I.5 Blood Derivatives

I.5.1 Time Period Described

Bio Products Laboratory (BPL) is the main centre for fractionation of plasma in the UK. In the fractionation process, plasma is pooled and separated into clotting factors (mainly factors VIII and IX), albumin and immunoglobulins.

In May 1998, BPL discontinued fractionation of UK fresh frozen plasma, in response to advice by the UK Committee on the Safety of Medicines. BPL now fractionates plasma imported from the USA, from which products will become available during 1999.

The following process description refers to 1997/98, the last full year of manufacture from UK plasma. BPL made available detailed production information that has been used in the analysis, but has not been reported here to protect commercial confidentiality.

I.5.2 Fractionation of Pooled Plasma

Plasma batches processed at BPL typically amount to 6400 kg, containing plasma from approximately 22,000 donations. This is based on 290 ml of plasma per donation.

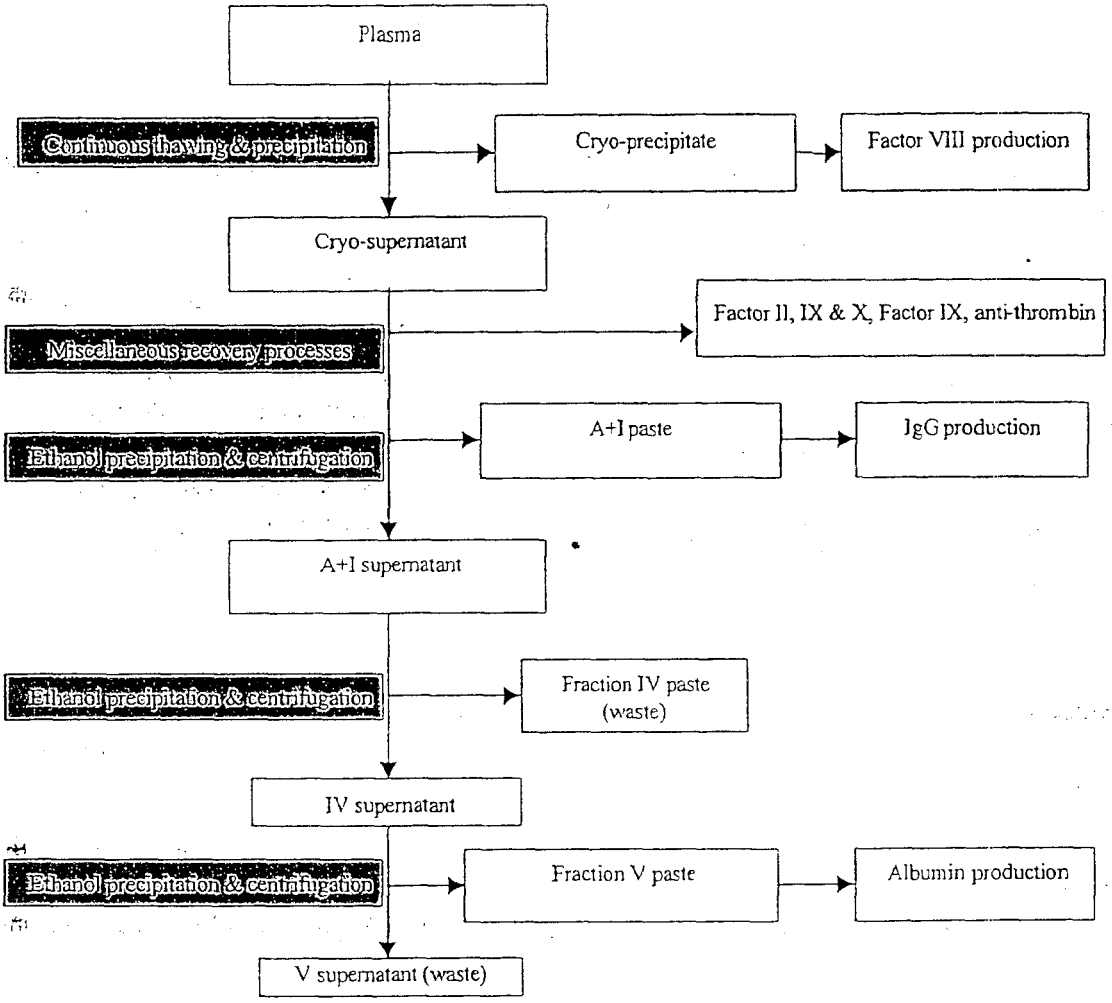
Fractionation of pooled plasma generates a range of intermediate products which, after storage and testing, may be used to prepare blood derivatives. The principal intermediates, and the associated derivatives, may be summarised as:

1. Cryoprecipitate - used to manufacture freeze-dried Factor VIII concentrates.
2. Cryosupernatant - used to manufacture freeze-dried Factor IX concentrates.
3. Fraction II precipitate - used to manufacture immunoglobulins.
4. Fraction V precipitate - used to manufacture albumin solutions.

The general production process is summarised on Figure I.5.1.

Note: This Appendix is included for completeness and reproduces relevant sections of Appendix I from the original February 1999 Final Report, and should be viewed in this context.

Figure I.5.1 Outline of Plasma Fractionation at BPL



I.5.3 Factor VIII

Factor VIII concentrate is a blood clotting agent given to people with haemophilia A. It is produced from the cryoprecipitate fraction of plasma.

One unit (iu) of factor VIII is approximately the amount contained in 1 ml of normal plasma. BPL produces approximately 100 million iu per year, representing approximately 60% of UK requirements.

Note: This Appendix is included for completeness and reproduces relevant sections of Appendix I from the original February 1999 Final Report, and should be viewed in this context.

At BPL there are two production processes, yielding products of different purity:

- Factor VIII Type 8Y - an intermediate purity product. This is produced by heparin precipitation of an extract of the cryoprecipitate fraction, followed by sterilising filtration, filling and freeze-drying, and then virus inactivation by heat treatment of the freeze-dried product. The product is formulated without added plasma protein stabilisers, and filled as a 250 iu or 500 iu dose in 50 ml vials. One 500 iu vial of Factor VIII type 8Y contains approximately 160 mg protein, of which <0.2 mg will be factor VIII (most being fibrinogen and fibronectin).
- Replanate - a high purity product. This is recovered from an extract of the cryoprecipitate fraction, after solvent/detergent virus inactivation, by sequential purification on highly specific monoclonal antibody and ion exchange gel chromatography columns. The process generates virtually homogeneous factor VIII, at very low protein concentration, necessitating product formulation in human albumin solution at a concentration of 50 or 100 iu/ml, and filled as a 250 iu, 500 iu or 1000 iu dose in 30 ml vials. One 500 iu vial of Replenate contains approximately 100 mg protein, of which <0.2 mg will be factor VIII (the remainder being albumin).

I.5.4 Factor IX

Factor IX concentrate (Replenine) is a blood clotting agent given to people with haemophilia B (Christmas disease). It is produced by ion exchange and affinity chromatography from cryosupernatant, which is the portion of plasma left once the cryoprecipitate fraction has been removed.

One unit (iu) of factor IX is approximately the amount contained in 1 ml of normal plasma. Factor IX is formulated as Replenine, consisting of 30 ml vials, each containing a nominal 500 iu factor IX for reconstitution in 10 ml sterilised water for injections. One 500 iu dose of Replenine contains approximately 4 mg protein, of which 2 mg will be factor IX.

I.5.5 Albumin

Albumin is produced from the fraction V paste generated by ethanol fractionation of cryo-supernatant. Successive batches of albumin are therefore derived from either a single plasma batch (22,000 donations) or from the combined Fraction V paste from two separate plasma batches (44,000 donations).

The albumin manufacturing process may be summarised as filtration of the re-dissolved fraction V, removal of impurities by anion exchange chromatography and bulk heat treatment. The albumin bulk is then formulated to two concentrations, sterilised by membrane filtration and filled in a range of dose sizes:

- Zenalb 4.5%: 500ml (22.5g of albumin); 250ml (11.25g); 100ml (5.6g).
- Zenalb 20%: 100ml (20g of albumin); 50ml (10g).

The product is subjected to a second heat-treatment in its final container (60°C, 10 hours).

Note: This Appendix is included for completeness and reproduces relevant sections of Appendix I from the original February 1999 Final Report, and should be viewed in this context.

Albumin may be transfused directly into patients for:

- Maintaining blood volume in the treatment of burns and serious accidents.
- Treating albumin deficiency (e.g. due to liver disease).
- Part of plasma replacement therapy in various chronic diseases.

Albumin is also used as an additive in formulating other medical products such as**:

- Factor VIII - human and recombinant.
- Hemopoietic factors.
- Interferons.
- Streptokinase.
- Some vaccines.

***Note: since issue of the 1999 report, an additional risk has been identified from the use of albumin as a carrier in certain in vivo imaging agents.*

I.5.6 Immunoglobulins

Normal human immunoglobulin (NHIG) (also known as gamma globulin, IgG) consists of antibodies derived from normal human plasma. It is manufactured in two forms:

- A preparation for intravenous (i/v) infusion - either freeze-dried (Vigam S) or in solution (Vigam Liquid) in 5g and 2.5g presentations. This is formulated as a 5% solution of immunoglobulin, also containing 2% human albumin (presently derived from a different plasma pool) and 2.4g sucrose. Each batch is therefore derived from approximately 44,000 plasma donations. It is used to treat primary antibody deficiency, ITP (see Section I.7.6) and other conditions.
- A preparation for intramuscular (i/m) injection. This is formulated as a 17% solution, in 250 mg and 750 mg dose sizes. It is used primarily for pre-travel prophylaxis against Hepatitis A, although the use of hepatitis A vaccine is gradually removing this need. It is also used to ameliorate the effects of measles and rubella infections in individuals at increased risk.

Hyperimmune globulins are specific immunoglobulins derived from the plasma of individuals exposed to previous infection or who have had immunisation. They are manufactured from small batches of specially collected plasma, and prepared in i/m form, typically in 250 mg dose sizes. They are used to confer passive immunity against infectious diseases such as tetanus, rabies, zoster, hepatitis B.

Anti rhesus factor immunoglobulin (Anti-D) is a type of hyperimmune globulin. It is given to RhD- mothers carrying RhD+ babies to prevent haemolytic disease of the newborn (HDN), (Section I.7.5).

Note: This Appendix is included for completeness and reproduces relevant sections of Appendix 1 from the original February 1999 Final Report, and should be viewed in this context.

I.6 Blood Transfusions

I.6.1 Data Sources

The National Blood Service (NBS) in England & North Wales maintains data on blood components issued to hospitals, but information on usage by patients is not readily available. Data for a 12 month period 1996/7 has been used in this report.

The Scottish National Blood Transfusion Service (SNBTS) has a database containing information on blood components transfused into patients. This covers about 30% of all red cell units used in Scotland. In the absence of better data, this is assumed to be a representative sample of transfusions into 30% of the Scottish population of 5 million, i.e. about 1.5 million people. Data from this source for 1995 has been used where necessary to supplement the NBS data. This introduces uncertainties due to the different patient populations.

Differences in transfusion rates between the NBS and SNBTS may occur due in part to intensive therapies for specific diseases and treatment of overseas patients, occurring particularly in London. The general growth in blood component use may also contribute to observed differences.

I.6.2 Blood Component Losses

Some donated blood may not be transfused into patients. Possible reasons include:

- Donations screened out during processing because of positive (or inconclusive) tests for HIV, hepatitis A and B or syphilis, or processing problems (see Section I.3.5).
- Blood not used in hospitals due to going out of date before it is needed, resulting from storage and handling problems and the need to maintain stock for emergencies. The shelf life for red cells is 35 days; for platelets 5 days and for FFP 1 year. The NBS wastage rate is estimated as 0.6% for red cells, 10% for platelets and negligible for FFP and cryoprecipitate. A survey of hospital blood stocks indicated than less than 2.8% of red cells are not transfused.
- Blood not transfused after the unit is opened, either due to use of a part unit for neonates or transfusions stopped before a complete unit is transferred. These cause exposure of the patient to additional donors, but transfuse less than a full unit. In the absence of any data, this contribution is assumed negligible.

The level of product loss probably varies widely between hospitals. For this study, it is assumed that 97% of red cells and 95% of platelets are transfused into patients.

I.6.3 Whole Blood

Modern medicine rarely requires whole blood transfusions, and the large volume of plasma increases the risk of hyper volaemia and cardiac failure.

Whole blood is only used when both red cell and volume deficit occur simultaneously, e.g. in acute blood loss. Another requirements for whole blood is in massive transfusion, defined as replacing the

Note: This Appendix is included for completeness and reproduces relevant sections of Appendix I from the original February 1999 Final Report, and should be viewed in this context.

patient's whole blood in less than 24 hours. However, this is more commonly supplied by red cells combined with platelets, FFP and non-plasma fluids.

Exchange transfusions of whole blood may be used as a treatment for haemolytic disease of the newborn (HDN) but this is now rare. Exchange transfusions are also used to treat high levels of bilirubin associated with prematurity.

Autologous transfusions may use whole blood, but are a small proportion of the units collected (1000 out of 2.2 million units processed).

The small quantities of whole blood transfused are therefore included in the analysis of red cell transfusions.

I.6.4 Red Cells

Products transfused as 'red cells' include:

- Plasma-reduced blood.
- Red cells in additive solution.
- Red cells with buffy coat removed.
- Red cells leucocyte depleted.

Red cells may be transfused to replace acute blood loss or to correct anaemia (see Sections I.7.1 to I.7.3).

In the SNBTS database in 1995, 13,000 individual patients received red cells (including whole blood), out of a covered population of 1.5 million. This is an average of 8.7×10^{-3} red cell transfusions per person year.

The SNBTS database also shows a total of 58,000 units of red cells were transfused into the 13,000 patients in 1995. This gives a mean of 4.5 units per transfused patient. The distribution of units transfused per patient-year has a modal value of 2 units. Approximately 7% of transfused patients received more than 10 units in the year.

In the NBS, 2.2 million red cell units per year were issued to hospitals in 1996/7. Based on a recent survey, NBS estimate that less than 2.8% of red cell units issued are not transfused. Assuming 2.8% losses, this gives 2.1 million red cell units per year are transfused. Using the SNBTS average of 4.5 units per transfusion suggests 480,000 red cell transfusions per year. Among the 51.8 million population of England & Wales, this is 9.2×10^{-3} red cell transfusions per person year. This is very close to the SNBTS transfusion rate.

A rounded figure of 400,000 red cell transfusions per year with an average of 5 units per transfusion is used for the present study.

Note: This Appendix is included for completeness and reproduces relevant sections of Appendix I from the original February 1999 Final Report, and should be viewed in this context.

I.6.5 Platelets

Platelets are used to treat or prevent haemorrhage in patients with severe thrombocytopenia (Section I.7.5).

In the SNBTS database in 1995, 1,600 patients received platelets, out of a covered population of 1.5 million. This is an average of 1.1×10^{-3} platelet transfusions per person per year.

The SNBTS database also gives a total of 23,500 individual donations of platelets transfused into the 1,600 patients in 1995. This gives a mean of 15 platelet donations per transfused patient. The distribution of individual donations transfused per patient-year has a modal value of 5 donations. Approximately 20% of transfused patients received more than 15 donations in the year. In the SNBTS, the mean of 15 donations per transfused patient corresponds to about 3 adult therapeutic doses of 5 donated units each.

In the NBS, 225,500 adult therapeutic doses of platelets per year were issued to hospitals in 1996/7. Assuming 5% losses, it is estimated that 214,200 therapeutic doses per year are transfused. Using the SNBTS average of 3 therapeutic doses per patient suggests 71,000 patients receive platelet transfusions per year. Among the 51.8 million population of England & Wales, this is 1.4×10^{-3} platelet transfusions per person year. This is 60% higher than for the SNBTS, assumed to result from the different patient populations. It is consistent with the pattern within the English regions, where significant variation in demand for platelets is seen between regions, with generally much higher demand in the London and Southeast. The factor of 1.6 may indicate the uncertainty range on the estimate.

The present study uses a rounded figure of 70,000 patients receiving platelet transfusions per year, with an average of 3 therapeutic doses being transfused to each patient. Within the NBS, each therapeutic dose is derived from 4 individual donations, or only 1 if apheresis platelets are used.

I.6.6 Plasma

Plasma may be transfused for the following reasons:

- Disseminated intravascular coagulation (DIC) (see Section I.7.6).
- Severe liver disease.
- Correction of coagulation disorders associated with massive blood transfusion.
- Reversal of oral anticoagulation where it has caused significant bleeding.
- Replacement therapy of some rare congenital factor deficiencies.
- Bleeding in haemorrhagic disease of the newborn.
- Thrombotic thrombocytopenic purpura (with plasma exchange).
- Depletion of coagulation factors following thrombolysis.

Note: This Appendix is included for completeness and reproduces relevant sections of Appendix I from the original February 1999 Final Report, and should be viewed in this context.

In the SNBTS database in 1995, 1,400 patients received plasma, out of a covered population of 1.5 million. This is an average of 9.3×10^{-4} plasma transfusions per person year.

The SNBTS database also gives a mean of 4.6 units per transfused patient, and a modal value of 2 units. Assuming a mean unit size of 200 ml in the SNBTS, these give a mean of 920 ml per transfusion. NBS assume 3 x 300 ml units per transfusion, based on judgements by doctors, giving a total of 900 ml per transfusion, which is consistent with the SNBTS data.

NBS centres issued the equivalent of 350,000 individual units of FFP during 1996/7. As this is a frozen component with a 12 month shelf life, wastage is probably no more than 2%. On the above basis, assuming an average of 3 units (each 300 ml) this equates to 114,000 patients treated. Among the 51.8 million population of England & Wales, this is 2.2×10^{-3} plasma transfusions per person year. This is more than a factor of 2 higher than for the SNBTS, and this difference is assumed to result from the different case mix. The factor of 2 may indicate the uncertainty range on the estimate.

The figure of 114,000 plasma transfusions per year with an average of 3 x 300 ml units per transfusion is used for the present study.

I.6.7 Summary of Transfused Components

Table I.6.1 summarises the above estimates of blood components transfused in England & Wales.

Table I.6.1 Summary of Transfused Components

Component	Units Transfused (per year)	Units Transfused (per patient)	Patients Transfused (per year)
Red cells/ whole blood*	2,000,000	5	400,000
Platelets#	210,000	3	70,000
Plasma (FFP)*	340,000	3	114,000

* Unit derived from 1 blood donation
\# Adult therapeutic dose derived from 4 donations

I.7 References

Heye et al (1994): *Creutzfeldt-Jakob Disease and Blood Transfusion*", Lancet, vol 343, p298.

Sullivan (1998): Presentation to Blood Safety & Screening Conference - *TSEs: Perception and Reality*, Washington, February 1998.

Note: This Appendix is included for completeness and reproduces relevant sections of Appendix I from the original February 1999 Final Report, and should be viewed in this context.

APPENDIX II

INFECTIVITY OF BLOOD

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II. INFECTIVITY OF BLOOD

II.1 Introduction

II.1.1 Outline of Appendix

The purpose of this appendix is to estimate the level of infectivity in blood from donors infected with variant Creutzfeldt-Jacob disease (CJD), and the proportions of infectivity that might remain in the various blood products. The appendix gives a brief outline of CJD, and reviews the available evidence on the level of infectivity in blood and blood components. From the little quantitative information that is available, it makes initial estimates of infectivity in blood.

II.1.2 Units of Measurement

Infectivity is presented in this study in infectious dose units (ID_{50}), defined as the dose that would cause infection of 50% of the exposed population. Infectivity concentration in blood products is measured in terms of ID_{50} per ml of product. Annual risks from infectivity will be measured in terms of ID_{50} per year. The relevant pathway for infection is via blood transfusion or blood product injection into humans. Hence, infectivity is always measured in human intravenous (i/v) ID_{50} units, unless otherwise stated.

Experimental estimation of the ID_{50} requires measurement of infection rates in a series of dilutions and this necessitates large numbers of experimental animals, particularly if the level of infectivity is low. Most of the studies on which the estimates in this report have been based reported infectivity in infectious units (IU), rather than ID_{50} units. An infectious unit (IU) is the minimal amount of infectivity with a 100% probability of infecting a recipient (Brown, 2001). It is measured by recording the number of animals infected by a certain volume of test material. Therefore if 2 animals are infected from a pool of 2ml of sample, the infectivity is 1 IU/ml, regardless of the number of animals between which the dose was divided. Whereas the ID_{50} is the dose that is expected to result in infection of 50% of the animals inoculated. An exact conversion from IU to ID_{50} is not possible but for the purpose of this report, an approximate conversion of 1 IU = 2 ID_{50} is used.

II.2 Introduction to CJD

II.2.1 TSEs

CJD, BSE and scrapie are all varieties of transmissible spongiform encephalopathy (TSE) occurring in humans, cattle and sheep respectively. The distinctive feature of all TSEs is the development of sponge-like holes in brain tissue, resulting in a deteriorating mental condition and eventually death. The diseases develop slowly without external symptoms for several years, and death typically follows soon after the onset of clinical symptoms. The diseases can be transmitted from one person to another and even from one species to another, if sufficient infected tissues are transplanted, inoculated or eaten.

Despite extensive research, there are still many gaps in our knowledge about the nature of TSEs. Experimental research takes a long time to produce results because the diseases themselves develop so slowly. Most research concerns scrapie in laboratory mice, and it is unclear how far its conclusions might apply to TSEs in other species. Theoretical research is difficult because of lack of knowledge about the infective agent. Epidemiological studies are difficult because of the long incubation period, and the fact that there is at present no way to test for the disease except by removing a sample of infected tissue, which cannot normally be done until the patient is dead.

As a result, no one knows how many people may be incubating the disease already, and unless a simple test is developed this knowledge is unlikely to be gained until the majority of those infected are already dead or dying. This makes it extremely difficult to develop a public health policy to minimise further infections and to care for those already infected.

II.2.2 The Infective Agent

The nature of the agent that causes infection with TSEs is unclear. It is unlike a conventional virus or bacteria, as it stimulates no immune response in the host, and is resistant to inactivation by heat, chemical disinfection or radiation.

The dominant theory is that the agent is an abnormal form of the prion protein (PrP). In healthy animals, the normal form of the prion protein is present in many organs and tissues, including the brain. Its function is unknown. When an animal is infected with TSE, the abnormal form of PrP progressively accumulates. It is not clear whether this is the cause of the symptoms, or a result of some other unknown cause. The prion theory assumes that abnormal prion is able to induce neighbouring prion to distort similarly. In this way, the infection is able to increase slowly in the body, and can pass through, as yet uncertain, routes from the site of entry to the brain, which is the only organ in which the accumulation of prions is known to cause damage.

According to this theory, there is little genetic involvement in the disease, as the prion protein distorts after the molecules have been formed. The gene encoding prion protein has been identified, and it has been possible to develop laboratory mice without the gene, and these are completely resistant to inoculations of scrapie. However, the prion gene structure does influence the susceptibility of the host to infection and the incubation time for development of disease. Other genetic factors also influence susceptibility, incubation time and other characteristics of the disease.

There are several other theories about the nature of the infective agent, as the concept of a replicating protein containing no nucleic acid is not universally accepted. One theory is that it is an unconventional virus, resistant to conventional methods of inactivation. It is possible that other agents, producing similar effects, would lead to risks similar to those estimated using the prion theory.

II.2.3 The Development of the Disease

Tests with mouse-adapted scrapie have revealed that intracerebral inoculation directly into the brain establishes infection there directly, although symptoms may not appear for several months or even years. Once spongiform damage has reached certain critical areas of the brain, clinical symptoms become apparent. Following intracerebral inoculation, infectivity also enters the blood stream and lympho-reticular system, as for peripheral inoculation (see below).

Peripheral inoculation (e.g. intravenous) causes infectivity to be dispersed widely and rapidly in the blood stream, but it is removed in a few hours, possibly by the spleen. There follows a period in which the inoculum can be detected but there is no apparent replication of infectivity. Early replication of infectivity may be detected in the lympho-reticular system (particularly the spleen and lymph nodes), but this is variable (Hunter *et al* 2002). The route for the spread of infectivity from the periphery to the brain is not known, but may involve lymphoid cells and/or nerve fibres. Although infectivity and abnormal PrP has been shown to accumulate in the lymphoid tissues of animals with TSE's, the role of circulating lymphoid cells (notably white cells) in the pathogenesis of the disease and the contribution of white cells to the total infectivity in whole blood is far from clear (Raeber 2001).

Following experimental intragastric infection of a cow (e.g. after eating infected tissue), the first organ in which infectivity is detected is the lymph node (Peyer's patches) in the wall of the small intestine. Although the infectious agent persists in this organ for some time, it has not yet been established whether it replicates there.

II.2.4 CJD

Most cases of CJD (about 85%) are sporadic, with no known cause, possibly due to the random change of prion within the patient. Sporadic CJD occurs mainly among people aged 55-80. It produces a deteriorating mental condition, involving loss of memory and mental faculties, typically leading to death within 6 months of the onset of symptoms. At present, the infection is impossible to detect before symptoms develop; the disease is difficult to confirm before death occurs; and there is no known cure. There are approximately 50 cases of sCJD in the UK per year.

Sporadic CJD is known to be transmissible if infected tissues from someone with the disease are implanted in another person. This iatrogenic transmission has been documented where tissues used in medical or surgical procedures (e.g. human growth hormone treatment, corneal transplants or brain surgery) have later been found to be from donors incubating CJD. However, very few of the CJD cases (less than 1%) result from this form of transmission.

Genetic variations in the PrP gene may give some degree of resistance to the disease, as some genotypes are more prevalent among CJD patients than in the general population but this may be the

result of the effect of the genotype on the incubation time, rather than absolute susceptibility. Some cases (about 14%) are familial, associated with mutations in the PrP gene.

There is no evidence that scrapie or BSE are the cause of the majority of CJD cases. CJD occurs at the rate of approximately 1 case per million people per year world-wide. It occurs in Australia and New Zealand, where there is no scrapie or BSE, at the same rate as in other countries.

II.2.5 BSE

Bovine spongiform encephalopathy (BSE) is a type of TSE occurring in cattle. It was first identified in 1986. Most known cases of the disease have occurred in Great Britain, although some cases have occurred in other parts of Europe, possibly due to export of infected animals or feed. The number of cases in the UK reached a peak in 1992-93, and has since declined very significantly.

The main source of the infection appears to have been via meat and bone meal (MBM), which was used to provide protein in cattle feed. MBM is obtained by rendering residues from carcasses of animals, including both cattle and sheep. A change in the method of rendering, which occurred mainly in England in the early 1980s, may have allowed prion protein from scrapie-infected sheep brains to enter the cattle food chain. Once established, rendering of cattle-brains may have recycled the infectivity. This theory is supported by the fact that the ban on feeding ruminant-derived protein to ruminants, which was introduced in 1988, was followed 5 years later (the typical incubation period) by a decline in the number of BSE cases.

II.2.6 Variant CJD

In 1996, a number of cases of CJD were identified in the UK which differed from the other types of CJD. They occurred in younger patients (aged 16-53), produced different symptoms, and had a different pattern of lesions in the brain. This variation is refered to as variant Creutzfeldt-Jakob disease (vCJD). Characteristic aspects of vCJD are similar to those of BSE in cattle and different from sporadic CJD and other TSEs, indicating that a common agent is the cause.

The most likely cause of vCJD is consumption of beef products containing the BSE infective agent before those tissues most likely to contain infectivity were banned from human food in 1989. Most vCJD cases occurred in the UK (five cases have been reported in France, one in the Republic of Ireland, one in Italy, one in the USA and one in Canada).

There have been 130 UK cases of definite and probable vCJD of which eight are still alive (as at 3 February 2003).

There is a statistically significant rising trend in numbers of vCJD cases. Analysis by the Public Health Laboratory Service (PHLS) statisticians of data up to December 2001 shows that the trend continues to be significant at an increasing rate of 21 per cent per year for onsets and 23 percent per year for deaths. There is no evidence as yet of a significant departure from the ongoing upward trend but the analysis indicates that these values are also consistent with an epidemic reaching its peak. It is not yet possible to predict from them how many people might be infected with vCJD. Estimates from risk analysis are highly uncertain and vary between a few hundred and over a hundred thousand.

The distribution of infectivity in the body in vCJD patients differs from that of other forms of CJD in that it is much more widely spread. Infectivity has been demonstrated in the spleen and tonsils of patients with vCJD (Bruce *et al* 2001) and the disease-associated abnormal prion protein has been demonstrated in the tonsil, spleen and lymph nodes of such patients (Wadsworth *et al* 2001). The abnormal protein has also been shown to be present in the appendix of vCJD patients at autopsy and of 2 undiagnosed vCJD individuals up to 2 years before the onset of symptoms (Hilton *et al* 2002). In contrast, most evidence indicates that in sporadic CJD the infectivity is only found in the nervous system (Hill *et al* 1999). The role of circulating white cells in the pathogenesis of the disease is uncertain (Raeber 2001).