to Blood Transfusion, 14th October 1983, item 3.1.2). It was agreed that the situation would be reviewed. Other surrogate tests were not yet adapted for large-scale screening of blood donations.

5.23 Proposals for a study based on the screening of 50,000 blood donor samples for anti-HBc at the North London and Bristol RTCs with additional tests being carried out on positive reactors was considered, (Appendix 49, minutes of the meeting of the Central Committee for Research and Development, 28th February 1984, item 4.1).

A request for funding to implement this study was not put forward since the causative virus of AIDS was isolated and a specific antibody test was being investigated in the USA and France.

5.24 Nevertheless, it is worth commenting that surrogate testing had been carried out by the RTCs with testing for HBsAg and syphilis.

Appendix 50 shows the incidence of HBsAg in new donors from 1979 to 1988 which has been compiled from the DHSS national statistics on the BTS. The initial rate was approximately 1 in 1000. This was reduced in 1981, probably after the decision not to collect blood from prisoners in H.M. Prisons since it was found that these persons had an increased incidence of HBsAg

positivity. In 1984 to 1986 there was a significant reduction in the incidence of HBsAg and, arguably, this was due to the self-exclusion of donors following the introduction of the first AIDS leaflet. The subsequent rise may be due to some donors ignoring the advice given for self-exclusion or other unknown factors. However, it has not reached the pre 1984 levels.

Similar data for syphilis testing cannot be given since the information is not collected on a national basis.

5.3 Tests for anti-HIV on Blood Donations

This topic will be described in two sections since activities involving the administrative aspects of introducing this test ran parallel with the evaluation of a number of anti-HIV test kits.

5.31 Arrangements for the introduction of Blood Donor Screening

5.311 The Expert Advisory Group on AIDS (EAGA) held its first meeting on 29th January 1985. At this meeting, (Appendix 51, items 18-23) the availability of the AIDS screening test was discussed. A Sub-Group was established with Dr. Smithies (DHSS) in the chair to consider the various aspects of screening tests for AIDS.

5.312 The Working Party (known as the Screening Test Sub-Group) had terms of reference 'to advise the EAGA on the introduction of a test for antibody to AIDS related virus.' This virus was, at that time, called HTLV III but for simplicity throughout this text it will be referred to by its later name of human immunodeficiency virus (HIV). The Working Party considered both arrangements for introduction of the test and the evaluation of existing test kit systems.

5.313 With respect to administrative arrangements for the introduction of tests on donations the reports of the Working Party were fully discussed at the meeting of the EAGA on 22nd April 1985 (Appendix 52, paper EAGA(3)2). Comments at EAGA are summarised below (Appendix 53, minutes of EAGA 22nd April 1985).

5.3131 The importance of availability of internationally acceptable reference method.

5.3132 Blood donors must be told that their donations would be screened and they must be given the opportunity to withdraw.

5.3133 The appropriate time to inform the donor was following a validated test had been performed and the confirmed result was positive. From the legal, ethical and public health point of view, the donor **must** be told the result.

5.3134 It was necessary to ensure that before any screening test was introduced into the BTS that it was reliable and that proper validation of the results was available.

5.3135 That RTDs should devise an agreed procedure for all RTCs to follow was agreed and necessary meetings to achieve this should be set-up quickly.

5.312 Informal discussions took place within the BTS and a Working Party of the RTDs was convened on 11th July 1985. Their report (Appendix 54) was presented to EAGA on 30th July 1985. Appendices to this report includes the final text of a revised leaflet to be sent to donors informing them that a test for AIDS antibody would be performed on their blood; a proforma for donors

to sign at the blood collection session to agree to this test being performed on their blood and the draft of a letter to be sent to donors who were confirmed anti-HIV positive for them to make arrangements for counselling at the RTC. In the report itself details were given on the action to be taken on the receipt of results of screening and confirmatory tests. RTDs were advised to make arrangements with their respective RHAs for the introduction of routine screening whilst evaluations on the tests were being performed. RHAs had been advised in February 1985 that such tests would be introduced and requested by DHSS to make appropriate financial allocations.

This paper also drew attention to two matters which the NBTS considered to be important before the routine introduction of screening tests in the NBTS, namely:

5.3121 that reference centres were established to perform confirmatory tests on samples of blood from donors found positive by the screening tests.

5.3122 that separate venues were established for testing samples of blood from persons who may consider themselves at

risk from AIDS who were not potential blood donors.

It was considered that if such persons attended blood collection sessions in order to obtain the results of the anti-HIV test on their blood this may, in fact, have the effect of making the blood supply less safe. There was the possibility that some of these donations would be collected in the period between the onset of infection and the appearance of antibody in the blood. Such donations could be infectious but would give negative results in the anti-HIV test.

These persons were currently being encouraged to self-exclude as donors as a result of the leaflet or the information contained in the leaflet being distributed to all donors.

5.313 With respect to the establishment of reference centres for confirmatory testing, EAGA were informed at their meeting on 30th July 1985 (Appendix 55, item 7.3.1) that 22 PHLS

laboratories could perform ELISA testing and 7 were ready to do confirmatory tests.

5.314 Regional General Managers were asked in a letter dated 30th July 1985 from DHSS by G.A. Hart to arrange for facilities for testing outside the Blood Transfusion Service (Appendix 56).

5.315 By October 1985 RTCs had made the necessary arrangements and had trained staff for the introduction of anti-HIV testing and provision for confirmatory testing and alternative venues had been established.

5.314 Routine screening of all blood donations for anti-HIV commenced in all RTCs in the UK on 14th October 1985. The testing was accompanied by the issue of a new leaflet to donors advising them that a test for AIDS would be performed on their blood and they were asked to sign to agree to this procedure (Appendix 54).

5.4 Evaluation of anti-HIV Test kits

5.41 At the first meeting of the Screening Test Sub-Group on 15th February 1985 consideration was given to various matters concerned with the introduction of an anti-HIV screening test. These are summarised in Appendix 57. Two papers

by S. Budiansky (Appendices 58 and 59 were tabled. In these articles problems with the test in the USA were noted, particularly the prevalence of false positive results.

5.42 It was stated at the meeting that in the absence of statutory controls the DHSS had invited companies developing test kits to take part in a Departmental evaluation. Initially, tests would be undertaken by the PHLS and a follow up evaluation would be undertaken in RTCs and laboratories serving STD clinics.

Drs. Gunson and McClelland were asked to consider the feasibility of arranging a collection of a sufficient number of donor samples and preparing aliquots from them for the BTS phase of the study.

Appendix 60, note of the meeting of the Screening Test Sub-Group, 15th February 1985.

5.43 At the next meeting of the Screening Test Sub-Group on 1st March 1985 it was proposed that 10 RTCs would each collect 1,000 samples from blood donors and would divide these each into 10 aliquots. These would be sent to the co-ordinating centre which later was nominated as

the North Western RTC in Manchester. (Appendix 61 note of a meeting of the Screening Test Sub-Group, 1st March 1985).

5.44 The Central Virus Laboratory of the PHLS evaluated the available tests and had recommended that two tests appeared particularly suitable for use in screening blood donations (Appendix 62).

5.45 The study in the NBTS on 10,400 samples using these two test kits was conducted at the North London and Manchester RTCs during July 1985 to assess how these tests could be incorporated into the work pattern of RTCs. Recommendations were made with respect to the optimum way to analyse results, the use of controls and the importance of repeat testing to detect those results which, whilst not positive according to the manufacturers' cut-off value were clearly distinguishable from the bulk of negative results. Appendix 63). This important information was available to RTCs prior to the commencement of routine testing.

5.46 The steps taken during the six months prior to the introduction of routine anti-HIV screening of blood donations were reviewed by the author at a National AIDS Conference in 1986 (Appendix 64).

5.5 With financial support from, initially the Department of Health and subsequently the Medical Research Council, regular monitoring of anti-HIV testing of blood donations and blood donors has been performed. Monthly reports surveying the results of anti-HIV screening of blood donations in the UK and the use of quality control tests have been sent to all RTCs and other interested parties. A summary report for the Medical Research Council and the Department of Health for the years 1985-1989 can be found in Appendix 65.

5.6 The author conducted a survey on the anti-HIV screening of blood donations for the Committee of Experts in Blood Transfusion and Immunohaematology of the Council of Europe. The dates for commencement of testing of blood donations in member countries and the observers (USA, Australia and Canada) are given in Appendix 66.

5.7 The seropositivity rates for 1985-1988 for the member countries of the Council of Europe are shown in Appendix 67. It will be noted that there is a wide variation in the rates, and in general the results for Southern European countries are approximately 10 times higher than those countries in Northern Europe. In general, the seropositivity rates have declined in most countries during this period. This is not surprising since anti-HIV positive donors are withdrawn and those which are

anti-HIV negative are rebled. It can be seen that the HIV seropositivity rate for blood donations in the UK falls into the group of countries with the lower incidence of anti-HIV positives. This probably reflects the incidence of AIDS in the UK but, in part, is probably due also to the actions taken by the NBTS.

REFERENCES

Aach R.D. et al. N.E.J. Med. 1981; 304: 989-94

Alter H.J. et al. In Vyas G.N., Cohen S.N., Schmit D. Viral hepatitis 1978: 359-369

Alter H.J. et al. JAMA 1981; 245: 630-4

Alter H.J. Proc. XVIII ISBT Congress, Munich 1984; p.19

Ammann, A.J. Cowan, M.W., Wara, D.W. et al 1983 Lancet 1:956

Biggs, Rosemary and Spooner, Rosemary J.D. (1977) Brit. J. Haemt. 36:447

Blumberg, B.S., Alter, H.J. and Visnich, S. (1965) J. Amer. Med. Ass. 191:541

Blumberg, B.S., Sutwick, A.L. and London, W.T. (1968) Bull. N.Y. Acad. Med. 44:1566

Chataing B. International Forum, Vox Sanguins 1983; 44: 62-64

Friedman, S.L. et al. Annals of Internal Medicine (1987) 107: 137

Gillon et al (1988) Vox Sang. 54: 148

Gitnick G. Ann Rev. Med. 1984; 35: 265-78

Gunson, H.H. Health Trends, November 1986

Hornbrook M.C. et al, N.E.J. Med. 1982; 307: 1315-21

Khan R.A. N.E.J. med. 1982; 307: 844-5

Koretz R.L., Suffin S.C., Gitwick G.L. Gastroenterol 1976, 71: 797-803

Koziol, Deloris E., et al. Ann In. Med. 1986; 104: 488-495

Lenes, B.A. et al. N.E.J. Med. 1983; 308: 723

Poole, Judith G. and Shannon Angela E. (1965) New Eng. J. Med. 273: 1443

Poole, Judith G. (1970) Bibl. Haemat. (Basel) 34:23