OPINION ON
THE RISK QUANTIFICATION FOR CJD TRANSMISSION VIA SUBSTANCES OF HUMAN ORIGIN
Adopted by
The Scientific Committee on Medicinal Products and Medical Devices
On 21 October 1998

1- TERMS OF REFERENCE AND OPINION

Context of the questions

In the mid-1990s, the spread of Bovine Spongiform Encephalopathy (BSE) to epidemic proportions in the United Kingdom created considerable concern among national and Community health and agriculture authorities. This worry was exacerbated in 1996 when the Creutzfeldt-Jakob Disease Surveillance Unit in Edinburgh identified a new variant of the disease (nvCJD) that exhibited characteristics linking it to BSE infected animals. Given compelling indications that BSE and nvCJD were caused by the same infectious agent, it was surmised that the probable cause was ingestion of bovine products containing the causative organism of BSE in cattle.

This supposition raised serious concerns about the possibility that blood and blood products might transmit the agent responsible for new variant CJD and led to precautionary measures for industrially manufactured plasma-derived products being recommended by the Committee for Proprietary Medicinal Products (CPMP) of the European Agency for the Evaluation of Medicinal Products (EMEA).

Concerned about the potential impact of any link between the BSE causative agent and nvCJD on public health and in particular on the safety of blood and blood products, the Commission Services submitted a series of questions for deliberation by the Scientific Committee on Medicinal Products and Medical Devices (SCMPMD). These questions sought an opinion on the magnitude of risks, with respect to manufactured plasma-derivatives, and human tissues including cells and organs. They addressed: the pathways for transmission; the level of risk per unit of intake from different blood components and manufactured plasma derivatives; changes in risk as a function of quantity, individual characteristics, and duration of exposure; reasons for donor deferrals; risk-benefit considerations; and future models necessary for the on-going assessment of transmission risk.

Methodology

In order to develop a broadly based opinion on the topics presented by the European Commission the Scientific Committee on Medicinal Products and Medical Devices convened a working group consisting of experts in the fields of virology, experimental research in transmissible spongiform encephalopathies (TSE), epidemiology of Creutzfeldt-Jakob disease (CJD) and the new variant of Creutzfeldt-Jakob disease (nvCJD), clinical care of CJD and nvCJD patients, pharmacology, blood transfusions and blood products, as well as risk assessment of medicinal products. The working

group has reviewed the relevant literature published to date and evaluated the opinions of other bodies (e.g. CPMP, SEAC, national authorities) presented so far. The draft opinion was reviewed and agreed by the SCMPMD at its plenary meeting of 21 October 1998. As far as new information may become available in the future a revision of this opinion may become necessary.
The questions posed by the Commission (original text in Italics) and the comprehensive answers of the SCMPMD, a review of the literature, and an extensive discussion of the relevant topics, taking into account all data known to the working group, follow. For the sake of readability, references to published literature are omitted from the section "Questions and answers", but are extensively given in the subsequent chapters. A list of abbreviations in the Annex may be found useful by readers not familiar with this field.

Questions and answers

The Scientific Committees advising the Commission should be asked to provide an opinion on the nature and magnitude of risks with respect to CJD and nvCJD associated with substances of human origin. This includes blood, blood components, manufactured plasma-derivatives, human tissues, including cells and organs.

The opinion should address the following:

1.1. *What are the pathways for transmission via these human substances or the industrially manufactured products derived from them and the associated dose-effect relationship?*

It has been proven that transmission of CJD from one individual to another occurred by certain human tissues or products derived from them. These tissues and products are: cornea, dura mater, growth hormone and gonadotropin prepared from human cadaveric pituitary glands. In addition, transmission of CJD by silver electrodes used for stereotactic electroencephalography and by neurosurgical instruments has been described in single cases. In all these cases, the carrier of infectivity (i.e. tissues, instruments) was derived from or in close contact with the central nervous system of individuals infected with CJD. Whether those individuals suffered from overt CJD or were still in the incubation period is not always known. Transmissions occurred only after invasive procedures in the recipient (i.e. i.m. or s.c. application of pituitary hormones, use of tissues or instruments during surgery).

There is no evidence that CJD transmissions with other human organs, tissues or cells (including blood, blood components or manufactured plasma-derivatives) did occur. Epidemiological studies (surveillance, case-control-studies, population studies) did not detect a link between CJD and the administration of blood and blood products. The evaluation of all relevant data, including those which demonstrate a low TSE infectivity (1 to 10 infectious units (IU) per ml in contrast to $>10^5$ IU per ml with blood-borne viruses like HIV, HCV and HBV) in the blood of some animals in model systems (predominantly inbred rodents) leads to the opinion that transmission of CJD by blood and blood products either does not occur or does not contribute to the CJD epidemiology. Although a hazard cannot be excluded a real risk is not recognisable.

The transmission of CJD by contaminated electrodes showed that even extremely small quantities of the CJD agent may be sufficient for infection following intracerebral inoculation, as these transmissions occurred after cleaning and sterilisation of the electrodes. Other data on dose-effect relationships in humans are not available. Results of animal experiments are discussed in the response to question 3.

Few is known about the relative efficiencies of various routes of infection by the BSE or the nvCJD agent, but the epidemic spread of the disease in cattle and its transmission to other species presumably through food, suggests that the oral route may be efficient. With respect to transmissions via human substances, experimental and epidemiological experience with nvCJD is just emerging and, therefore, very limited. As with CJD firm conclusions cannot be drawn.

In general, the length of the incubation period seems not only to depend on the size of the inoculum, but also on the site of administration with inoculation in the central nervous system causing shorter incubation times and those in the periphery causing longer incubation times. It has been deduced from animal experiments that the most efficient way of transmission is by intracerebral (i.c.) inoculation followed by the intravenous (i.v.) route of administration. Intraperitoneal (i.p.), subcutaneous (s.c.) and intramuscular (i.m.) administration seem to be gradually less effective. In some TSE epizootics, transmissions are sustained by the oral route (p.o.). The efficiencies of different routes of administration differ by orders of magnitude, e.g. in a mouse scrapie model, the efficiencies of i.c. versus s.c. administration may differ by a factor of 100.000. An extrapolation to the human situation may be difficult, but there are also indications that intracerebral inoculation (e.g. by contaminated electrodes) is the most efficient way of infection.

1.2. ***Per unit of intake: What is the level of risk of transmission of CJD/nvCJD:***

a) from blood or plasma transfusion?
b) from human plasma for fractionation?
c) from virus inactivated human plasma or plasma products?
d) by transfusion of platelets?
e) from infusion of albumin?
f) by clotting factor concentrates, particularly to the haemophilia population?

As outlined in the answer to question 1, an evaluation of all available data does not lead to the recognition of a risk of CJD transmission by blood or blood products. As the experience with nvCJD is limited, the same statement cannot be made for nvCJD.

The blood products, cited in this question, need to be separated into two groups, namely blood components which contain cellular material, are less refined and are not pooled, and manufactured plasma-derivatives which are highly refined from plasma pools containing cellular material only as contaminants. Measurements of blood-borne infectivity in TSE infected laboratory rodents indicated that at least half of the low level infectivity associated with blood is recovered in white blood cells but that significant infectivity may remain in the plasma perhaps as cell debris. The rodent data, but the rodent data alone suggest that blood components (erythrocytes concentrates,

thrombocytes concentrates, plasma for transfusion) may contain infectivity and, therefore, pose a theoretical risk. However, this theoretical risk has never been substantiated.

Per definition, plasma for fractionation is not administered directly to patients, but subjected to processing and, mainly as a consequence of the transmission of hepatitis viruses and HIV, to treatment for virus inactivation. The methods used for virus inactivation (predominantly heat and solvent/detergent) do not influence profoundly the infectivity of TSE agents. However, the fractionation process contributes to an elimination of the TSE agent leading to a gradual loss of infectivity with additional fractionation steps. Therefore, the theoretical level of infectivity may decrease in the sequence of coagulation factor VIII > immunoglobulins > albumin. Again, the theoretical risk has never been substantiated. Even in haemophiliacs treated with high amounts of factor VIII cases with CJD are not known, although plasma of individuals at risk for CJD or developing CJD post donation appears to be often incorporated into plasma pools for fractionation. The frequency of such plasma donations being incorporated into plasma pools for fractionation may be estimated from the calculated prevalence of individuals incubating CJD (30 per million donors, calculated from the incidence (1 per million population) times average incubation period (30 years)) and has been experienced with recall policies in some Member States and in the USA. This lack of infectivity may be explained, if it exists at all, by a very low level of infectivity in blood which will be removed during the manufacture of the plasma-derivatives.

1.3. Do the above risk estimates change as a function of:

a) Quantity of intake?
b) Characteristics of the individuals concerned (age, sex, medical history, etc.)?
c) duration of exposure and reiteration of exposure

a) Quantity of intake

Quantity of intake of infectious material is generally one of the most important risk factors. Animal experiments clearly indicate that as for other micro-organisms titration of the TSE agent can be performed. Both incubation time and number of affected animals depend upon infectious intake, whatever the route of administration. On the basis of such titration experiments, a "Lethal Dose 50%" can be calculated. It should be noted that, for high doses, incubation time cannot be reduced under a certain limit that is characteristic of the strain of TSE agent and the host animal.

b) Characteristics of the individuals concerned (age, sex, medical history)

Although there are reports in the scientific literature of a slight effect of histocompatibility antigens, age and sex of the recipient in animal models of TSE, none of the epidemiological studies conducted in humans to date have identified any clinical or biological criterion that could be associated with risk. However, in hGH-related CJD the age at the onset of the disease may be associated with the duration of the clinical disease. Also it is an unexplained anomaly, that all nvCJD cases have, in marked contrast with classical CJD, occurred in young adults.

Nevertheless, one should remind that homozygosity at codon 129 of the gene coding for the protein (PrP) which is altered invariantly in TSEs is the major determinant for genetic susceptibility to iatrogenic and sporadic CJD and to nvCJD.

Moreover, epidemiology and search for risk factors have been and will be enhanced by broad international co-operative investigations covering high numbers of patients and controls.

c) Duration of exposure and reiteration of exposure.

Duration of exposure is directly linked to the quantity of intake (see above).
Animal experiments and neurosurgery-related iatrogenic cases of CJD indicate that a single exposure to TSE agent is sufficient to induce disease. The effects of reiteration have not yet been investigated except recently when it was demonstrated that suboptimal doses of 263K scrapie agent induce infection and disease in hamster after repeated oral exposure. Reiteration of exposure should be considered as a risk factor.

1.4. In addition to those presented by the Commission in its proposal for a Council Recommendation on the "Suitability of blood and plasma donors and the screening of donated blood in the European Community" such as,

- ***Creutzfeldt Jakob Disease (CJD) (persons on whose family this has occurred)***
- ***Cornea/dura mater transplantation recipient***
- ***Pituitary hormone of human origin (e.g. growth hormone) recipient***

what are additional grounds for the deferral (temporary or permanent) for blood and plasma donors, tissue donors and organ donors?

1.4.1. Donor deferral

In addition to the donor exclusion criteria proposed by the Commission, the Council in its Recommendation 98/463/EC on the suitability of blood and plasma donors and the screening of donated blood in the European Community (O.J. L203 21.07.98, p. 14) expanded these to include donors who have, or have a history of, TSEs, or history thereof in the genetic family.

The CPMP's "Note for Guidance on plasma-derived medicinal products" (CPMP/BWP/269/95 rev. 2) requests the implementation of a standard operating procedure describing the mutual information system between the plasma collection centres and the manufacturing fractionation facility with the aim of informing each other inter alia when a donor has developed or is found to have a risk factor for CJD. The SCMPMD supports this recommendation.

No distinction has been made in either of these documents between the deferral criteria related to the classical forms of CJD and those related to nvCJD.
The SCMPMD considered other possible exclusion criteria for (permanent) deferral of blood and plasma donors:

1.4.1.1 Previous transfusion of whole blood or blood components (red cell and platelet concentrates, plasma for transfusion).

The permanent exclusion of donors who in the past have received transfusions of whole blood or blood components is already mandatory in France to reduce the risk of alloimmunisation in recipients. Deferral of persons assumed to be at risk for TSE disease due to prior exposure to blood or blood products would prevent the propagation of the disease through subsequent donations presuming that either CJD or nvCJD can be transmitted by blood or blood products. Recognising that this could lead to the exclusion of a substantial number (estimated to vary between 5% and 10% of the donor base) of first time and repeat donors, a thorough evaluation of the consequences of such deferrals on the supply of blood and blood components is needed before a decision is taken on the implementation of this measure. The experience compiled in France since 1997 could be of valuable assistance.

1.4.1.2. Positive result of validated test for TSE infectivity in donor blood.

When a validated test for TSE infectivity in donor blood becomes available, it should be implemented in routine donor screening as soon as possible and donors found to be positive should be excluded from donation. Member States should, in the interest of public health, warrant availability of TSE tests for blood screening in collaboration with possible patent holders.

Guidelines or recommendations regarding the exclusion of tissue and organ donors with TSE risk need to be adopted by the European Community particularly in view of article 152 of the treaty of Amsterdam. The SCMPMD recommends that exclusion criteria as established for blood donors should also apply to tissue and organ donors.

1.4.2 Leukodepletion

Leukodepletion of all blood units was introduced in France in 1997 in order to reduce HLA immunisation of recipients and the risk of transmission of intracellular micro-organisms such as CMV and HTLV. In addition, there is evidence that through leukodepletion certain immunomodulatory effects of blood components are prevented, which decreases post-operative infections. This practice has been introduced recently in Ireland, Luxembourg, Portugal and the UK as a precautionary measure following the identification of nvCJD.
The arguments that have been presented for implementing this practice in the context of nvCJD are:
i) White blood cells are suspected to be involved in the transport of the CJD and nvCJD agent via blood.
ii) "Recycling" i.e. the spread of the agent among blood recipients may be reduced.
iii) The possibility of species adaptation of TSE agent strains may be diminished.

It is not yet clear what proportion of the blood-borne infectivity is distributed into white blood cells and which subtype classes of white blood cells (T-, B- or dendritic cells) carry infectivity for CJD or nvCJD. In addition, the efficacy of filters used in the process of leukodepletion to adequately remove any of the relevant subsets of white blood cells has not been established. Furthermore, there is little or no evidence to date that CJD/nvCJD is spread by blood transfusion and therefore comprehensive leukodepletion to prevent CJD/nvCJD transmission is not yet based on epidemiological or firm experimental data.

1.4.3. Recall of plasma products

In the European Community the policy concerning the withdrawal of plasma derivatives if a donor to a plasma pool for fractionation is subsequently suspected on having TSE has been worded as follows (CPMP report 22/11/1995, CPMP 846/95 and CPMP/201/98 of 25 February 1998):

- Regarding classical CJD: *"no recall should be initiated because it is not scientifically justified"*
- Regarding nvCJD: *"Given the lack of specific information on nvCJD, as a precautionary measure it would be prudent to withdraw batches of plasma-derived medicinal products from the market if a donor to a plasma pool is subsequently strongly suspected, by the reference centre, of having nvCJD"*.

The SCMPMD endorses the present policy. In the USA, the FDA, which until recently required recall of plasma derivatives when a donor to the plasma pool was suspected of CJD has now adopted a policy similar to that defined by the CPMP.

1.5. On what risk-benefit considerations should decision makers choose what level of risk is acceptable in relation to the administration of these substances and products?

Prior to any consideration of risk-benefit, decision makers must recognize that most blood components and manufactured plasma-derivatives are indispensable for clinical treatment and enhancing the quality of life of those suffering from blood disorders, and are still irreplaceable with the exception of factor VIII and factor IX and that production of these cloned factors will not be able to meet demand for at least another five years.

The risks associated with the transfusion of blood components or the administration of plasma derivatives are mostly known from long experience with these products. The problem of blood group connected incompatibility has been overcome, in principle, by testing donors and recipients but one should realise that incompatibility reactions, most often deadly, still occur at a frequency of 1:1,000 to 1:10,000 in industrialised countries. Another intractable problem has been bacterial contaminations of blood components which still cause a significant number of deaths per year in industrialised countries (e.g. in France annually seven deaths on average).

The risk of transmission of viral diseases is widely debated. The existence of post-transfusion hepatitis has been known for decades. The risk of transmission of HIV by blood products was recognised one year after the first description of AIDS. The subsequent introduction of screening tests for markers of infections with HBV, HIV and HCV has drastically reduced the risk of transmission of those viruses. The introduction of effective virus inactivation steps into the manufacturing process of plasma derivatives has further diminished the residual risk of transmission of HBV, HIV and HCV to virtually zero. However, the risk of transmission of those viruses by blood components for transfusion is still measurable and is estimated to be in the range of 1:1.000.000 for HIV, 1:500.000 for HBV and 1:120.000 for HCV (based on studies in Germany). Other viruses are still transmitted by blood components (Parvovirus B19, Cytomegalo-

virus (CMV) and others) or plasma derived products (B19, hepatitis A virus) but have not generated much public concern as the diseases they cause are most often not severe or are limited to small number of persons at special risk (e.g. leukaemia patients).

The risks just described are quantifiable. There are possibilities and, therefore, also efforts to reduce the risks (e.g. testing for viruses with highly sensitive nucleic acid amplification techniques, leukodepletion for removal of cell associated viruses like CMV and for reduction of immune reactions). The effectiveness of such measures can be quantified by measuring the reduction of risks. In contrast, the risk of transmission of CJD by blood components and manufactured plasma-derivatives is, despite the experience of several decades, not measurable, i.e. not quantifiable, and therefore theoretical.

From a scientific point of view a risk can be subjected to a risk-benefit evaluation if the risk is real and, at least approximately, quantifiable. Then, the effectiveness of any measure can be checked. Such an approach seems to be adequate when alternatives to the considered medicinal products are not available. If equally efficacious alternatives (with satisfying quality, sufficient supply etc.) are available, then even theoretical, unquantifiable risks may be considered. However, one should have in mind that other theoretical risks may become recognised also for the alternative products with time.

1.6. What models should be developed for the continuous assessment of the risk of transmission of CJD/nvCJD in the above?

At the present state of knowledge several broad approaches are deemed necessary for assessing the risk of transmission of CJD/nvCJD via substances of human origin.

1.6.1 One of the most important contributions for assessing the risk of transmission could be the development of a simple readily available ex vivo diagnostic test for preclinical nvCJD/CJD.

1.6.2 Further development of simple experimental models able to detect and quantify efficiently infectivity in any substance of human origin (tissues, blood, blood components, manufactured plasma-derivatives).

1.6.3 Accelerate investigation of the pathogenesis of model TSE diseases in readily accessible laboratory rodents. Extend these findings to higher or alternative species to establish which components of the pathogenesis are likely to be generalisable and can therefore be extended to humans and agricultural animals.

1.6.4 Community-wide surveillance systems for the identification of CJD patients should be established or continued. These systems should include: notification of suspected cases to and confirmation of the clinical and neuropathological diagnosis of CJD by national reference centres, collection of data and epidemiological studies on risk factors.

1.6.5 Surveillance systems capable of detecting directly the presence of infectivity in human tissues during the preclinical stages. Several methodologies are worth of consideration: for instance, the use of appendices (an organ highly rich in lym-

phoid tissue) from routine appendectomies. This is a tissue available in many thousands a year and, thus, a way of sampling a large number of people. This approach may be useful in assessing the prevalence of preclinical nvCJD as appendectomy is performed mainly in the second or third decade of life, an age at which most cases of nvCJD occur. Use of tonsils from tonsillectomies is also recommended, although its origin in mostly younger people limits the possibility of its usefulness. Consideration could be given to sampling other lymphoid tissues which may become available from surgical interventions. However, there are a number of ethical issues (e.g. patient's permission, patient notification, data confidentiality) which have to be resolved until studies can be initiated.

1.6.6 Efforts should be undertaken to detect, despite the problem of long incubation periods, pairs of donors and recipients who both developed CJD/nvCJD. Such studies should include retrospective examinations of selected populations, e.g. look back studies of haemophiliacs.

1.6.7 Establishment of a system of traceability of donors and recipients in blood transfusion, plasma-derivatives and organ and tissue transplantation could be an essential element of surveillance.

1.6.8 Although at present almost all cases of nvCJD have occurred in one of the Member States of the European Community (i.e. the UK), it is possible that in the coming years nvCJD will occur in other countries of the EC. In the context of the precautionary measure already executed in the UK, namely to stop the use of local plasma for fractionation, it would be relevant to define a common (EC) policy (regarding the ban of plasma) if and how in the interest of public health other Member States will need to react in the event that the epidemiology of nvCJD changes.

2 - CLINICAL PICTURE OF HUMAN TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

The following syndromes are subsumed under the term "human transmissible spongiform encephalopathies" (hTSE):

- kuru
- sporadic Creutzfeldt-Jakob disease (CJD)
- iatrogenic CJD
- new variant CJD (nvCJD)
- familial CJD
- Gerstmann-Sträussler-Scheinker syndrome (GSS)
- fatal familial insomnia (FFI)

The major clinical symptoms of these syndromes are mental deterioration, cerebellar dysfunctions, involuntary movements, missensations and psychiatric alterations. Histological hallmarks are spongiform degeneration of the central nervous system (appearance of numerous vacuoles in the neuropil), astrocytic gliosis (increase of astro-

cytes in number and size) and deposition of a protein, called prion protein (PrP^{res}), which is the protease resistant form of a cellular protein (PrP^{c}). PrP^{c} is coded by a cellular gene designated PRNP. PrP^{res} is pathognomonic for the whole of this group of illnesses. These diseases lead inevitably to the death of the patients, usually after a duration of six weeks to a couple of years depending on the type of the disease.

The diagnosis of the disease can be strongly suspected on clinical grounds, e.g. by a characteristic electroencephalogram in a number of cases, but it can be only confirmed by histological or biochemical investigations of brain material after biopsy or autopsy. Other diagnostic tools, e.g. detection of brain proteins in the cerebrospinal fluid or in the peripheral blood, are under development (Hsich 1996, Otto 1998). A specific immune response, often basis for a diagnostic test, has not been observed. Tests indicative of the disease before the onset of clinical symptoms do not yet exist.

2.1. Studies on transmission of hTSEs and their limitations

The clinical and histological picture of hTSEs resembles that of a disease in sheep known since more than 200 years which is called "scrapie". Since 1938, reports of the transmission of scrapie to sheep or goats by the inoculation of a number of tissues collected from diseased sheep have been published (Cuillé 1938, Pattison 1960). In 1961, the successful transmission of scrapie to laboratory mice was described (Chandler 1961). Stimulated by those successful transmissions of an animal disease closely related to hTSE attempts to transmit hTSEs have been undertaken. Transmissions to apes as well as New and Old World monkeys have been reported since 1966 (Gajdusek 1966, Gibbs 1968, reviewed in Gibbs 1972). In 1975, the first report appeared which described the transmission of CJD to a small laboratory animal, the guinea pig (Manuelidis 1975). Later on, transmission of hTSE to hamsters, mice and rats has been reported (Manuelidis 1977, Tateishi 1979). The by far most effective way of transmission was the direct inoculation of brain material of an affected donor into the brain of an indicator animal (intracerebral route of transmission).

The experimental transmissibility of scrapie is in congruence with the observation that this disease seems to spread horizontally within flocks of sheep and goats. However, the exact route of natural transmission is not yet elucidated. Some forms of hTSEs (kuru, iatrogenic CJD, see below) are also easily explained by the transmission of an infectious agent, others (familial and sporadic forms) not.

The elucidation of the routes of transmission of hTSEs can be approached by epidemiological and experimental studies. Both suffer from certain limitations which will be discussed before observations with different forms of hTSEs are described.

The rarity of hTSEs and the often very long incubation times counted in years and decades instead of weeks or months as in ordinary infectious diseases are the most important factors which impede a straightforward interpretation of epidemiological data. For example, the lack of recognized hTSEs in certain populations which are thought to be possibly at risk may also be explained by intercurrent causes of death which give insufficient time for the development of a hTSE. Statistically powerful case control studies take long times until a sufficient number of cases are collected.

Transmission studies in animals suffer from a phenomenon which is called "species barrier". It reflects the observation that an animal species challenged by a TSE agent isolated from another species is less sensitive than the originally infected species. Nevertheless, experimentation with small laboratory animals like mice, hamsters and guinea pigs as recipients is often preferred as they are available in high numbers (e.g. as needed for titrations) and as they can be easily kept in a laboratory setting. However, it has always to be kept in mind that those experiments may underestimate the amount of infectivity in the material under study due to this species barrier. Another factor also contributing to an underestimation is the limited sample volume which can be administered to small laboratory animals (e.g. only 30 to 50 µl for intracerebral administration).

Another aspect of the species barrier is the observation that the incubation period of the second and later passages in a species is often drastically reduced compared to the incubation period after primary inoculation with material from another species. It means that there is some adaptation of the infectious agent to its new host. This fact raises the question of whether or not data obtained in one species can be easily extrapolated to another species. Experience over the last decades has shown that the factors which influence the infectivity distribution in animals are: the strain of agent, the host species and sometimes even the breed of the host, the route of inoculation and the dose. As a consequence it is important to investigate diverse models of TSE disease for points of convergence as it is these features that are held in common that can be most confidently generalized to other species such as humans and cattle.

The limitations of the epidemiological studies (rarity of hTSEs, length of the incubation period) as well as of the experimental transmission studies (species barrier, relevance of animal models) have always to be considered when the observations with the different forms of hTSEs are discussed.

2.1.1. Kuru

The transmissibility of hTSEs was first recognized during studies of kuru, a disease which was indigenous to the Fore peoples inhabiting the Central Highlands of Papua-New Guinea. The disease was apparently spread by the practice of endocannibalism which was performed as a rite of respect and mourning for the dead. Since the cessation of cannibalism in the 1950's the disease has nearly disappeared. The only individuals that still develop clinical kuru are those who were already alive before the cessation of cannibalism. As they were most probably infected when cannibalism was still practiced the current cases only occur in individuals in their mid-40s or older, thus indicating an incubation time in excess of forty years.

The disease could be transmitted experimentally to different species of old and new world monkeys and apes. These studies showed that the brain contains the highest amount of infectivity and that transmission experiments were only occasionally successful when organs outside the central nervous system (e.g. spleen and lymph node) were used (Asher 1976). In overall ten attempts infectivity could not be found in serum or blood (Asher 1976). Transmissions occurred after intracerebral and other types of invasive peripheral inoculations, but not by administration through a nasogastric tube (four attempts with chimpanzees, Asher 1976). Therefore, it is speculated that, in hu-

mans, transmission may not have occurred through ingestion of infected brain tissue during the cannibalistic rite, but rather through skin injuries during the preparation of the tissues for cannibalism. However, in this interpretation the species barrier between human and chimpanzees is not sufficiently taken into account. Therefore, transmission by eating infected tissues is a possibility which is in agreement with the observation that at least in some animal TSEs (like transmissible mink encephalopathy (TME) and bovine spongiform encephalopathy (BSE)) the disease is spread by the oral route. Clinically, kuru is characterized by a preponderance of cerebellar symptoms while mental deterioration becomes obvious only later in the course of the disease. In histological sections, relatively large deposits of PrP (so called "kuru plaques") can be observed regularly.

2.1.2 Sporadic CJD

The sporadic form of CJD is the most common type of hTSEs. Clinically, it is characterized by an early onset of mental dysfunctions, e.g. memory loss or behavioral abnormalities. However, in a substantial proportion of cases (15% in a large study of confirmed CJD cases, Brown 1994) cerebellar symptoms are the first signs of the disease. In many cases, a characteristic electroencephalogram (periodic triphasic waves) can be observed during the course of the disease. In histological investigations of brain tissue, deposits of PrP are not necessarily obvious, but can be found after immunochemical staining. The disease can be transmitted to many primate species (Asher 1976), but also to non-primates (cats, guinea pigs, mice and hamsters), however less regularly (Brown 1994). The most effective route of transmission is intracerebral inoculation, although other ways of peripheral invasive administration (intravenous, intramuscular, subcutaneous and others) may also be effective. These transmission studies (mainly with squirrel monkeys as recipients) show that the highest infectivity is found in the brain, followed by spinal cord, cerebrospinal fluid and eye. Occasionally, infectivity is also found in lung, liver, kidney, intestine, spleen (3 of 31 attempts) and lymph node (3 of 15 attempts) (Brown 1994). Epidemiological surveys on risk factors and experimental studies on the infectivity of blood will be dealt with later in the text.

Some recent findings suggest a link between clinical presentation and neuropathological changes on the one hand and genetic factors like polymorphism at codon 129 and the PrP glycosylation pattern on the other hand (Parchi 1996). Homozygosity for methionine at codon 129 and a type I PrP was found in patients with ”classical” CJD of short duration, myoclonus and periodic sharp wave complexes (PSWCs) in electroencephalograms (EEG). Patients with type II PrP were either homozygous or heterozygous and were distinct in their clinical and neuropathological presentation, showing more often cerebellar signs and a longer duration of the disease. PSWCs in EEG were only exceptionally seen. It has been suggested that the cerebellar presentation in patients with hGH related CJD may be caused by a distinct strain of the agent.

2.1.3 Iatrogenic CJD

It was soon recognized that CJD can be transmitted by medical treatments. In 1974, the possible transmission of CJD by a cornea transplant was described (Duffy 1974). Altogether, two well-documented cases of CJD transmissions by cornea transplants were reported worldwide in the literature (Duffy 1974, Heckmann 1997). Onsets of

disease after transplantation of 18 months and 30 years were reported. In a third case, no reference to the disease of the donor was given (Uchiyama 1994). Also, silver electrodes, used for stereotactic electroencephalograms during neurosurgery, transmitted CJD to two patients, although the electrodes have been cleaned and sterilized by 70% ethanol and formaldehyde vapor between their use (Bernoulli 1977). No other transmissions by either of these routes has been reported. However, two greater episodes of CJD transmissions occurred by the use of dura mater transplants and by the administration of hormones prepared from human cadaveric pituitary glands.

More than 80 cases of growth hormone related Creutzfeldt-Jakob disease have been reported in the UK, in USA and in France. Estimated mean incubation time is 8.9 years in France, and more than 10 years in the UK and in the US. Clinical and neuropathological symptoms are identical in all cohorts and consist mainly in cerebellar ataxia. Main lesions were in cerebellum and basal ganglia. Duration of the disease was significantly longer than those observed in sporadic CJD. Genotyping for codon 129 of the PRNP gene, the main genetic determinant for susceptibility to CJD, shows that homozygosity is significantly more often seen in patients. For example, in the French cohort, among 50 patients that have been genotyped, 4 were heterozygous (8%), 13 were Val/Val (26%), and 33 Met/Met (66%) as compared with a control population (individuals treated with hGH but not developing CJD) where the distribution is Val/Val (14%), Met/Met (36%) and Met/Val (50%). Kinetics of appearance of hGH-related CJD indicates that the mean incubation time in heterozygous individuals is significantly longer than in homozygous individuals (11 years versus 8.95 years).

Gonadotropin was also prepared from cadaveric human pituitaries but has had a far more limited use than human growth hormone. Nevertheless, there have also been four transmissions traceable to gonadotropin use, all in Australia.

Dura mater is both collected from the brain cavity, providing ample opportunity for contamination with high titer infectivity, and when used for duraplasty, is transplanted back to the brain cavity providing an optimal route of exposure. This is reflected in very short incubation times of less than two years for some patients, though incubation times are typically 5 to 10 years or more. The maximum incubation time for this route that has been reported to date is 16 years. Most, but not all, of the dura mater transmissions have been associated with use of a single product manufactured in large batches by a single manufacturer. Large batch sizes also contributed to wide contamination of hGH. To date, ~60 cases have been traced to this source. Most and perhaps all of these cases originated from product manufactured prior to 1987 when batch processing was abandoned and a one hour incubation in 1N NaOH was added to the manufacturing process. The total number of transmissions world wide may be much greater as evidenced by the recent discovery of 43 cases in Japan in response to a questionnaire sent to neurologic, psychiatric and neuropathologic institutions (Centers for Disease Control 1998). This was not an exhaustive survey and other countries utilizing the same brand of dura mater to a similar extent would be expected to have been similarly affected. To date, most of the transmissions have occurred in codon 129 homozygous individuals. This leaves open the possibility of another wave of cases from this same exposure among heterozygous recipients requiring more time to manifest the disease. Transmission have also occurred after the use of dura mater in peripheral, non-CNS surgeries.

Only a small percentage (1 or 2%) of the dura mater-associated transmissions that have been recognized to date have been associated with single donor lots and these transmissions might be reduced even further, if the brains of all donors were screened for the absence of PrP amyloid and the dura itself were treated with 1N NaOH before use. Where possible dura mater should be replaced with lower risk materials. One study found that autologous tissues, fascia lata and galea-periost, and a synthetic material were equal to or superior to dura mater for some uses. However, dura was considered essential to other procedures and it was recommended that access should not be restricted where superior efficacy has been proven.

The observation that cases of iatrogenic CJD differ from cases of sporadic CJD by a preponderance of cerebellar symptoms led to the conclusion that a peripheral route of CJD transmission is characterized by an early onset of cerebellar symptoms. If this conclusion is correct, then the 15% or more of sporadic CJD cases that also present with cerebellar symptoms can also be presumed to have originated from peripheral exposures. On the other hand, such a conclusion may not be justified due to the variability in the expression of clinical symptoms.

2.1.4 New variant CJD

A new variant of CJD was first described in 1996 (Will, 1996, Will 1997). In a number of aspects, this new variant differs from sporadic CJD. The age distribution is the most obvious difference. While sporadic CJD occurs mainly in the elderly with a peak of incidence at an age of 70 (Holman 1996), all cases of nvCJD (until Oct 1998 29 cases in UK and 1 case in France) occurred in persons with an age below 50 (Creutzfeldt-Jakob disease surveillance in the UK, Sixth annual report 1997) . The first symptoms in persons with nvCJD are very often psychiatric alterations and sensory disturbances. The histological picture is characterized by relatively large and abundant deposits of PrP (kuru-like plaques) surrounded by prominent vacuoles ("florid“ plaques) in the neuropil. In contrast to sporadic CJD PrP deposits have been found in peripheral lymphoid organs (tonsils, spleen) in limited studies (Hill 1997). A number of arguments link this disease with bovine spongiform encephalopathy (BSE): the nearly exclusive occurrence of nvCJD in the country with the by far most prominent epizootic of BSE, the peculiar clinical presentation, the molecular similarity of the nvCJD- and BSE-associated PrPs (Collinge 1996) and the biological properties of the infectious agents (Bruce 1997).

2.1.5 Familial hTSEs

Three types of CJD, namely familial CJD, GSS (Gerstmann 1936) and FFI (Lugaresi 1986) are hereditary diseases. They are associated with mutations in the normal cellular gene which codes for the prion protein. The sites of mutations are linked to different clinical presentations which led to the description of the different syndromes. For example, in GSS the duration of the disease is counted in years rather than in months as in sporadic CJD. In FFI, the most impressive symptom is the inability of the patients to sleep. Also in hereditary hTSE, the disease can be transmitted to animals although the success rate is lower, sometimes considerably, depending on the type of mutation (Brown 1994).

2.2. Nature of the transmissible agent

hTSEs are a unique group of diseases in so far as they are hereditary on one hand and infectious on the other hand. The nature of the infectious agent is not known. Two hypotheses have been put forward. One hypothesis supports the idea that the infectious agent is an unconventional virus which is unusually stable against a number of inactivating treatments and which has an unusually long incubation period counted in years or even decades. The other hypothesis favors the idea that the infectious agent is solely of a proteinacious nature lacking any genetic material. During the last decade evidence accumulated in support of the latter interpretation, but there is still experimental data which is difficult to explain with the protein-only hypothesis. An exact knowledge of the nature of the infectious agent would help to estimate the tissue distribution of the infectivity and would alleviate the assessment of the risk associated with the transplantation or transfusion of human tissues or blood. In fact, a number of studies (Manuelidis 1978a, Diringer1984) was aimed to prove the virus hypothesis by the demonstration of a "viraemia" as a viraemia was believed to be an essential part in the course of a virus disease.

2.3. Section Summary

In summary, hTSEs are a group of diseases which affect seriously the central nervous system. They can be transmitted to animals, most easily with brain material of affected individuals, but also, though less effectively, by other organs (including spleen and lymph nodes). Transmission from man to man by medicinal treatments is also proven. In every episode instruments, products or tissues were involved which have been derived from or have been in close contact to the brain or the eye of a person suffering from or developing an hTSE (hormones prepared from cadaveric pituitary glands, dura mater, cornea, electrodes). Nowadays, the risk of hTSE transmission by the iatrogenic route is minimized by a number of measures: licensed growth hormones are now produced by recombinant technology, dura mater is replaced by other connective tissues or by synthetic material or is treated with agents which inactivate TSE infectivity (sodium hydroxide), electrodes are replaced by disposable material.

The question remains whether other human substances also bear the risk of hTSE transmission. In the next two sections emphasis is given to estimate the possible risk posed by blood and plasma-derived products.

3 - EPIDEMIOLOGY OF SPORADIC CJD

Patients affected by Creutzfeldt-Jakob disease were reported from many countries all around the world. The overall mortality rate in Europe in a prospective studies covering the years 1993 to 1995 is 0.69 (with 0.56 in Italy and 1.37 in Austria as extremes with the high prevalence in Austria most probably explained by the high autopsy rate in this country, Will 1998). In many surveillance systems, the age distribution pattern is uniform. In age-specific incidence, a rise in the age group between 60 and 70 and a decline in the elderly was found (Will 1998). CJD may occur in people younger than 30. However, in this age group it is an absolute rarity. There is a slight over-representation of females in age and sex specific incidence figures (male:female 1:1.4, Will 1998). In a study from the United States, CJD death rates in individuals of African

origin are consistently lower than in Caucasians (0.37 vs. 1.01, Holman 1996). While apart from iatrogenic cases approximately 10% of all CJD cases are familial (see above), 90% of CJD cases occur seemingly without an apparent risk factor and are therefore designated as sporadic CJD. There were some case reports on coincidental CJD in a husband and a wife or in neighbours (Brown 1998a, Will 1982). Such reports are extremely rare and a contact to another CJD patient could not be found as a risk factor in large epidemiological studies (van Duijn 1998).

In the published case reports on CJD in health professionals (histopathology technicians, dermatologist, physician, pathologist, neurosurgeon, orthopedic surgeon) no proven link to exposure to CJD could be established (Berger 1993, Weber 1993). Health professionals do not seem to be at a greater risk than the normal population (van Duijn 1998).

In sporadic CJD, the phenotypic expression of the disease may be linked to the polymorphism at the codon 129. In contrast to the normal population, 69% of the CJD patients were linked to homozygosity for methionine at codon 129 (vs. 42% of the controls), whereas 15% are heterozygous and 16% homozygous for valine (vs. 45% resp. 13%, German CJD study, unpublished). Homozygosity on codon 129 is considered as a genetic predisposing factor.

3.1. Case-control studies

Previous case-control studies differed in the design, case ascertainement methods and the selection of the controls. No risk factor was shown to be constant in each study. The history of blood transfusions was assessed in some studies (Kondo 1982, Davanipour 1985, Harries-Jones 1988, Esmonde 1993), no significant risk could be established. Previous studies were limited by small size of investigated collectives. The only significant finding in a recent multicentric European case control study was the family history of dementia and no significantly increased risk of CJD related to past medical history including surgery and blood transfusions was shown (van Duijn 1998). However, the use of hospital control subjects in this study may have led to the higher exposure frequencies for medical disorders, surgery and blood transfusions in controls. No association with blood transfusions was found in a British case-control study (Esmonde 1993). The frequency of blood transfusion history was similar in cases and in age, sex and hospital matched controls (14% vs. 19%). There was no evidence of an increased incidence of CJD in localities where multiple donors resided at the time of blood donation. The clinical picture in 16 CJD patients with a history of a blood transfusion was consistent with sporadic CJD. It was distinct from those in recipients of human cadaver derived human growth hormone who present clinically with progressive cerebellar syndrome. An odd ratio of 0.6 for previous blood transfusions was found in a study by Davanipour, using hospital controls (Davanipour 1985). In the study of Kondo, only one case and three controls had a history of blood transfusion five years before onset of the disease (Kondo 1982).

3.2. Case reports

In a follow-up of one CJD patient who was a frequent blood donor, none of the blood recipients developed CJD. Eighteen blood recipients have died of a non-neurological

disorder, nine were alive at the time of the investigation (Heye 1994). The time periods for follow-up ranged from 1 to 22 years after blood transfusion. A look back study was performed by the CDC and the American Red Cross in 178 recipients of blood units derived from donors who developed CJD. Nine of these recipients lived between 13 and 24 years after transfusion and 41 more than 5 years. No CJD was revealed in any of these recipients (Sullivan 1997, Evatt 1998). However, only single cases (one in the study of Heye and two in the study of Sullivan) with a time period of more than 20 years after blood transfusion were followed.

"Transfusion-associated" CJD has been claimed for four patients with a medical history of blood transfusions in the past five years prior to the onset of the illness (Klein 1993). The clinical presentation of these patients with prominent cerebellar ataxia was consistent with a peripheral inoculation route (see above). On the other hand, more than 1/3 of the sporadic CJD patients present clinically with cerebellar syndrome as was shown in large studies (Brown 1994). Recent correspondence with the senior author (L.Dumble) clarified that the donors have not been identified and that, therefore, their status with respect to CJD is not known. This report adds no new information to what is known from the case-control studies discussed above. It does not present pairs of donors and recipients who both have developed CJD.

In a further case report, a CJD patient had a history of a liver transplant two years prior to the illness (Créange 1995). The liver donor has died from a non-neurological condition, a kidney recipient form the same donor remains healthy at the time of publication. However, one of the donors whose plasma was used in the preparation of albumin that was transfused to the liver recipient has died from CJD three years after the donation. A causal link between these two cases is intriguing, but difficult to be accepted mainly because of the discrepancy between the presumed short incubation time for CJD in the liver patient and the low amount of infectivity in the inoculum as to be judged by the lack of CJD cases in other recipients of the same batch of albumin.

3.3. Special population studies

If CJD can be transmitted by blood products special populations which depend on continuous application of blood products (like hemophiliacs who need coagulation factors and patients with thalassaemia or sickle cell disease who need blood transfusions) should be at higher risk for CJD. No CJD in such groups of patients has been reported or found so far (Operskalski 1995, Holman 1996). The Centers for Disease Control (CDC) in the USA have examined tissues of the central nervous system from 30 patients with severe hemophilia who died with CNS symptoms since 1983. These patients did receive clotting factor concentrates for 15 to 23 years (and other blood products even longer). In this group of patients no evidence for CJD was found (Evatt 1998). Furthermore, in a cohort of 101 hemophilia A patients, 76 of which lived 11 to 17 years after receiving more than 100 units of cryprecipitate (a non-purified factor VIII preparation) no development of CJD was observed (Evatt 1998).

However, the average lifespan in these patient groups may be too short for the development of CJD due to intercurrent causes of death although hemophiliacs not infected by HIV may have a close to normal life expectancy.

Case reports and studies of special populations did not reveal any case of CJD transmission by blood or blood products. This lack of evidence does not necessarily prove a lack of transmission, but it should be realized that the transmissions by cornea transplants, dura mater transplants and cadaveric pituitary hormones have been alerted to by case reports (references).

4 - EXPERIMENTAL STUDIES ON TSE INFECTIVITY IN BLOOD AND IN ITS COMPONENTS

The studies published in the literature are usually of the following type. Tissues are collected from naturally or artificially infected "donor animals". In many instances the time course of the disease is studied by sacrificing groups of donor animals at certain points in time after infection. After some preparatory steps (homogenization, enrichment etc.) the material is injected into "indicator animals", usually intracerebrally, sometimes also by additional routes (intraperitoneal, intravenous, subcutaneous). The indicator animals are checked for clinical signs of TSE. A histopathological investigation was not or could not be performed in all instances. The effects of reiteration of inoculations have not yet been investigated except recently (Diringer 1998) when it was demonstrated that suboptimal doses of 263K scrapie agent induce infection and disease in hamster after repeated oral exposure.

In most experiments, the amount of infectivity is estimated on the basis of the incubation period (time between infection and onset of clinical symptoms or death) in the indicator animals. The correlation between infectivity titer and incubation time is described by a biphasic curve (e.g. Dickinson et al. 1969) which is steep at high titers (a difference in incubation time by a few days indicates a profound change in titer) and shallow at low titers (a difference by many days indicates a subtle change in titer). Therefore, graphs in which incubation time in indicator animals is plotted against the time course in infected donor animals (some of such graphs are presented in this report) give an impression which overestimates low titers.

An additional indication for the amount of infectivity is the percentage of animals developing the disease in a group of animals treated with the same amounts of the same material. However, classical titrations where the material is given at different dilutions to sufficiently sized groups of indicator animals and where calculations of the titer can be performed with accepted statistical methods are rarely performed.

As already mentioned, a major problem is presented by the species barrier. Therefore, experiments in which donor and indicator animals are of the same species should be more sensitive than experiments in which donor and indicator animals are of different species. In the discussion below, experiments will be grouped according to this criterion.

Another difficulty may arise from the way of inoculation. In instances, e.g. scrapie, where the natural route of infection is not known, it cannot be mimicked in experiments. However, also in instances where the oral route is the most probable route of natural infection, this route is not been used in experiments aimed to estimate the titers of infectivity in different tissues (with the exception of the BSE study (Wells 1996, Wells 1998)). Instead, the most efficient routes (intracerebral or intraperitoneal) are

usually used for the infection of donor animals. It is at least questionable whether, in those experiments, the time course of infectivity in different tissues reflects the distribution in naturally infected animals. It is easily conceivable that during inoculation blood vessels may be opened allowing the inoculum to enter the blood stream. The detection in blood of infectivity shortly after inoculation (0.5 to 18 hrs. post inoculation, Field 1968) may be explained by such a mechanism. In the discussion below, an additional grouping is performed as to whether the donor animals are naturally or artificially infected.

Inoculations are usually performed with high amounts of infectivity (most often brain homogenates). Under such conditions, small amounts of infectivity in blood may be explained by the presence of the inoculum during clearance of the site of inoculation, may be after phagocytosis by monocytes (Casaccia 1989). This explanation may not only hold true for time points shortly after inoculation but also for later time points as it was recently shown that an inoculum may persist for a very long time period (over two years in mice, Race 1998). Quantitative calculations which prove or disprove this possibility have not been performed and cannot be performed on the basis of published data.

In evaluating published data one has to have in mind that, predominantly in early studies, the extreme tenacity of TSE agents was not sufficiently taken into account and that, therefore, contaminations were not rigorously excluded. Contamination is a possible explanation if, in a given study, the titer of a certain tissue which can be estimated from incubation time in and percentage of infected indicator animals is far outside the range deduced from the whole of the studies for the same tissue, and if such a study cannot be repeated. With this argumentation, the isolation of the scrapie agent from the serum of a naturally infected ram (Gibbs 1965) could be regarded as contamination as the infectivity titer in serum, in the same study, seems to be close to the titer in brain, and as this study could not be repeated (Hadlow 1982).

4.1. Studies of blood infectivity in naturally infected animals without crossing species barriers

As the pathogenesis of sporadic and familial CJD is not known, it is difficult to decide what type of animal model would best reflect the situation in man. In a first approximation, studies with naturally infected animals may be preferred although there are some indications that those infections occur by the oral route (e.g. TME, BSE, possibly scrapie in sheep and goat). It may be difficult to imagine that sporadic CJD is also caused by an oral infection.

The most sensitive way to study infectivity in tissues including blood would be the use of indicator animals of the same species. Unfortunately, such studies neither have been published nor are known to the group drafting this report.

4.2. Studies of blood infectivity in naturally infected animals crossing species barriers (Table 1)

There are only two studies looking for TSE infectivity in blood of naturally infected animals. No infectivity could be found in serum or in the blood clot of scrapie infected

goats and sheep (Hadlow et al. 1980, Hadlow et al. 1982). There is an additional anecdotal report on the isolation of the scrapie agent from the serum of a naturally infected ram (Gibbs 1965). As outlined above, there is reason to believe that this isolation may be due to a contamination.

A similar experimental situation is given when human blood or blood components are tested for CJD infectivity. Such experiments are discussed in a separate section.

4.3. Studies of blood infectivity in artificially infected animals crossing species barrier (Table 2)

Only two studies of this type, with diminished sensitivity and with limited relevance for the human situation, has been published. No infectivity could be found in blood clots from scrapie infected goats during a time course study (Hadlow et al. 1974). Also no infectivity has been detected in buffy coat of cattle infected orally with the BSE agent up to 18 months post inoculation (Wells 1996, Wells 1998). This study is not yet completed. In both studies, mice were used as indicator animals.

4.4. Studies of blood infectivity in artificially infected animals without crossing species barriers (Table 3)

The majority of studies belongs to this category. It is characterized by a relatively high sensitivity on one hand and diminished relevance for the human situation on the other hand.

Two studies (Pattison et al. 1962, Pattison et al. 1964) with goats as donor and indicator animals could not demonstrate any infectivity in blood (fig. 1).
Studies in minks, inoculated with the TME agent, did not reveal any infectivity in serum (Marsh 1969) or any other component of blood (Marsh 1973). It may be of special interest that, during a time course study, purified and concentrated lymphocytes from peripheral blood did not transmit the disease while spleen and mesenteric lymph nodes were clearly positive. In another time course study in mink, inoculated with the Idaho strain of TME, only at one occasion, 28 weeks after inoculation and four weeks before the onset of clinical symptoms a low level of infectivity was detected in serum, but not in bone marrow (Hadlow 1987). Lymph nodes and spleen were clearly positive. The cellular components of peripheral blood (e.g. buffy coat) were not tested in this study.

The infectivity of blood of scrapie infected hamsters was investigated by two groups (Diringer 1984, Casaccia 1989) by very similar approaches using identical methods to concentrate the scrapie agent from whole blood. Both groups find infectivity in their preparations. However, there is no congruence in the time course (fig. 2, fig. 3). Casaccias findings indicate that infectivity is highest shortly after inoculation and that it is steadily decreasing afterwards (fig. 3). This picture could be explained by the steady clearance of the inoculum, administered intraperitoneally, during an extended period of time. That the inoculum can persist for a long time at least in mice, has been recently demonstrated (Race 1998).
When time course studies were performed in scrapie infected mice, during later stages of the incubation period sometimes a small amount of infectivity could be detected in

the blood (Dickinson et al. 1969), sometimes not (Eklund et al. 1967). This may depend on the mouse strain used (Dickinson et al. 1969). Also Clarke (1967) detected infectivity in the blood of diseased mice, but he argued that the amount of infectivity he found may depend on whether the blood samples were contaminated with other bodily cells or not. Field's (1968) finding of infectivity in blood shortly after inoculation was discussed earlier in the text.

After CJD having been transmitted to small laboratory animals (Manuelidis 1975) such model systems were also used in a search for infectivity in blood. The result obtained with guinea pigs inoculated with the CJD agent adapted to this species is puzzling: infectivity appears and disappears irregularly during the incubation period (Manuelidis 1978a, see fig. 4). A consistent picture is obtained with mice inoculated with the agent from an "atypical case" of CJD (Tateishi 1980, Kuroda 1983) which is nowadays known to be a case of Gerstmann-Sträussler-Scheinker syndrome. In this model a low level of infectivity, after a lag period, but afterwards increasing with incubation time, is found in buffy coats (fig. 5). In a closely related system, Doi (1991) could not demonstrate infectivity testing whole blood in which leukocytes are diluted approximately 1:100 compared to buffy coat.

4.5. Studies of blood infectivity in CJD patients (Table 4)

In a small number of studies, data is presented which should demonstrate the presence of the CJD agent in the blood of CJD patients (Manuelidis 1985, Tateishi 1985, Tamai 1992, Deslys 1994). These papers are difficult to evaluate as they do not report experimental details (Deslys 1994) or as the presented data is conflicting (Manuelidis 1985, Tateishi 1985, Tamai 1992).

One paper (Tateishi 1985) claims to find CJD infectivity in blood clot of one CJD patient. In addition, an amount of infectivity in urine is reported which is similar to that found in brain in contrast to all other published experimental studies in which urine is not infectious. The same author did not succeed in demonstrating infectivity in the blood of two other CJD patients (Tateishi 1985).
In another paper (Tamai 1992), plasma of a pregnant woman with CJD is found non-infectious, but after a three-fold concentration an amount of infectivity like that in the brain of the same patient is reported. However, it has to be concluded from all other studies that dilution of the brain of a clinical ill patient or animal by a factor of 3 will not prevent the detection of CJD infectivity. While patient's leukocytes are negative, a small amount of infectivity is described for leukocytes in cord blood.

Some doubt has to be shed on the third paper (Manuelidis 1985), as the same group reported not only transmission from the blood of CJD patients, but also from a patient with an unclear neurological disease (Manuelidis 1978b), from patients with Alzheimer's disease (Manuelidis 1988) and from healthy individuals (Manuelidis 1988, Manuelidis 1993). Those results could not be repeated (Godec 1992, Godec 1994). A possible explanation may be the occurrence of an late-onset wasting disease associated with Clostridium difficile in the hamsters used for the experiments (Rohwer 1992).

These case reports are challenged by a long-lasting series of experiments in which the infectivity of quite a number of tissues (including blood) of Kuru and CJD patients has

been tested not exclusively, but predominantly in monkeys and apes (Asher 1976, Brown 1994). A TSE infectivity could neither be demonstrated in blood nor in serum nor in leukocytes although it has to be assumed that the species barrier between humans and other primates is relatively low. One has to realize that even in this series the number of patients studied is small but exceeds the number of patients studied in all of the aforementioned case reports together. In a remarkable experiment, units of blood of three different CJD patients (exceeding 300 ml) were transfused into three chimpanzees (Gajdusek 1977). None of these animals has developed a TSE after an observation period of more than 20 years (Brown 1994, Brown 1997b).

4.6. Section conclusion

There is no convincing data demonstrating the presence of TSE infectivity in the blood of Kuru patients and patients with sporadic CJD. The few reports claiming such an infectivity contain severe inconsistencies. The number of patients studied is very small, therefore, it is advisable to extent such investigations. The problem of the species barrier inherent to all those studies may be overcome by the use of mice which express the mouse-adapted human version of the prion protein gene (PRNP), the major determinant of the species barrier. In first experiments using this mouse system, infectivity in the blood of two sporadic and one familial CJD case could not be demonstrated (Prusiner, personal communication June 1998). Whether the situation in patients with iatrogenic CJD is different from those with sporadic CJD cannot be concluded from the single one-case study published so far (Deslys 1994). Studies on the infectivity in blood of patients with nvCJD have not yet been performed.

An evaluation of all animal experiments has to take into account, that the results depend on several factors: strain and sometimes breed of the host animal, type of TSE agent, its dose and its route of administration (Asher 1976). Therefore, an extrapolation from animal experiments onto the situation in men may be difficult. A conservative conclusion may be, that in animal models, if at all, a low infectivity in blood may be measurable in late stages of the incubation period and during clinical illness. This is best demonstrable in rodents (Clarke 1967, Dickinson 1969, Tateishi 1980, Kuroda 1983). Such an interpretation is in congruence with very recent data which has been obtained under the condition of limiting dilution (Rohwer, personal communication June 1998). With such an experimental design, infection is achieved with a single infectious unit, so that it is highly improbable that the demonstration of infectivity in the blood of an infected animal merely reflects the re-isolation of the inoculum. Also under such conditions, a small amount of infectivity can be demonstrated in the blood of diseased hamsters (also a rodent). It is estimated from the results that the maximum titer of infectivity of the TSE agent in blood of rodents is in the range of 1 to 10 infectious units (IU) per ml, far below the titers of viruses which undoubtedly can be transmitted by blood (10^5 to 10^{12} IU/ml).

The analysis of animal experiments may shed some light on the widely debated role of peripheral leukocytes in the pathogenesis of TSEs. Recently, in experiments using mice with mutations affecting different functions of the immune system a crucial role of B lymphocyte functions in TSE pathogenesis could be demonstrated (Klein 1997). It is also argued that peripheral lymphocytes may carry infectivity as TSE infectivity is easily detected in lymphatic organs like peripheral lymph nodes and spleen and as there

might be an exchange of lymphocytes between those lymphatic organs and the peripheral blood. However, in animal experiments in which TSE infectivity in blood is demonstrable this infectivity never parallels the infectivity in spleen (Eklund 1967, Kuroda 1983, Casaccia 1989) what has to expected if lymphocytes carrying infectivity are exchanged between lymphoid organs and peripheral blood. A lacking correlation between infectivity in spleen and peripheral blood is also supported by the recently reported observation, that lymphocytes isolated from spleens of infected mice carry TSE infectivity, while lymphocytes isolated from the peripheral blood of the same mice do not (Aguzzi, personal information June 1998). This observation confirms identical results with TME infected minks (Marsh 1973).

5 - IMPLICATIONS FOR BLOOD AND BLOOD PRODUCTS

Although there is as yet no evidence that sporadic, familial or iatrogenic CJD and nvCJD are transmitted by blood transfusion or via plasma derived medicinal products, this does not equate to the absence of risk of such transmission. As a consequence various precautionary measures have been introduced (or may be considered for implementation in the future) to secure the safety of blood and blood products. As with blood borne infectious diseases these safety measures can be directed at donor exclusion, screening of donations for the agent, inactivation or removal of the agent, recall of products when one or more donors subsequent to donation develops CJD (or nvCJD), and exchange of implicated products between countries. Furthermore, limiting the pool size of plasma for fractionation and substitution with alternative products is discussed.

5.1. Donor suitability

5.1.1. Blood and plasma donors

According to "Council Recommendation of 29 June 1998 on the suitability of blood and plasma donors and the screening of donated blood in the European Community" permanent deferral from donation of blood or plasma should be instituted for prospective donors which have, or have a history of :

- TSEs (or history thereof in the genetic family),
- Cornea/dura mater transplantation recipient,
- Pituitary hormone of human origin (e.g. human growth hormone) recipient.

In the "Guide on the preparation, use and quality assurance of blood components"(version 1997) of the Council of Europe it is stated that "all individuals who have in the past been treated with extracts derived from human pituitary glands, have been recipients of dura mater grafts or who have a family history of CJD are debarred from donation". The World Health Organization (WHO) recommends to permanently exclude individuals suffering from classical CJD, GGS, FFI or dementia from blood and plasma donation. Individuals treated with extracts derived from human pituitary glands (growth hormone and gonadotropin) or who have been recipient of dura mater grafts as well as donors with a family history of classical CJD, GSS or FFI should also be excluded because they are considered to be at risk.

The CPMP "Note for Guidance on plasma-derived medicinal products" requests a standard operating procedure describing the mutual information system between the plasma collection and the manufacturing fractionation facility with the aim to inform each other *inter alia* when an donor develops CJD or is found to have a risk factor for CJD.

Similar donor deferral criteria are recommended by the FDA in the US. The latter recommendations distinguish "familial risks" (i.e. persons with two or more genetically related family members - who are not infected by the iatrogenic route) and "possible familial risk" (i.e. persons with only one known genetically related family member with CJD). As a single case in a family is most likely sporadic rather than due to genetic mutation, the risk that CJD is involved is decreased. When laboratory testing (gene sequencing) confirms the risk of TSE, the donor must be permanently deferred from donation.

There is still discussion ongoing (in the USA) about the need to exclude donors which have had dura mater implants, notably when this material is not pooled and when the donor from which the dura mater is derived, was free of clinical symptoms of TSE and the dura mater at autopsy did not reveal CJD.

In France permanent donor deferral includes persons which have received blood components in the past. The main argument to institute this safety measure was to reduce the incidence of alloimmunisation in recipients. Subsequently the potential reduction of the risk of TSE-transmission was mentioned.

In summary, it appears that in the EC and in other countries (notably the USA) the deferral criteria for donors as regards to TSE risk are rather similar although there are some differences concerning previous cornea transplantation and transfusion of blood components in the past.

5.1.2. Tissue and organ donors

There are no official guidelines or recommendations regarding the exclusion of tissue and organ donors with TSE (risk) adopted by the European Community, or prepared by the Council of Europe or the WHO. In general, in member countries of the EC as well as in the USA the same exclusion criteria as described for blood donors are applied for tissue and organ donors. There appears to be agreement that once CJD is diagnosed a tissue or organ donor should be deferred from donation.

In the UK exclusion criteria for potential cornea donors include CJD, recipients of human pituitary-derived growth hormone, unexplained neurological diseases, central nervous system diseases of unknown cause (including multiple sclerosis, motor neurone diseases, Parkinson's disease), active viral diseases, some ocular conditions and haematological malignancies (Allan 1997).

With regard to the use of dura mater the FDA/TSE Advisory Committee concluded recently that transplantation is safe when dura mater is sterilised using incubation in sodium hydroxide during one hour. Although it has been proposed that before transplantation donor tissue should also be tested for proteinase resistant protein, this re-

quirement was opposed because such testing is not feasible as long as the test is not FDA approved.

5.2. Screening tests for CJD/nvCJD

There are currently no (surrogate) tests which could be used to screen blood donations for CJD or nvCJD. In the absence of cases which demonstrate the transmission of CJD and nvCJD by blood, it is not possible to conclude whether or not application of the currently used screening tests for detection of viral diseases transmitted by blood, affect the risk of transmission of CJD and nvCJD in recipients of blood components and plasma derivatives. It has been reported that using monoclonal antibodies various strains of PrP^{Sc} can be detected and distinguished from normal PrP (PrP^{c}) (Korth 1997, Safar 1998). It is still too early to know if these monoclonal antibodies may be used to develop a screening test for blood, organ and tissue donors.
For tissue and organ donors the situation is similar to blood donors except for dura mater material which may be tested for proteinase resistant protein although there is as yet no licensed test available.

5.3. Inactivation/Removal of the agent from blood and plasma

The infectivity of various cellular blood components and plasma derivatives has been studied through ”spiking” human blood with the scrapie agent and by intracerebral, intravenous and intraperitoneal inoculation of a mouse-adapted strain of human CJD using hamsters and mice as assay animals. The data of such studies suggest that when CJD is present (at low concentration) in blood of infected animals, partitioning into various cellular and plasma compartments occurs.

In cellular blood components CJD is present in leukocytes and platelets while in plasma CJD is recovered from cryoprecipitate and fractions I,II and III of the Cohn fractionation method, but not in fractions IV and V (albumin, Dormont 1996, Brown 1998). Various studies are currently performed to find out if the infectivity in blood and plasma can be reduced or eliminated.

5.4. Leukodepletion of blood and blood cells

The argument that leukodepletion, a process which removes the vast majority of leukocytes from blood and blood components, may decrease CJD-infectivity of blood is derived from two different types of observations. Firstly, Klein (1997) has demonstrated that mice lacking mature B-lymphocytes do not develop clinical symptoms of scrapie when inoculated with infectious material outside the brain (i.e. intravenously and intraperitoneal). Secondly, PrP^{Sc} has been detected in tonsils and appendices of patients with nvCJD (Hill 1997, Hilton 1998). As infectivity could be present in circulating lymphocytes leukodepletion would be a practical way to reduce the risk of nvCJD although in view of a number of uncertainties the impact of leukodepletion is difficult to assess.

In July 1998 the UK-Government has decided that, following recommendations by the Spongiform Encephalopathy Advisory Committee (SEAC), the use of leukodepletion for all blood for transfusion should be extended as soon as practically possible. Earlier (in September 1997) routine leukodepletion of blood for transfusion was already insti-

tuted in France to prevent CMV transmission and alloimmunisation through transfusion of donor blood. Subsequently other countries like Portugal, Ireland and Luxembourg have introduced leukodepletion of all donor units.

There are however a number of uncertainties regarding the effectiveness of this measure to reduce the potential risk of transmission of nvCJD. Firstly, the necessary degree of leukocyte reduction cannot be ascertained as yet since the infective titre of nvCJD in blood, if it is present at all, is not known. In addition, the subclass of white blood cells which may carry the nvCJD agent has not been determined. The standard for leukodepleted blood commonly adopted by the member states of the Council of Europe is less than one million leukocytes in 90% of units. In practice, reduction of the total leukocyte number to below 5 million in all units appears possible. This can be achieved through filtration and apheresis. Filtration is commonly applied to freshly processed red cell and platelet concentrates as well as plasma for transfusion, and occasionally also to whole blood. During apheresis, which is routinely used to collect platelets and plasma, platelets and leukocytes are separated by density or through in-line filtration.

Secondly, side effects of leukodepleted blood units, although infrequent, include allergic reactions and rarely hypotensive reactions in patients using angiotensin converting enzyme inhibitors. Recently in the USA some patients receiving filtered red cell concentrates developed the so-called ”red eye syndrome”; the origin of this side-effect, which was only transient, is unclear. It should be noted that until now filtered blood and blood components have been used predominantly in selected patient groups. It is therefore possible that upon introduction of routine leukodepletion more and other side effects appear. Bedside filtration of blood units does not provide well controlled leukocyte depleted products.

Thirdly, cell-associated PrP^{Sc}, which appears to be resistant to removal from cells by proteases, is likely to remain stable during filtration although this has not been demonstrated yet. Fragmentation of cells during storage and filtration is possible and may affect infectivity of filtered blood. In addition, the distribution of infectivity between cellular components and plasma has not yet firmly established leaving open the possibility that a significant proportion of infectivity resides in plasma. Finally, data on the removal of subsets of leukocytes (i.e. B-lymphocytes, T-lymphocytes, dendritic cells) are still limited. It may therefore be concluded that additional studies are required to ascertain the need and safety of leukodepletion to reduce the possibility of CJD-transmissions.

Among the benefits of leukodepletion should be mentioned the prevention of transfusion-associated CMV and alloimmunisation in recipients of blood transfusions. Whether or not prevention of cancer recurrence and post-operative infections occurs by the use of leukodepleted blood is still controversial, but further studies are scheduled to elucidate these potential benefits.

Inactivation/Removal of infectivity from plasma-derivatives

The manufacturing of plasma products includes a variety of partitioning, precipitation and virus inactivation steps. It is still unknown if and to what extent each of these steps is able to reduce CJD-infectivity of plasma and plasma products. Preliminary results

indicate that some partitioning, (nano)filtration and purification (chromatography) techniques are likely to reduce infectivity, but this needs still confirmation (Tateishi 1985, Dormont 1996, Brown 1998).

5.5. Recall and blockade of plasma products

"Late notification" refers to the situation when a donor is diagnosed with CJD, and a review of previous donations identified that plasma had already entered a plasma pool which was used for the fractionation. In the USA, until recently manufacturers were required to quarantine implicated pools and to recall product. During the last years this has led to a number of recalls of plasma derivatives such as factor VIII-concentrate, albumin, and immunoglobulins. In September 1998 the FDA published its decision to change this policy which is now similar to that of the CPMP (see below).

In Europe a different approach with respect to "late notifications" has emerged. The CPMP concluded in 1995 that in the absence of evidence of any risk associated with plasma products quarantine of implicated pools and recall of products was scientifically not justified and would be likely to lead to shortage of essential products. The solution proposed by the FDA to avoid shortage, i.e. recall + quarantine + recirculation if needed, was considered practically not feasible. With regard to products recalled by the FDA, the CPMP urges the manufacturers concerned to immediately inform the competent authorities in the EU and provide a complete list of implicated product batches (CPMP report 22/11/1995, CPMP 846/95).

Following reports of nvCJD in the UK and evidence that it represents the human form of Bovine Spongiform Encephalopathy (BSE), in February 1998 the CPMP issued new recommendations which state that:
"Given the lack of specific information on nvCJD, as a precautionary measure it would be prudent to withdraw batches of plasma-derived medicinal products from the market if a donor to a plasma pool is subsequently strongly suspected, by de reference centre, of having nvCJD.
Since a recall involving albumin used as excipient has the potential to cause major and widespread supply difficulties for essential products, manufacturers should avoid using, as an excipient , albumin derived from countries where a number of nvCJD cases have occurred."

At the same time in the UK the Secretary of State, following recommendations of the UK Haemophilia Directors to provide funding for recombinant factor VIII and advice by the Committee for the Safety of Medicines (CSM), decided that recombinant factor VIII would be made available for treatment of all newly diagnosed haemophilia A patients and for those under the age of 16 years. It was also decided that CSM should undertake a product by product review of the theoretical risk of transmission and the supply position. This review has not yet been published. Furthermore, anticipating the outcome of the review, the fractionation centres in Elstree and Edinburgh have been permitted to import plasma for fractionation.

5.6. Product exchange

The WHO states that batches of plasma derivatives withdrawn from use in one country should not be exported to another country (WHO, 1997).

5.7. Pool size of plasma for fractionation

The pool size of plasma for fractionation varies from 1,000 to 10,000 units when source (apheresis) plasma is used; for recovered plasma the pool size is also quite variable (30,000 to 60,000 donor units). The question has been raised if reduction of the pool size may decrease the risk of CJD transmission.

The probability that pooled donor plasma will contain a donation from an individual with a disease has been analyzed for a range of disorders and different pool sizes (Lynch 1996). Using this analysis, the probability that a CJD patient contributes to a pool of 10,000 donors is 0.8%; if the pool size increases to 100,000 donors, the probability increases to 7.6%. However it is unlikely that a person with symptoms of CJD will donate blood. Using mathematical modelling Brown (1998a) concludes that the chance of contracting CJD from a pooled blood product to which a patient with CJD has contributed should be extremely small, no matter what the size of the donor pool is. Limitation of the pool size is not likely to reduce such a risk because the infectivity does not saturate the pool.

5.8. Substitution with autologous transfusion and alternative plasma products

Using autologous blood transfusion (either predeposit or peroperatively), notably during elective surgical procedures, the exposure to allogenic donor blood may be reduced. In addition, the injection of haematopoetic growth factors like thrombopoietin and erythropoietin may limit the usage of platelet concentrates respectively red cell concentrates in various circumstances such as during haemodialysis and during chemotherapy.

Use of recombinant plasma products, when available, could be considered an alternative for a number of patients with clotting factor deficiencies such as haemophilia A and B. However, it should be kept in mind that several of the recombinant products contain albumin as a stabiliser.

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7 - ACKNOWLEDGEMENTS

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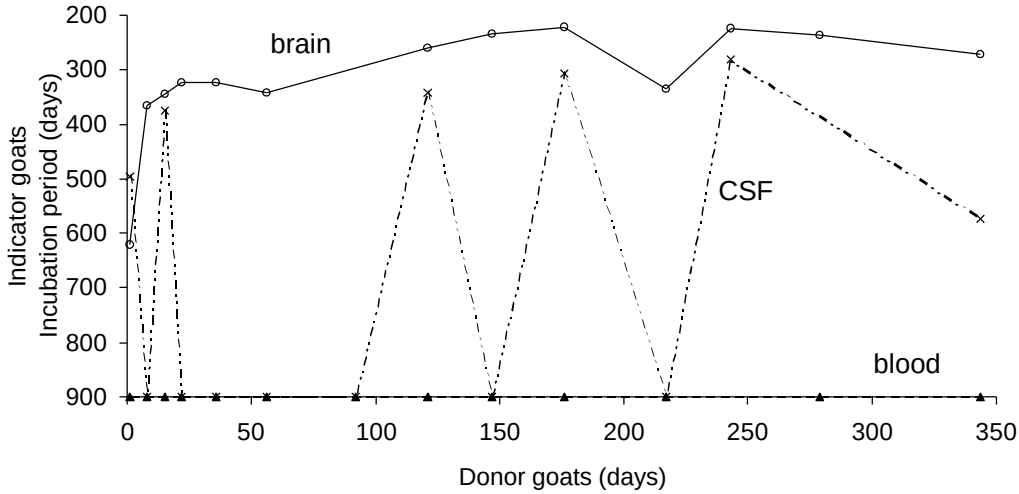
GLOSSARY

AIDS: Acquired Immune Deficiency Syndrome
BSE: Bovine Spongiform Encephalopathy
CJD: Creutzfeldt-Jakob Disease
CMV: Cytomegalovirus
CNS: Central Nervous System
CPMP: Committee for Proprietary Medicinal Products
CSM: Committee for the Safety of Medicines (UK)
EC: European Community
EEC: Electroencephalogram
EMEA: European Agency for the Evaluation of Medicinal Products
FDA: Food and Drug Administration (USA)
FFI: Fatal Familial Insomnia
GSS: Gerstmann-Sträussler-Scheinker Syndrome
HBV: Hepatitis B Virus
HCV: Hepatitis C Virus
hGH-related CJD: Human Growth Hormone- related CJD
HIV: Human Immunodeficiency Virus
HLA: Human Leukocyte Antigens
HTLV: Human T-Cell Leukemia Virus
hTSE: Human Transmissible Spongiform Encephalopathy
IU: Infectious Unit
nvCJD: new variant of the Creutzfeldt-Jakob Disease
PRNP gene: Human Prion Protein Gene
PrP: Prion Protein
PSWCs: Periodic Sharp Wave Complexes
SCMPMD: Scientific Committee on Medicinal Products and Medical Devices
SEAC: Spongiform Encephalopathy Advisory Committee (United Kingdom)
TME: Transmissible Mink Encephalopathy
TSE Transmissible Spongiform Encephalopathy
WHO: World Health Organization

Fig. 1: Tissue infectivity in scrapie goats



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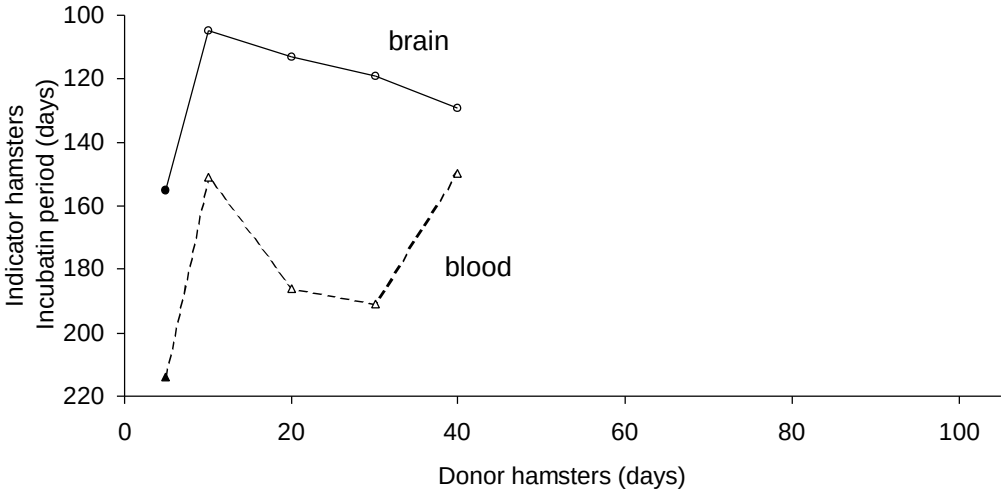
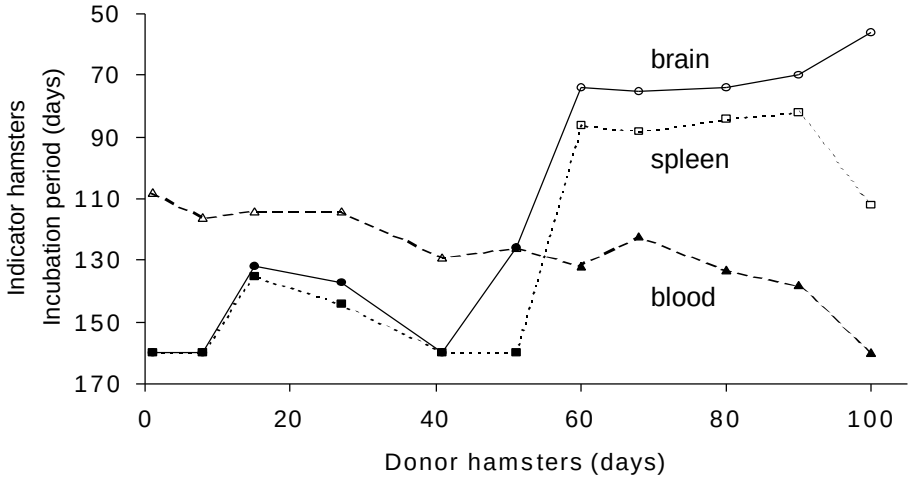
Donor goats: i.c.
Indicator goats: i.c. 1 ml whole blood

Fig. 2: Infectivity of P_{215S} in scrapie hamsters



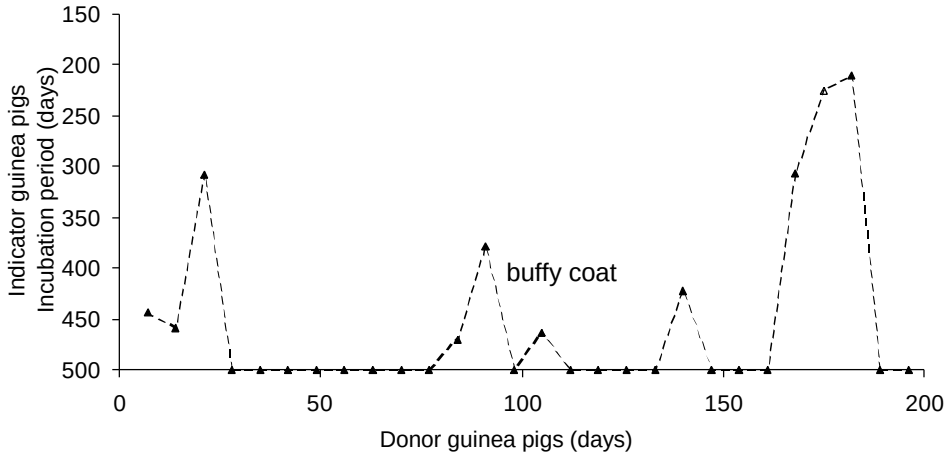
Diringer, H. Sustained viremia in experimental hamster scrapie. Arch.Virol. 82,105-109,1984
Donor hamsters: i.p.
Indicator hamsters: i.c. P_{215S} (equivalent to 2 ml blood)

Fig. 3: Infectivity of P_{215S} in scrapie hamsters



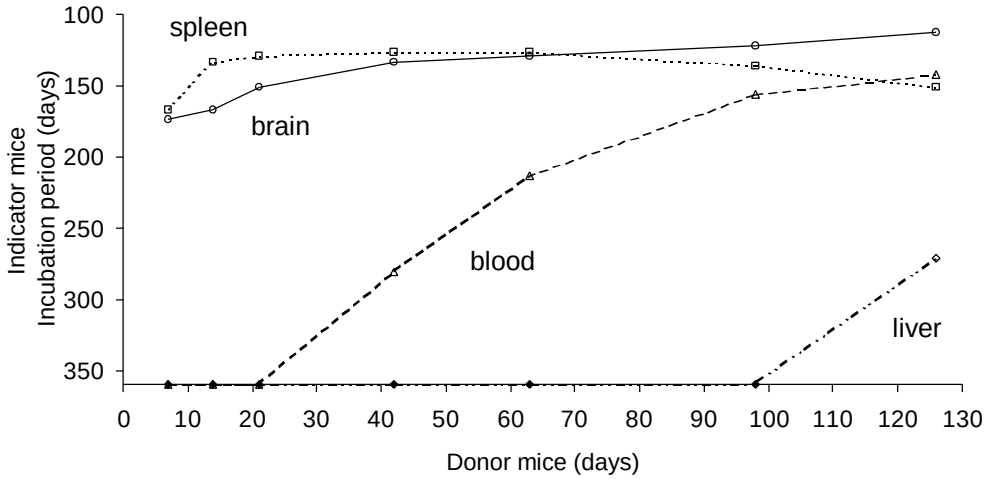
Casaccia, P. et al. Levels of infectivity in the blood throughout the incubation period of hamsters peripherally injected with scrapie. Arch.Virol. 108, 145-149, 1989
Donor hamsters: i.p.
Indicator hamsters: i.c. P_{215S} (equivalent to 0.2 ml blood)

Fig. 4. Infectivity of buffy coat in CJD Guinea pigs



Manuelidis, E.E., Gorgacz, E.J., Manuelidis, L. Viremia in experimental Creutzfeldt-Jakob disease. Science 200, 1069-1071, 1978
Donor guinea pigs: i.c
Indicator guinea pigs: 0.1 ml i.c.+s.c.+i.m.+i.p. buffy coat

Fig. 5: Tissue infectivity in GSS mice



Kuroda, Y., Gibbs. C.J. Jr., Amyx, H.L., Gajdusek, D.C. Creutzfeldt-Jakob disease in mice: persistent viremia and preferential replication of virus in low-density lymphocytes. Infect.Imm. 41, 154-161, 1983
Donor mice: i.c.
Indicator mice: i.c. buffy coat, i.p. serum and erythrocytes

Table 1: Studies with naturally infected animals crossing the species barrier

author	TSE strain, route of administration	donor species	tissue	indicator amount of material, route of administration	animal species	remarks
Gibbs, C.J.Jr. (Gajdusek, D.C.), 1965	scrapie (natural infection)	sheep (ram)	serum		Swiss mice (NIH general purpose stock)	from transmission data: infectivity similar to that in brain not reproducible (Hadlow et al. 1982)
Hadlow, W.J. (1980)	Goats (living with scrapie infected Suffolk sheep) with natural scrapie (clinically affected)	3 goats (Nubian x Toggenburg breeding)	serum and blood clot	30 µl i.c. (tenfold dilutions, 10 mice per dilution)	Swiss mice	no infectivity in serum and blood clot
Hadlow, W.J. (1982)	Sheep naturally exposed to scrapie (young sheep, infected, but not clinically ill as well as diseased sheep)	Suffolk sheep	blood clot, serum	30 µl i.c. (undiluted and tenfold dilutions, 10 mice per dilution)	Swiss mice	no infectivity in serum and blood clot

Table 2: Studies with artificially infected animals crossing the species barrier

author	TSE strain, route of administration	donor species	tissue	indicator amount of material, route of administration	animal species	remarks
Hadlow, W.J. (1974)	scrapie Chandler strain (derived from Cheviot sheep) i.c.:$10^{7.3}$ mouse i.c.LD_{50} s.c.:$10^{7.7}$ mouse i.c.LD_{50} i.c.:$10^{6.6}$ mouse i.c. LD_{50}	goats (Saanen breeding)	10% blood clot (whole blood?)	30 µl i.c.	Swiss mice	exps. include time course studies, no infectivity found in blood clots

author	TSE strain, route of administration	donor species	tissue	indicator amount of material, route of administration	animal species	remarks
Wells, G.A.H. (1996) Wells, G.A.H. (1998)	BSE (homogenate of brain stems of 75 cases of BSE) 100 g single oral dose	Friesan/ Holstein male calves	10% buffy coat	20 µl i.c. and 100 µl i.p.	RIII mice or C57Bl-J6 mice	time course study, no infectivity in buffy coat up to 22 months post inoculation, study not yet completed

Table 3: Studies with artificially infected animals not crossing species barriers

author	TSE strain, route of administration	donor species	tissue	indicator amount of material, route of administration	animal species	remarks
Pattison, I.H. (1962)	goat adapted scrapie (passaged 3x in goats) (1 ml sup. (1500 rpm, 15 min) 10% brain susp.) i.c.	goat	whole blood	1 ml i.c.	goat	time course study, no transmission with blood (see fig. 1)
Pattison I.H. (1964)	scrapie affected goats (no further details, as in Pattison 1962?)	cross bred goat	1. whole blood 2. blood cells (mainly red blood cells) 3. serum (each pools of two animals)	1a. 1 ml (0.5 ml?) i.c. 1b. 3 x 5 ml s.c. (weekly intervals) 2. 1 ml (0.5 ml?) i.c. 3. 1 ml (0.5 ml?) i.c.	cross bred goat	no transmission
Marsh, R.F. (1969)	TME 1. 1 ml 10% brain of naturally infected mink (Hayward, Wis.) i.m. 2. 0.1 ml 10% brain (2nd passage) i.c.	mink (Genetics - Dep., Univ. of Wisconsin)	serum	1 ml s.c. (diluted in tenfold steps)	mink	no infectivity in serum, but infectivity in a series of tissues including spleen

author	TSE strain, route of administration	donor species	tissue	indicator amount of material, route of administration	animal species	remarks
Marsh, R.F. (1973)	TME 1. late clinical stage 2. 10^5 LD_{50} i.c.	mink	1. whole blood, plasma, 10% red blood cells, 10% platelets, white blood cells ($1.7x10^7$/ml), cultured lymphocytes ($1.5x10^7$/ml), PHA stimulated lymphocytes ($1.5x10^7$/ml) 2. lymphocytes ($2x10^7$/ml)	0.1 ml i.c.	mink	1. no infectivity in listed preparations 2. time course study, no infectivity in lymphocytes, but in spleen and peripheral lymph nodes
Hadlow, W.J. (1987)	TME agent Idaho strain, second mink passage, 10^3 LD_{50} s.c.	royal pastel mink	undiluted serum	100 µl i.c. (2 animals)	royal pastel mink	time course study, serum only at one occasion (28 wks. after inoculation) positive in 1/2 animals (during secondary spread from brain?)

author	TSE strain, route of administration	donor species	tissue	indicator amount of material, route of administration	animal species	remarks
Clarke, M.C. (1967)	Chandler strain (mice) Chandler and Fisher strain (rats)	weaned white mice (B.S.V.S.) Wistar rats	serum from blood pools of animals with advanced clinical signs 1. blood from chest cavity after severing carotid and brachial vessels 2. blood from heart puncture	50 µl i.c. (mice and rats)	weaned white mice (B.S.V.S.) Wistar rats	more transmissions in exp. 1 than in exp. 2 (contamination with tissue?)
Eklund, C.M. (Hadlow, J.) 1967	scrapie Chandler strain (fourth mouse passage), $10^{5.7}$ LD_{50} s.c. (50 µl, 1% mouse brain susp.)	Swiss mice	blood clot serum (pools of three mice)	30 µl i.c. (10-fold dilutions, six mice per dilution)	Swiss mice	time course study, no transmission with blood
Field, E.J. (1968)	scrapie (Chandler) (50µl 10% brain suspension) 1. i.c. 2. i.p.	mice	blood collected from chest cavity 1. serum 2. whole blood	1. 50µl i.c. 2. 50µl i.c.	mice	time course for the first 18 hours p.i., procedure 2 more successful (association of the agent with cells?)
Dickinson, A.G. et al. (1969)	ME7 scrapie (fourth passage in mice, last two in C57BL) 20 µl s.c. and 10 µl i.p. (1% brain susp. after 50 g, 5 min)	C57BL (s7) or SM (s7) or LM (s7) or VM (p7) (s7, p7: sinc alleles)	blood (cardiac puncture)	20 µl i.c. (10^{-1} saline susp. centrifuged 300g for 10 min.)	C57BL weanling mice (11 to 15 mice per sample)	time-course study, blood occasionally positive (3/12 time points, 1 or 2 of 11 to 15 mice)

author	TSE strain, route of administration	donor species	tissue	indicator amount of material, route of administration	animal species	remarks
Diringer, H. (1984)	scrapie 263K (100µl 1% brain homogenate) i.p.	CLAC hamsters	blood concentrate P_{215S} (1:1 diluted, sonicated in 1% sarkosyl, centrifuged 20min 22000g, supernatant pelleted 120min 215000g)	50µl (equivalent to 2ml blood) i.c.	hamsters	time-course study (see fig. 2)
Casaccia, P. et al. (Pocchiari, M.), 1989	scrapie 263K (50µl 10% brain homogenate) i.p.	golden Syrian hamster	blood concentrate (1:1 diluted, sonicated in 1% sarkosyl, centrifuged 20min 22000g, supernatant pelleted 120min 215000g)	50µl (equivalent to 0.2ml blood) i.c.	weanling hamsters	time-course study (infectivity phagocytized by monocytes, cellular turnover) see fig. 3
Manuelidis, E.E. (1978)	CJD (54yr female) 5x serially transmitted in guinea pigs (100µl 1% brain homogenate), i.c.	guinea pigs (Hartley strain)	buffy coat from 8ml blood (centrifuged, frozen, cut 0.5cm at each side)	altogether 0.4ml (i.c. and s.c. and i.m. and i.p., 0.1ml each) (equivalent to 6.4ml blood)	guinea pigs	time-course study (see fig. 4)

author	TSE strain, route of administration	donor species	tissue	indicator amount of material, route of administration	animal species	remarks
Tateishi, J. et al. (1980)	CJD, serially transmitted in mice (same strain as Kuroda 1983? No details) (10µl 15% brain homogenate) i.c.	mice (different strains?)	whole blood	10µl i.c.	mice (different strains?)	2/10 indicator mice positive (material from diseased animals?)
Kuroda, Y. et al. (Gajdusek, D.C.), 1983	Fu strain of "CJD" (atypical case = GSS) 2x serially transmitted in mice (30µl sup. 10% brain homogenate), i.c.	weanling BALB/c mice	buffy coat (blood centrifuged, frozen, cut 0.5cm at each side) serum erythrocytes	30µl buffy coat, i.c. 100µl serum, i.p. (?) 100µl erythrocytes, i.p. (?)	weanling BALB/c mice	time-course study (see fig. 5)
Doi, T. (1991)	"CJD Fukuoka 1" strain (same as Kuroda 1983), 5x serially transmitted $10^{5.5}$ LD_{50} i.c. (20 µl)	ddY mice	whole blood (cardiac puncture)	20 µl whole blood (undiluted? 20% homogenate?) i.c.	ddY mice (720 days)	no infectivity in blood

Table 4: Studies with human blood crossing the species barrier

author	TSE strain, route of administration	donor species	tissue	indicator amount of material, route of administration	animal species	remarks
Asher, D.M. et al. (Gajdusek, D.C.), 1976	Kuru, CJD natural infection	human	serum/blood (pools of three or four patients)	?	animals	no transmission after at least three years 0/10 kuru 0/3 CJD
Gajdusek, D.C. (1977)	Kuru natural infection	human	blood	i.c.?	monkeys	no transmissions, no experimental details

author	TSE strain, route of administration	donor species	tissue	indicator amount of material, route of administration	animal species	remarks
Asher, D.M. et al. (Gajdusek D.C.), 1985	CJD (brain infectious in animals)	human	1. 450ml whole blood 2. 0.2ml blood 3. separated lymphocytes	1. i.v. (3 animals) 2. i.c. (2 animals) 3. i.c. (1 animal)	animals	no transmissions, no further experimental details
Brown, P. (Gajdusek, C.D.), 1994	Spongiform encephalopathy (natural infection, positive transmission with brain tissues)	human	1. whole blood (including units of blood) 2. leukocytes 3. serum	inoculation	primates (mostly squirrel monkeys)	no transmission 1: 0/7 2: 0/3 3: 0/2
Manuelidis, E.E. et al. (1985)	2 CJD patients (second case only with "subtle microscopic changes)		buffy coat (centrifuged, frozen, cut 0.5cm at each side, 50% crude homogenate)	i.c.	1. 4 guinea pigs 2. 5 hamster, 5 guinea pigs	ad 1: 1 guinea pig positive, brain transmitted to 1 of 6 guinea pigs ad 2: hamsters positive, guinea pigs negative
Tateishi, J. (1985)	CJD patient	human	1. blood clot 2. cornea (10% homogenate) 3. urine 4. brain (10% homogenate)	20µl i.c.	CF1 mice	ad 1: 2/13 ad 2: 1/6 ad 3: 5/10 ad 4: 7/10 (blood of two other CJD patients: negative)

author	TSE strain, route of administration	donor species	tissue	indicator amount of material, route of administration	animal species	remarks
Tamai, Y. (1992)	1 pregnant CJD patient (brain infectious in animals)	human	1. patient's erythrocytes 2. patient's leukocytes 3. patient's plasma (3x concentrated) 4. cord erythrocytes 5. cord leukocytes 6. cord plasma	i.c. (no volume given)	BALB/c mice	ad 3: 3/8 positive ad 5: 1/10 positive all others negative , also non-concentrated plasma
Deslys, J.-P. et al. (1994)	iatrogenic CJD (hGH)	human	buffy coat	no information	hamster	no experimental details