ADVANCING PRION SCIENCE: Guidance for the National Prion Research Program

Rick Erdtmann
Laura B. Sivitz,
Editors

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PRION SCIENCE

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Committee on Transmissible Spongiform Encephalopathies:
Assessment of Relevant Science

Rick Erdtmann and Laura B. Sivitz, *Editors*

Medical Follow-up Agency

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The serpent has been a symbol of long life, healing, and knowledge among almost all cultures and religions since the beginning of recorded history. The serpent adopted as a logotype by the Institute of Medicine is a relief carving from ancient Greece, now held by the Staatliche Museen in Berlin.

COVER: The cover photograph, provided by Dr. David Asher, is a histopathology slide of brain tissue from a patient with a prion disease. Stained with the chemicals eosin (red) and hematoxylin (blue), the magnified tissue manifests microscopic holes (white circles) that illustrate why prion-infected tissue is described as spongiform. This report aims to guide scientists beyond histopathology toward new strategies to diagnose prion diseases noninvasively, rapidly, and early.

"Knowing is not enough; we must apply.
Willing is not enough; we must do."
—Goethe

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COMMITTEE ON TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES: ASSESSMENT OF RELEVANT SCIENCE

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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by our coordinator, **Morton N. Swartz,** Chief, Jackson Firm of Medical Service, and Chief Emeritus, Infectious Disease Unit, Massachusetts General Hospital; and our monitor, **Linda Cork,** Professor and Chair of Comparative Medicine, Stanford University School of Medicine. Appointed by the National Research Council and the Institute of Medicine, Drs. Swartz and Cork were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Preface

Why is the U.S. government concerned about prion diseases? Known scientifically by the descriptive term *transmissible spongiform encephalopathies* (TSEs), these diseases do not currently represent significant public health problems in the United States. While it brings incalculable grief to affected families, Creutzfeldt-Jakob disease (CJD), the primary human prion disease, causes only 1 in 10,000 annual deaths worldwide, and there is no evidence that this rate is growing. Bovine spongiform encephalopathy (BSE), the epidemic "mad cow" disease in Europe, has yet to be detected in the United States.[1]

Nevertheless, several compelling reasons exist for focusing greater research efforts on prion diseases. First, the sudden appearance of BSE in the United Kingdom in the mid-1980s represented a massive and unforeseeable contamination of the bovine and human food supplies. Hundreds of thousands of cattle died, and the infectious agent unexpectedly crossed the species-barrier to humans. In the past decade, more than one hundred young adults have developed a variant of CJD from exposure to BSE. The social, political, and economic impacts of those epidemics of cattle and human diseases in the United Kingdom and continental Europe have been enormous. Consequently, a number of policies have been instituted to exclude BSE from the United States and to limit its spread, should it enter the country.

[1]EDITORS' NOTE: After this report was completed, the first U.S. case of BSE was identified in Washington State and was announced to the public on December 23, 2003.

In addition to concerns over the possible introduction of BSE into the United States and the occurrence of cases of variant CJD, the presence in this country of a TSE of deer and elk, chronic wasting disease (CWD), has caused alarm. Might this disease spread to cows or humans? There is no evidence that it has, as yet, but European colleagues who have suffered through the BSE crisis are astonished at the paucity of attention that the United States has directed at CWD. They have told me, "You may be sitting on a time-bomb."

The second rationale for expanding prion research in the United States is that the studies may provide insights into the pathogenesis of common neurodegenerative diseases, such as Alzheimer's, Parkinson's, and many hereditary neurodegenerative diseases. This is because the abnormal processing of altered neuronal proteins appears to be a feature of a variety of brain diseases, including TSEs. Prions are believed to be abnormally folded proteins that can replicate by converting normal prion protein into the altered conformation associated with disease. This generally insoluble, pathogenic isoform collects in the brain and spinal cord. Studies of the cellular transport of altered proteins, such as prions, could have broad pathogenetic implications.

All prion diseases have long incubation periods extending for years or decades, cause progressive and uniformly fatal neurological degeneration lasting for months, induce pathological changes limited to the nervous system, and evoke no inflammation or immune response. The idea that a disease of this nature might be transmissible is revolutionary. Moreover, no RNA or DNA has been implicated thus far in the process of prion replication—a stunning affront to the central dogmas of biology. Unlocking the secrets of TSEs could advance a new disease paradigm that would help scientists develop treatments for a variety of neurological diseases that afflict millions of people.

The charge to the Committee on Transmissible Spongiform Encephalopathies: Assessment of Relevant Science emphasized sensitive and specific diagnostic methods. This emphasis stemmed from concerns about the safety of blood and meat products and the wish to detect prion infections during the incubation period of CJD, BSE, or CWD. Diagnosis of infectious diseases has traditionally relied on the sensitive surrogate marker of antibodies formed by the host. As noted, however, prions, which are isoforms of a normal host protein, usually do not evoke an antibody response. Newer, highly sensitive tools to detect infectious agents in blood and spinal fluid use polymerase chain reaction to amplify nucleic acids, but this method is inapplicable to prion diseases because nucleic acid does not appear to be involved.

Although tests are available to detect altered prion proteins in tissue obtained at death or by biopsy, a blood or spinal fluid test is needed to

diagnose TSEs antemortem. Achieving the necessary level of acuity in such a test will probably require an innovative technology, not simply incremental improvements to known methods of protein detection. More knowledge about the structure of prions and their normal cellular counterparts, about isoform conversion, the cellular trafficking of prions, pathogenesis, and other basic aspects of TSEs will probably be prerequisite to devising a diagnostic method that increases sensitivity and specificity exponentially.

Another issue that the committee was asked to address, the infrastructure for TSE research in the United States, poses special problems. First, a limited number of investigators and laboratories here are dedicated to studying TSEs. Second, the usual investigator-initiated grants to universities or research institutes are ill suited for supporting the quantization and characterization of TSEs because this research generally involves animal hosts with incubation periods of months or years. Initial grants usually require renewal—and results—after only 2 or 3 years, when many TSE studies are still in a preliminary stage. Third, laboratories and animal-holding facilities require varying degrees of complex and expensive biological containment equipment. Fourth, few host institutions can afford to commit faculty positions and facility construction to TSE research. As a consequence of these challenges, new funding methods or the further expansion of government laboratories will be needed to meet the goal of increasing the number of U.S. investigations into prion diseases.

In January 2003, our committee published an interim report that dealt primarily with basic biomedical research on TSEs, diagnostics, research infrastructure, and risks to the U.S. military. This final report has new chapters and recommendations on testing blood for evidence of TSEs, on TSE surveillance in the United States, and on strategies for TSE prevention and treatment. This report also updates and expands upon the information from the interim report. The broad array of topics discussed here will be of interest not only to the scientific and medical communities, but also to a range of readers who want to learn about the multidimensional impact of the bizarre, fascinating, and deadly maladies collectively called prion diseases.

Richard T. Johnson, M.D.
Chair

Acknowledgments

The Committee on Transmissible Spongiform Encephalopathies is grateful for the many people who contributed to this report. We first thank the sponsor of the study that has culminated with this report—the Medical Research and Materiel Command of the U.S. Department of Defense—for requesting advice from the Institute of Medicine regarding the National Prion Research Program. We specifically convey our thanks to COL Ken Bertram, director of the Congressionally Directed Medical Research Program; to LTC Calvin Carpenter, our point of contact for this study; to COL Scott Severin, deputy director of the DOD Veterinary Service Activity; to CDR Rebecca Sparks, deputy director of the Armed Services Blood Program; and to LTC Ruth Sylvester, operations director for the Armed Services Blood Program.

We greatly appreciate and benefited from the expert technical advice that our six standing consultants provided throughout the study. Their willingness to travel long distances to committee meetings without remuneration reflects their dedication to advancing prion science. In addition, Dr. David Asher kindly provided the photograph on the cover of this report—a histopathology slide of brain tissue from a patient with spongiform encephalopathy—and Dr. Pierluigi Gambetti provided the slides of normal and infected brain tissue that appear in Chapter 4.

We also extend our appreciation to the many invited guest speakers who attended our meetings to share their expertise through both formal presentations and participation in committee discussions. We encourage readers to look at Appendix A, which provides each speaker's name and the

topic he or she addressed. These individuals provided a significant body of information for us to draw upon as we formulated the discussion and recommendations in this report.

We give special thanks to our committee chair, Dr. Richard Johnson, for planning the five meetings and for his insightful guidance and direction to both the committee and the staff.

The IOM's Office of Reports and Communication deserves special thanks for its assistance to the study staff. This report would not have come together as it did without Bronwyn Schrecker's help navigating the review process and Jennifer Bitticks's efficient facilitation of the production process. In addition, the report benefited significantly from Rona Briere's exceptionally detailed and thoughtful copyediting; Alisa Decatur's specialized copyediting of references; Will Mason's skill at preparing the figures and creating the illustration in Figure 2-1; and the designers at National Academies Press who created the cover of this report. We are grateful to Janice Mehler, the associate director of the National Academies' Report Review Committee, for facilitating the extensive review process. Last but not least, Andrea Cohen's expert assistance in tracking the financial aspects of the study enabled us to complete it within budget guidelines.

We also thank the TSE researchers and experts beyond our standing consultants who provided information or artwork for this report: Dr. Bruce Caughey, for his technical assistance to both the committee and the staff; Dr. Michael Coulthart, for providing information about Canada's TSE surveillance program; Dr. Linda Detwiler, for her technical assistance; Dr. Dominique Dormont, for providing information on therapeutic strategies; Dr. Cedric Govaerts, for providing models of prion structure; Dr. James Ironside, for providing microphotographs of brain tissue; and Dr. Holger Wille, for providing electron micrographs of scrapie-associated fibrils and of a two-dimensional crystal of PrP 27–30.

In addition, we thank the publishers and authors who graciously allowed us to reprint their illustrations in this report.

Finally, we thank our staff at the IOM: Study Director Rick Erdtmann, Research Associate Laura Sivitz, and Senior Project Assistant Reine Homawoo. They have done an outstanding job planning the committee meetings, providing us with background literature, keeping the study on track, drafting and editing this report, and facilitating its dissemination.

Abbreviations and Acronyms

AAFES	Army and Air Force Exchange Service
AIDS	acquired immune deficiency syndrome
AMR	advanced meat recovery
APHIS	Animal and Plant Health Inspection Agency, U.S. Department of Agriculture
ASBP	Armed Services Blood Program
BSE	bovine spongiform encephalopathy
CBER	Center for Biologics Evaluation and Research, U.S. Food and Drug Administration
CDC	Centers for Disease Control and Prevention
CDMRP	Congressionally Directed Medical Research Program
CFIA	Canadian Food Inspection Agency
CFR	Code of Federal Regulations
CIE	capillary immunoelectrophoresis
CJD	Creutzfeldt-Jakob disease
CNS	central nervous system
CSF	cerebrospinal fluid
CT	computed tomography
CVM	Center for Veterinary Medicine, U.S. Department of Agriculture
CWD	chronic wasting disease

DHHS	U.S. Department of Health and Human Services
DOD	U.S. Department of Defense
DOI	U.S. Department of the Interior
EC	European Commission
EEG	electroencephalography
ELISA	enzyme-linked immunosorbent assay
EU	European Union
FCS	fluorescent correlation spectroscopy
FDA	U.S. Food and Drug Administration
FFI	fatal familial insomnia
FLAIR	fluid attenuated inversion recovery
FSIS	Food Safety and Inspection Service, U.S. Department of Agriculture
GAO	General Accounting Office
GPI	glycosyl phosphatidylinositol
GSS	Gerstmann-Sträussler-Scheinker disease
HFSP	Human Frontier Science Program
HIV	human immunodeficiency virus
i.c.	intracerebral(ly)
iCJD	iatrogenic Creutzfeldt-Jakob disease
ID_{50}	a dose that infects 50 percent of the population exposed to the infectious agent
IGIV	immune globulin to be given intravenously
IHC	immunohistochemistry
IMAC	immobilized metal ion affinity chromatography
IOM	Institute of Medicine
i.p.	intraperitoneal(ly)
IU	infectious unit
i.v.	intravenous(ly)
kDa	kilodalton(s)
LCGE	laser-assisted capillary gap electrophoresis
LD_{50}	a dose that is lethal to 50 percent of the population exposed to an infectious agent
µg	micrograms
MRI	magnetic resonance imaging

MRMC	Medical Research and Materiel Command, U.S. Army
MUFS	multispectral ultraviolet fluorescence spectroscopy
NaPTA	sodium phosphotungstate
NASS	National Agricultural Statistics Service, U.S. Department of Agriculture
NIH	National Institutes of Health
nm	nanometer
NMR	nuclear magnetic resonance
NPDPSC	National Prion Disease Pathology Surveillance Center
NPRP	National Prion Research Program (DOD) and National Prion Research Project (congressional language)
nvCJD	new variant Creutzfeldt-Jakob disease
NVSL	National Veterinary Services Laboratories
PCR	polymerase chain reaction
pg	picograms
PK	proteinase K, an enzyme that digests cellular PrP
PMCA	protein misfolding cyclic amplification
PRNP	prion protein gene in humans
Prnp	prion protein gene in animals other than humans
PrP	prion protein
PrP^{C}	protease-sensitive cellular prion protein
PrP^{Sc}	protein associated with prion disease; has limited resistance to proteinase K
PrP^{res}	protease-resistant protein associated with prion disease
PrP^{CWD}	protein associated with chronic wasting disease
RIA	radioimmunoassay
sCJD	sporadic Creutzfeldt-Jakob disease
sFI	sporadic fatal insomnia
SRM	specified risk material
TME	transmissible mink encephalopathy
TSE	transmissible spongiform encephalopathy
USDA	U.S. Department of Agriculture
vCJD	variant Creutzfeldt-Jakob disease
VMRD	Veterinary Medical Research and Development Inc.
WHO	World Health Organization

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ADVANCING
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Executive Summary

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are inevitably fatal neurodegenerative diseases of long incubation affecting humans and animals. In this report, the Institute of Medicine's (IOM) Committee on Transmissible Spongiform Encephalopathies: Assessment of Relevant Science recommends research to close significant gaps in present knowledge of TSEs and techniques to strengthen the U.S. research infrastructure for studying these diseases. This report fulfills a request of the U.S. Army's Medical Research and Materiel Command for advice from the IOM on the most effective research agenda for the National Prion Research Program (NPRP), established by the U.S. Congress in 2002.

Unlike all other known infectious diseases, TSE infectivity appears to be associated with a misfolded form of a normal cellular protein. The misfolded protein is either the primary or exclusive component of a prion, the infectious agent of TSEs.

There is no cure, prophylaxis, or fail-safe antemortem diagnostic test for TSEs. To develop any of these tools to protect human and animal health, the committee determined, the scientific community must first answer fundamental questions about TSEs and prions. Therefore, the committee recommends that NPRP fund basic biomedical research on the structural features of prions; the molecular mechanisms of prion replication; the mechanisms of TSE pathogenesis; and the physiological function of prion protein, the normal form of the misfolded protein of prions. Moreover, the committee recommends that NPRP support research on the epidemiology and natural history of TSEs.

In addition to the lack of knowledge about TSEs, an impediment to the development of diagnostics and therapeutics for these diseases is the limited infrastructure available for studying them in the United States, the committee found. Consequently, this report includes several recommendations for augmenting the U.S. infrastructure for TSE research. Notably, the committee suggests that NPRP attract and train more investigators and provide grants of 5 to 7 years for research in animals (because prion diseases incubate for years). The report also recommends the expansion or upgrading of existing laboratories, animal facilities, and containment laboratories and the construction of new ones. Finally, the committee advises NPRP to support new or established repositories for collections of materials and animals that investigators around the country could borrow for their experiments.

As noted, no existing drugs are effective in treating TSEs, although many have been tried. NPRP should support research to develop new therapeutic agents, including antibodies, that would either block the conversion of normal prion protein to the abnormally folded form or disrupt the molecular mechanisms of TSE pathogenesis after conversion has occurred. The most promising approach appears to be the design of agents that attack a specific site on the target protein molecule. These same therapeutic agents may have applicability for detection as well.

The development of TSE diagnostics will require quantum leaps rather than marginal improvements of existing tools, the committee concluded. Existing tests are several orders of magnitude less sensitive than is optimal. Thus, the committee advises NPRP to support the development of truly novel methods and reagents that detect or bind to prions, including new antibodies, peptides, nucleic acids, synthetic derivatives, and chimeric molecules.

Because of the present limitations of prion-detection tools, it is unknown whether the blood of a human who has a prion disease is a vehicle for transmitting the disease to another individual. However, recent animal studies showed that two different TSE agents could be transmitted from TSE-infected sheep to uninfected sheep by the transfusion of sheep blood. Therefore, the committee recommends that NPRP support research to determine the risk of prion transmission through human blood.

The outbreak of the TSE called bovine spongiform encephalopathy (BSE), commonly known as mad cow disease, in the United Kingdom and the consequent emergence of variant Creutzfeldt-Jakob disease in humans demonstrated the importance of good surveillance for TSEs. The United States could strengthen its TSE surveillance systems through research into the natural history, prevalence, distribution, exposure and transmission characteristics, host susceptibility, and host range of TSEs, especially of chronic wasting disease (CWD), a TSE of elk and deer that is epidemic in this country. The committee recommends that NPRP support such studies.

Human TSEs in the United States are underrecognized and underreferred for definitive diagnosis, the committee found. It is recommended that NPRP support efforts to ensure that a greater proportion of suspected cases are identified and autopsied, because the only way to diagnose a TSE definitively at present is through a neuropathological exam.

The most effective strategy for managing the threat of TSEs is to avoid preventable exposure to the infectious agent, the committee concluded. U.S. measures to prevent the tissue of cattle infected with the agent of BSE from entering the food chain are not foolproof, however, the committee found. Therefore, the committee recommends that NPRP fund research to improve rapid, accurate, and affordable screening assays for detecting central nervous system (CNS) tissue in processed meat products, since prions reside primarily in CNS tissue. In addition, the committee notes that a review of U.S. policy on specified risk materials, the mammalian tissues most likely to contain TSE infectivity, would be appropriate. Finally, the committee highlights the potentially damaging economic effects of the discovery of even a single case of BSE in the United States.

Studies evaluating the human risks associated with the infectious agent of CWD should also be funded, the committee recommends. There is no evidence to conclude that CWD is transmissible to humans; however, the theoretical risk of infection led the committee to advise people to avoid exposure to CWD-contaminated meat and meat products. The wide range of practices for processing venison, the paucity of regulation or oversight in this area, and the many opportunities for spreading the CWD agent influenced the committee's conclusions in this regard.

There is a very small but unknown level of risk that U.S. forces and their dependents who were stationed in Europe during the 1980s and 1990s acquired a TSE, the committee determined. Consequently, the committee recommends that the occurrence of TSEs in this population be monitored through established data systems in the Department of Defense and the Department of Veterans Affairs.

Clearly, many unanswered questions remain regarding prions and TSEs. The recommendations in this report should provide a framework for research, especially basic research, leading to the development of effective diagnostics and therapeutics. Such research could advance scientific knowledge relevant to many neurodegenerative diseases in addition to TSEs. The reader is directed to the report summary for a more comprehensive and referenced discussion of all the committee's recommendations, only a portion of which are discussed above.

Summary

The 1985 outbreak of bovine spongiform encephalopathy, or mad cow disease, in the United Kingdom generated global awareness of a previously obscure set of neurodegenerative diseases called transmissible spongiform encephalopathies (TSEs). TSEs are caused by infectious agents. Yet, unlike all other known infectious diseases, TSE infectivity appears to be associated with an abnormally folded protein known as a prion (Prusiner, 1982). There is no cure, prophylaxis, or fail-safe antemortem diagnostic test for TSEs, often called prion diseases. Infected hosts incubate a TSE for months to decades, and their health declines rapidly after the onset of clinical symptoms; death invariably follows within a period of months.

Bovine spongiform encephalopathy (BSE) became an epidemic that affected hundreds of thousands of animals in the United Kingdom and inflicted economic harm on the country's cattle and beef industries. Cases of BSE have also been reported in continental Europe, Israel, Japan, Canada, and elsewhere.[1] Exposure to BSE-infected beef products has given rise to a fatal human neurodegenerative disease called variant Creutzfeldt-Jakob disease (vCJD), first identified in 1996 (Will et al., 1996). As of September 2, 2003, 140 definite or probable vCJD cases had been identified in the United Kingdom (National Creutzfeldt-Jakob Disease Surveillance Unit, 2003), and a handful of cases had been identified in other countries. Estimates of the total number of people who will contract vCJD as a result of the BSE epi-

[1]EDITORS' NOTE: After this report was completed, the first U.S. case of BSE was identified in Washington State and was announced to the public on December 23, 2003.

demic vary depending on assumptions regarding the incubation period, individual susceptibility, and the level of exposure. The incubation period for another human prion disease, kuru, is 4 to 40 years (Huillard d'Aignaux et al., 2002).

The origin of vCJD in prion-infected cattle raises the concern that chronic wasting disease (CWD), a prion disease spreading among North American deer and elk (Williams and Miller, 2002), could cause disease in people who consume venison from the affected regions.

The European Commission has spent millions of euros on research to develop better diagnostics for TSEs, especially BSE, with modest success. Commercial diagnostic tests have been developed for rapid postmortem BSE detection and are used throughout the United Kingdom and Europe. These tests cannot detect prions present at low levels, however. The lack of highly sensitive, accurate, and rapid tests has led to such controls as categorical importation bans and massive culling of herds to ensure the safety of beef products.

To date the U.S. Food and Drug Administration (FDA) has received no request to approve a rapid test for the detection of human TSEs (personal communication, D.M. Asher, FDA, July 1, 2003). However, the U.S. Department of Agriculture's (USDA) Center for Veterinary Biologics has approved the use of three rapid tests for the detection of CWD in cervids (personal communication, R. Hill, USDA APHIS Center for Veterinary Biologics, November 25, 2003).

ORIGINS OF THIS STUDY

The economic and health consequences of BSE and vCJD in Europe and the risk that U.S. military forces stationed abroad and their dependents could contract a TSE through infected beef or contaminated blood products led the U.S. Congress to pass a law establishing the National Prion Research Project (NPRP) in 2002 (U.S. Senate Committee on Appropriations, 2001). NPRP funds research on TSEs, with special emphasis on developing an antemortem diagnostic test.

Congress mandated that the U.S. Department of Defense (DOD) administer the new project, which was delegated to the Army's Medical Research and Materiel Command (MRMC). MRMC administers grants through a two-tiered process of external scientific peer review, followed by programmatic review by a multidisciplinary group of DOD and civilian experts called an integration panel. MRMC requested that the Institute of Medicine (IOM) produce two reports assessing present scientific knowledge about TSEs and recommending the highest-priority research for funding (Department of Army Contract DAMD17-02-C-0094, May 2002).

In June 2002, IOM formed the Committee on Transmissible Spongiform Encephalopathies: Assessment of Relevant Science to conduct this study. The committee was charged with evaluating the state of prion science, especially as regards research needs in the area of diagnostics. The members of the committee were asked to examine novel technologies that might advance diagnostics; evaluate the reagents and assays used in prion research and recommend improvements; evaluate the adequacy of the TSE research infrastructure in the United States with respect to the number of investigators, physical facilities, and training needs; suggest opportunities for collaboration with foreign investigators; evaluate the threat of TSEs to U.S. military forces with respect to their food supply, their blood supply, and any other factors; provide advice on public health policies or surveillance programs that require new research or that might affect the military; and recommend additional research on ways to reduce or prevent TSEs.

The committee's first report, released in January 2003, advised MRMC's integration panel on the most promising avenues of research for developing antemortem TSE diagnostic tests. It also recommended ways to remedy key shortcomings in the U.S. infrastructure for TSE research and evaluated the degree of risk posed by TSEs to U.S. military forces and their dependents stationed abroad. This, the committee's second report, recommends the highest-priority research in TSE surveillance, prevention, and therapy. It includes an updated version of the material from the first report, with an additional chapter on the unique challenges of detecting TSE infectivity in blood. This summary presents the committee's conclusions and recommendations, which are also listed in Table S-1.

Clearly, we believe all the recommendations in this report are important. Given that NPRP has a limited amount of resources, however, it can act only on a limited number of recommendations each fiscal year. Therefore, we prioritized our recommendations by placing them in one of three ranks, 1 being the highest (see Table S-1). We assigned approximately a third of the recommendations to each rank. The rankings are based on the following criteria:

- Impact on public health
- Impact on protecting animal health (mainly cattle)
- Impact on protecting the U.S. economy (BSE/CWD)
- Impact on advancing scientific understanding of prions (potential for breakthroughs)
- The need to accomplish first rather than second (stepwise progression of the science)
- Return on investment
- Likelihood of success (How feasible is it?)

In addition to prioritizing all of our recommendations, we indicate in the body of the report the most urgent and critical areas of research related to recommendations 3.1 and 6.4.

PRIONS AND PrP^{Sc}: DEFINITIONS AND USAGE

This report uses the terms prion and PrP^{Sc} interchangeably in reference to the protease-resistant protein associated with prion disease.[2] The committee is sensitive to the fact that such usage is controversial, however, so we want to explain our choice of words in the context of this controversy.

The proteins PrP^{Sc} and PrP^{C} are both encoded by the *PRNP* gene on chromosome 20 in humans. Like all proteins, PrP^{C} has a characteristic conformation. Under certain conditions, however, it folds into an abnormal shape that is associated with fatal neurodegeneration after a long incubation period. This misfolded protein is called PrP^{Sc}.

The committee believes that the preponderance of scientific evidence favors the hypothesis that prions, consisting of PrP^{Sc}, are associated with infection in TSEs. If prion aggregates are the infectious agent that causes TSEs and if a prion is the misfolded protein known as PrP^{Sc}, then by parallel reasoning, aggregates of PrP^{Sc} are the infectious agent of TSEs.

However, some reputable TSE experts believe that PrP^{Sc} alone may not be sufficient to cause a TSE infection, although the protein may be associated with and even necessary for such infection. A number of these investigators hypothesize that the infectious particle—the prion—may contain hidden nucleic acid, additional proteins, or some other additional, essential material. This camp uses the term prion to refer to TSE infectivity, but does not equate prion with PrP^{Sc}. To respect and recognize this point of view, this report often uses the phrase "the infectious agent of [TSE under discussion]" instead of the term prion or PrP^{Sc}.

The purpose of this report is not to resolve the controversy over the definition of a prion or to proffer the committee's opinion regarding the hypothesis that prions consist exclusively of the protein PrP^{Sc}. Rather, the purpose of this report is to call for additional research—especially studies of the fundamental molecular structures and mechanisms related to TSEs—so that disparate views may converge and advance prion science.

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[2]At times an additional term, PrP^{res}, is used synonymously with PrP^{Sc}. PrP^{res} is an abnormally folded prion protein that is highly resistant to digestion by the enzyme proteinase K (PK) and is strongly associated with prion disease. Unlike PrP^{res}, however, PrP^{Sc} demonstrates a gradient of resistance to PK. PrP^{Sc} is associated with infectious potential and with prion disease even in circumstances where it may be sensitive to PK digestion.

BASIC BIOMEDICAL RESEARCH

The committee determined that the main obstacle to developing sensitive, specific antemortem diagnostics for TSEs is the lack of knowledge about prions and their normal cellular isoform, PrP^C.

Recommendation 3.1:[3] Fund basic research to elucidate (1) the structural features of prions, (2) the molecular mechanisms of prion replication, (3) the mechanisms of pathogenesis of transmissible spongiform encephalopathies, and (4) the physiological function of PrP^C.

The committee believes basic research in these four areas will supply the knowledge required to advance TSE diagnostics more quickly than applied research alone. The report text describes specific gaps in knowledge that need to be filled in each of the four areas.

For instance, present models of prion conformation and tertiary structure are neither complete nor conclusive. Defining the structural differences between PrP isoforms could enable scientists to synthesize a PrP^{Sc}-specific antibody probe or aptamer. Defining the structures of PrP^C and PrP^{Sc} at the sites where they interact during binding and conformational change could support the development of molecules that would block those interactions.

Most experts believe the conversion of PrP^C to PrP^{Sc} and the accumulation of prions require the assistance of one or more molecules (Caughey, 2001) that may be easier to detect than prions themselves. These unidentified ancillary or chaperoning factors could serve as surrogate markers for prion detection and as drug targets for TSE therapeutics and prophylaxes.

Current gaps in knowledge of the pathogenesis of prion diseases prevent better characterization of diagnostic targets and strategies. The routes of transmission, the factors that influence host susceptibility, the lack of an immune response, the mechanisms of neuroinvasion, and the cause or causes of cellular toxicity all lack satisfactory explanations; filling these gaps would result in tests with greater sensitivity and specificity. In addition, isolating the multiprotein complexes that contain prions might make it possible to identify new cofactors important to the formation and stabilization of PrP^{Sc} and infectivity.

Understanding the normal role of PrP^C might also reveal associated molecules and pathways that would be appropriate detection targets for TSE diagnostics. Investigators should clarify whether the basis for nerve cell dysfunction and death in prion disease is related to the toxicity of PrP^{Sc}, to

[3]For ease of reference, the committee's recommendations are numbered according to the chapter of the report in which they appear. For instance, recommendation 3.1 is the first recommendation in Chapter 3.

the loss of function of PrP^{C} as a result of its conversion to PrP^{Sc} and its aggregation during a prion infection, or to other factors.

Finally, the committee concluded that too great a focus on applied rather than basic research would slow progress in TSE diagnostics.

TSE DIAGNOSTICS

Obstacles to Developing Antemortem Diagnostics for TSEs

Conventional methods used to diagnose most infectious diseases, such as malaria, tuberculosis, hepatitis, and AIDS, fail to detect prion diseases for numerous reasons. First, a prion is a host protein with an altered conformation such that the immune system does not recognize it as foreign and does not produce antibodies against it. Second, since a prion lacks DNA and RNA, it cannot be identified by molecular methods such as polymerase chain reaction and other nucleic acid–based tests, nor can it be identified by such customary methods as direct visualization under a light microscope, cultivation in the laboratory, or detection of specific antibodies or antigens by standard immunological methods. Moreover, prions are largely insoluble, distributed unevenly in body tissues, and found in a limited set of tissues by currently available tests. PrP^{Sc} is neurotropic, so it ultimately affects cells of nervous system tissues. Yet where and how PrP^{Sc} progresses through the body before its final assault on the nervous system are largely unclear, complicating the ability to locate and detect it.

In addition, the similarities between prions and the normal host cellular protein PrP^{C} pose a fundamental problem. Since it is normal to find PrP^{C} in healthy individuals, detection tests must differentiate between the normal and abnormal prion protein molecules. The most common strategy thus far has been to mix the test material with the proteinase K enzyme (PK), which digests normal prion protein but only a portion of the abnormally folded protein. Various techniques are then used to detect the residual PrP^{Sc} after digestion. This process incidentally reduces the small amount of original PrP^{Sc} captured, however, making the process less sensitive than the newer experimental methods that do not rely on PK digestion.

The fact that only small amounts of abnormal prion protein may be available for detection in accessible living tissues such as blood, urine, and cerebrospinal fluid challenges diagnosticians to develop a sufficiently sensitive test. Such a test must differentiate not only between normal and abnormal prion proteins, but also, for some purposes, between one or more strains of PrP^{Sc}—a challenge resulting from basic deficiencies in the understanding of prion strain diversity and the nature of strain variation. The ultimate objective of a prion detection test is to detect a single infectious unit while avoiding a false-positive test result.

Presently Available TSE Diagnostics

The diagnostic assays available today are generally used only after the death of an animal or person. These assays test brain tissue, where the greatest concentrations of prions are found during the terminal stage of disease. Standard histopathological and immunohistochemical techniques are used to view the tissue microscopically and identify characteristic vacuoles, plaques, or other abnormal features and staining associated with prion diseases. The standard confirmatory test is the Western blot.

Attempts made to date to develop accurate, rapid, and highly sensitive antemortem tests, especially for detecting prions early in the course of infection, have largely failed. Also, most tests still involve PK digestion, and the specificity and sensitivity of tests that do not use PK require further demonstration. Newer tests appear to have improved the limits of detection. As of this writing, however, most of the newer detection methods are experimental and have not been independently verified and reported. Moreover, the sensitivity of these tests must still be improved by several orders of magnitude if they are to reliably detect an infectious unit of prions.

Recommendation 4.1: Fund research to develop new assays most likely to achieve quantum leaps in the quality of prion detection tools, rather than incremental improvements to existing tests. Any efforts to improve existing tests should aim to increase their sensitivities by several orders of magnitude (at least 10^3). The optimal test should detect less than 1 infectious unit (IU) of PrP^{Sc} per unit of ultimate product used (e.g., 1 liter of blood or 100 grams of beef).

Recommendation 4.2: Fund research to improve in vitro techniques that amplify small amounts of PrP^{Sc} to enhance the sensitivities of diagnostic tests.

The Need for Novel Approaches to Developing TSE Diagnostics

Major improvements in TSE diagnostics must await the availability of novel testing techniques or of reagents designed to specifically target PrP^{Sc}.

Recommendation 4.3: Fund research to develop novel methods and reagents that detect or bind to prions, including new antibodies, peptides, nucleic acids, synthetic derivatives, and chimeric molecules. This research could lead not only to better diagnostics, but also to better therapeutic and prophylactic strategies.

Researchers have attempted a variety of novel ways to improve the sensitivities of tests for TSEs. The most promising of these techniques are summarized below.

Surrogate Markers

A strategy other than the direct detection of PrP^{Sc} is to detect surrogate markers of prion infection. Cells that have been injured by prion invasion may produce other unique proteins or protein mixes that can be detected. The committee determined that the rapidly expanding field of proteomics may offer new tools for the development of highly sensitive prion detection tests employing such surrogate markers. This strategy is being used successfully for the detection of certain cancers (Petricoin et al., 2002a,b), and the committee recommends that it be applied to TSEs.

Recommendation 4.4: Fund research to identify surrogate markers or signatures for the detection of prions or prion diseases.

Prion Amplification

As indicated in recommendation 4.2, the committee also sees promise in strategies for amplification of PrP^{Sc} material before further testing (Saborio et al., 2001). Analogous to the polymerase chain reaction technique for amplifying small amounts of DNA, these strategies could significantly boost the power of prion diagnostics.

Cell Culture Assays

In vitro culture systems have been used for prion detection with moderate success. Yet the committee believes these assays would hold great promise if a stable and robust cell culture assay were developed. Their speed and biological simplicity would make them highly effective in testing for TSEs.

Recommendation 4.5: Fund research to improve techniques for propagating prions in cultured cells and develop new in vitro cell systems as a means to assay and study prions.

Clinical Diagnostics

Although clinical criteria for the characterization of prion diseases have been established, they are adjunctive at present. Neuroimaging offers promise as a future clinical diagnostic tool for prion diseases. The committee concluded that newer magnetic resonance imaging techniques, positron emission tomography scanning applications, and multiphoton microscopy should be developed for antemortem detection of TSEs.

Recommendation 4.6: Fund research to develop functional imaging for the presence of PrP^{Sc} in brain tissue, leading to an early

diagnostic test similar to the imaging diagnostics being developed for Alzheimer's disease.

TESTING BLOOD FOR EVIDENCE OF TSEs

The ability to detect prions in blood would enable diagnosis and treatment of a TSE at an early stage of the disease, ideally before neuroinvasion. It would also allow a TSE-infected donor to be identified and deferred before his or her blood is taken and administered to others as whole blood, a blood product, or a blood derivative. In addition, a test for TSE infectivity in blood could allow donors currently deferred because of potential exposure to a TSE agent to reenter the donor pool. In animals, such a test would permit early recognition of infection and timely control procedures. The committee believes the development of a screening test for detecting prions in blood is desirable for all these reasons, but will be extremely challenging to accomplish.

The findings of TSE transmission studies in animals give reason to believe that prions can be found in blood (Brown, 2001). Most of those studies used the most sensitive form of in vivo assay, an injection of blood directly into the brain, to see whether the animals would become infected; some did. In addition, a limited number of studies showed that blood from infected animals could infect by the intravenous route. Those studies more closely simulate exposure by blood transfusion. Recent compelling studies in sheep demonstrated that both the BSE and scrapie agents could be transmitted to other sheep by blood transfusion (Hunter et al., 2002), supporting the hypothesis that blood can serve as reservoir for infectious prions.

Multiple transmission and case-control studies have failed to demonstrate that blood from patients with sporadic Creutzfeldt-Jakob disease (sCJD) is infectious (Brown et al., 1994). By contrast, investigations into the transmissibility of the vCJD agent through blood transfusion are just beginning to gain momentum. Because a significant amount of prions appears in the lymphoreticular system in vCJD cases but not in other varieties of human TSEs, the blood of vCJD-infected individuals may contain prions. The amount of vCJD prions that might be contained in blood and the amount that constitutes an IU are important public health questions. The committee concluded that there is a small but unknown level of risk of acquiring vCJD from blood products; therefore, more research to clarify the nature of that risk should be conducted.

Recommendation 5.1: Fund research (1) to determine the amount of sporadic Creutzfeldt-Jakob disease (sCJD) prions and variant Creutzfeldt-Jakob disease (vCJD) prions in human blood and (2) to

estimate the amount of PrP^{Sc} corresponding to one infectious unit of sCJD and vCJD prions in human blood.

The technical feat of developing a prototype test to detect low, and possibly changing, levels of prions in blood is an enormous challenge. But that is only one of the steps necessary to field a new commercial blood-screening test. To avoid false-positive test results, multiple testing schemes should be developed so that results can be confirmed or refuted. Stable, standard, and reliable testing reagents should be developed. Biotechnology companies must be properly structured to successfully mass-produce a novel test. Users will need to develop ethically sound counseling and notification policies for those tested, especially to deal with positive tests. Developers will need to demonstrate and document the test's performance rigorously enough to achieve FDA approval. Finally, a market for the product must exist, or be created, to attract commercial investment and manufacturing. These tasks are achievable with great resolve.

SURVEILLANCE FOR TSEs IN THE UNITED STATES

Surveillance for TSEs in humans and animals can permit the detection of potential outbreaks of known or new TSEs and the monitoring of those TSEs known to occur in the United States: CWD and scrapie in animals, and sCJD, iatrogenic CJD (iCJD), familial CJD, familial fatal insomnia, sporadic fatal insomnia, and Gerstmann-Sträussler-Scheinker disease in humans.

Surveillance for TSEs in Humans

The Centers for Disease Control and Prevention (CDC) monitors the U.S. population for human TSEs. It also conducts surveillance specifically for vCJD (Belay et al., 2003), even though there has never been a reported case of BSE in the United States. Human TSEs are reportable in 12 states.

Identification and Autopsy of TSE Cases

The diagnosis of TSE in humans almost always requires a neuropathological examination. These examinations are conducted for the U.S. population by the National Prion Disease Pathology Surveillance Center (NPDPSC). Yet at least half of the estimated total number of deaths caused by TSE in the United States are not autopsied and confirmed by laboratory examination (Gambetti, 2002; Holman et al., 1996). The committee concluded that the large percentage of undiagnosed TSE cases represents a major obstacle to thorough U.S. surveillance for human TSEs. Physicians

and public health officials need to identify more suspected TSE cases, and more of those cases should be autopsied and sent to NPDPSC for neuropathological examination.

As noted earlier, the knowledge that BSE can cross the species barrier into humans has generated concern that CWD, which is epidemic in U.S. deer and elk, could potentially infect humans as well. If so, the human form of CWD could manifest itself clinically in a form quite unlike that of the known human TSEs. Therefore, the committee believes it would be prudent for CDC to conduct TSE surveillance on all atypical cases of neurodegenerative disease.

Recommendation 6.1: Provide funds to promote an increase in the proportion of cases of human neurodegenerative disease, especially suspected cases of transmissible spongiform encephalopathy, that are recognized and autopsied.

Epidemiological Research on Human TSEs

Improving U.S. surveillance for human TSEs will also depend on information gleaned from epidemiological studies that help define the target population and hone survey instruments.

Recommendation 6.2: Provide funds to increase the number and diversity of epidemiological studies on human transmissible spongiform encephalopathies (TSEs) in the United States. In particular, support research to identify potential cases of variant Creutzfeldt-Jakob disease and new human TSEs possibly caused by the agent of chronic wasting disease.

Surveillance for TSEs in Animals

Chronic Wasting Disease

Although CWD has existed in deer and elk in Colorado and Wyoming since at least the 1960s (Miller et al., 2000; Williams and Young, 1980), the disease has apparently[4] spread to captive and free-ranging herds in 10 other states and two Canadian provinces since 1996 (Fischer and Nettles, 2003; Williams and Miller, 2002). Nationwide surveillance for CWD is improving thanks to increased awareness of the disease and the availability of more resources, including a network of USDA laboratories that conduct neuropathological examinations on possible and probable CWD cases.

[4]It is also possible that increased awareness of CWD has brought preexisting cases to light for the first time.

In addition, a multijurisdictional joint working group formed in response to a congressional mandate published a plan in 2002 for controlling the spread of CWD in the United States (CWD Task Force, 2002). The committee urges NPRP to support research proposals that complement the nationwide surveillance initiatives implemented by the joint working group.

Recommendation 6.3: Provide funds to support the development of a nationwide surveillance system for chronic wasting disease in the United States.

Bovine Spongiform Encephalopathy and Scrapie

USDA runs surveillance programs for the other two animal TSEs of concern in this country: BSE and scrapie. There is no evidence that humans can contract TSE from eating scrapie-infected sheep tissue or from contact with sheep. As discussed above, humans can contract vCJD from BSE-infected beef products, but no U.S. case of BSE has been reported to date.

USDA has continued to improve its BSE surveillance program, especially in response to concerns about the economic and health consequences of an outbreak of BSE in U.S. cattle. The program targets the subpopulations of adult cattle most likely to have BSE. USDA tested 19,990 cattle brains for the disease in 2002 and 11,152 brains during the first 4 months of 2003—dramatic increases over the 5,272 brains examined in 2001 (Animal and Plant Health Inspection Service, 2003).

Research to Improve U.S. Capabilities to Conduct TSE Surveillance

Many unanswered questions regarding the epidemiology, natural history, and prevalence of animal and human TSEs in the United States limit the nation's ability to conduct the best possible surveillance for these diseases. First and foremost, NPRP should fund laboratory and epidemiological research to determine the likelihood that the infectious agent of CWD (PrP^{CWD}) could infect humans. The transmissibility of PrP^{CWD} to nonhuman animal species, such as cattle, should also be studied further. Second, scientists must determine the characteristics of a host, an infectious agent, and the environment that affect an animal's susceptibility to a TSE. Third, the mechanisms of exposure to the infectious agent in nature, the routes of transmission, and the way the agent invades the host's central nervous system must be defined. Fourth, scientists must develop surveillance systems that are sensitive enough to identify new TSEs and new TSE phenotypes.

Recommendation 6.4: Provide funds to expand research on the natural history, prevalence, distribution, exposure and transmission characteristics, host susceptibility, and host range of transmis-

sible spongiform encephalopathies, especially chronic wasting disease.

ASSESSMENT OF STRATEGIES TO PREVENT AND TREAT TSEs

The only reliable strategy for protecting animals and humans from the infectious agents of TSEs is avoidance. To date, a few techniques applicable in specific circumstances have successfully removed or inactivated TSE agents from the principal avenues of transmission: food, animal feed, transplanted tissue, and blood products. No successful therapy has yet been developed. Therefore, USDA and FDA have enacted many regulations, some enforced more effectively than others, to prevent human and animal exposure to TSE agents.

Preventing TSEs from Entering the Food Chain

The U.S. formula for preventing exposure to TSEs through human food or animal feed has three elements (PL 107-9 Federal Inter-agency Working Group, 2003):

1. Do not import live animals or animal products possibly infected with a TSE agent.
2. In case a TSE agent penetrates U.S. borders, have measures in place that will prevent its spread.
3. Protect U.S. residents from possible exposure to TSE agents in food and other products that come partially or completely from potential carriers of TSE agents.

Policies to Prevent the Entry and Spread of BSE

The United States applies the above formula primarily to the infectious agent of BSE through four policies: the import ban, the feed ban, slaughter surveillance, and the ban on central nervous system (CNS) tissue and CNS-associated tissue in beef and beef products.

Most of these policies have been challenging to enforce. A 2002 report of the U.S. General Accounting Office highlights several policies, especially the feed ban, that lacked adequate enforcement at the time the report was researched (GAO, 2002); full compliance with some of these policies has yet to be achieved. By contrast, a BSE risk assessment published in 2001 concluded that U.S. policies make this country highly resistant to the introduction and spread of the BSE agent (Harvard Center for Risk Analysis and Tuskegee University Center for Computational Epidemiology, 2001).

Policy recommendations are beyond the scope of this committee's charge. However, further research could help beef processors detect ruminant CNS tissue in their products. USDA's Food Safety and Inspection Service (FSIS) tested end-product samples from 34 U.S. beef processors in 2002 and found that 35 percent of the samples contained CNS and CNS-associated tissues (FSIS, 2003). Consequently, FSIS issued a more stringent enforcement directive for the CNS tissue ban (FSIS, 2002). The most effective rapid CNS test available for beef processors cannot distinguish between CNS tissue from ruminants and that from other animals, such as pigs, which do not contract TSE (personal communication, D. Cliver, University of California, Davis, 2003).

Recommendation 7.1: Fund research to improve rapid, accurate, and affordable screening assays for central nervous system (CNS) tissue such that the assays can specifically identify CNS material from cattle in processed meat products.

Economic Impact of a Single BSE Case

The consequences of a single case of BSE discovered in a Canadian cow in May 2003 offered a glimpse of what the United States might experience if a case of BSE were to occur here. Separately, a federal interagency working group concluded that a single case of BSE in the United States could have a greater economic impact on this country than the BSE epidemic has had cumulatively on the United Kingdom (PL 107-9 Federal Inter-agency Working Group, 2003).

Multiple Opportunities for Human Exposure to the CWD Agent

While the United States has conducted three BSE risk assessments since 1991, it has conducted none to determine the risk of human exposure to the agent of CWD. It is possible, though unproven, that human consumption of deer or elk meat infected with the CWD agent could cause TSE. The processing of cervid meat is monitored only superficially, and processors generally make independent decisions about what precautions to take (Means, 2003). Moreover, some meat processors handle both game and beef, creating a theoretical opportunity for cross-contamination. The poorly understood risks of the CWD agent should be characterized scientifically.

Recommendation 7.2: Fund risk assessments that characterize the exposure of hunters, cervid processing establishments, and consumers to the infectious agent of chronic wasting disease.

Preventing TSE Transmission Through Blood Products and Transplants

Neither prions nor TSE infectivity has been detected in human blood, blood products, or blood derivatives. Yet these substances remain theoretical vehicles for the transmission of human TSE agents, especially the agent of vCJD, because scientists have transmitted natural scrapie and BSE to healthy sheep by blood transfusions (Hunter et al., 2002).

The FDA's Center for Biologics Evaluation and Research (CBER) manages blood safety issues for the U.S. population. As a result of lessons learned from the AIDS epidemic, CBER has tightened the entire framework for evaluating and ensuring blood safety (Hoots et al., 2001) and has instituted many policies to protect the blood supply from TSE infectivity.

In the absence of a test to screen blood donors for TSEs, the next-best approach is to inactivate or remove any prions in donated blood. A promising tool for removing prions from plasma products and their derivatives appears to be a filter with pores 15 nanometers in diameter, which experimentally removed enough prions in blood to eliminate infectivity (Tateishi et al., 2001). This filter should be validated further, however.

Certain steps in the commercial processing of blood into plasma and its derivatives appear to reduce but not eliminate TSE infectivity in blood (Brown et al., 1999; Trejo et al., 2003). The experimental use of gamma radiation to inactivate prions in blood also has reduced but not eliminated infectivity (Miekka et al., 2003).

Recommendation 7.3: Fund research to develop novel methods for removing prions from or inactivating prions in blood products and tissues in vitro, using physical, chemical, or immune mechanisms alone or in combination.

In contrast to blood, blood products, and blood derivatives, certain human tissues and hormones have been documented to transmit TSE agents from an infected human donor to a healthy human recipient. Present FDA regulations and guidance to industry have reduced the rate of this form of TSE transmission in the United States. The medical literature reports 267 human cases of iatrogenic transmission of TSE by tissues and hormones (Brown et al., 2000a)—all tragic cases, but small in number by global standards.

Inactivating Prions

The committee believes avoiding TSE infectivity is a preferred strategy, but is at times impractical. People working in research and reference laboratories, hospital operating rooms, and veterinary clinics may have little choice about their proximity to the TSE agent. Thus, it is important to

develop methods to inactivate the agent on surfaces. Researchers in this area have focused on inactivating or destroying prions, primarily PrP^{Sc}.

Standard biosafety methods that eliminate bacterial, viral, and fungal pathogens do not work with prions. Despite their relatively small size, prions are one of the most durable organic substances known. Even turning prion-containing animal tissue to ashes by heating it in a crucible at 600°C does not eliminate infectivity (Brown et al., 2000b).

Investigators have tried to inactivate prions using many physical, chemical, and radiological treatments, with just a few successes (Taylor, 2000). Combinations of steam heat and chemicals appear to work best. Some substances can reduce the prion load, but few can eliminate prions reliably and consistently. The inactivation of prions on glass or steel surfaces appears to be particularly difficult and is a major infection-control concern in hospitals, where surgeons could use certain instruments to operate on prion-infected tissue in patients with preclinical TSE. In the absence of reliable methods to clean instruments, disposal is the only dependable way to prevent contamination, and that is a very expensive option. Hospitals need less costly techniques for preventing the inadvertent spread of TSEs among patients.

Recommendation 7.4: Fund research to develop standard assays for the detection of PrP^{Sc} or TSE infectivity on the surfaces of reusable medical instruments and materials, as well as research to develop better methods to disinfect such instruments and materials.

The inactivation of prions contaminating a relatively small contained space, such as a laboratory work surface, operating room table, or surgical instrument, poses challenges. Far greater challenges are posed, however, by the inactivation of prions in a large contained space, such as an abattoir, or a large open space, such as a pasture. Knowledge of the natural degradation of prions in the environment is extremely limited. Standard methods for evaluating the presence and decay of TSEs in the natural environment are not available, and this has hampered our understanding of the threat posed by long-term environmental contamination by prions.

Recommendation 7.5: Fund research to develop standard test methods for detecting prion contamination in environmental samples.

The longevity of TSE infectivity specifically in soil has tremendous implications for the proper disposal of animals infected with a TSE agent and for these animals' offal and rendered material. For years, the burial of dead farm animals has been a common, acceptable practice. However, large-scale burial of animal material potentially contaminated by TSE agents poses a significant risk (Scientific Steering Committee, 2003). Present research on

this subject has not progressed far enough to provide a sufficient scientific foundation for policy making. Therefore, the United States should intensify research into disposal methods for prion-infected animals and animal tissues. Given that the United Kingdom and Europe need such methods urgently, this area of research appears ripe for international collaboration.

Recommendation 7.6: Fund research to identify safe, cost-effective disposal mechanisms for animals and rendered waste infected with agents of transmissible spongiform encephalopathies. This research would best be conducted with a multidisciplinary approach involving experts in such fields as prion biology, biochemistry, environmental engineering, and commercial disposal technology.

Since the destruction of prions is so difficult, an alternative approach to prevention is to change host resistance to prion infection. This is the concept of immunization, which has been so successful in combating other infectious diseases (CDC, 1999). As discussed above, prions are made from normal host protein that is misfolded, so the host does not recognize them as foreign or mobilize a typical immune response as it does with pathogens containing DNA or RNA. This stymies the use of vaccines to prevent TSEs because vaccines work by exploiting the body's natural immune response. Nevertheless, studies in mice show some marginal positive effects in prolonging life following prion challenge (Sigurdsson et al., 2002).

Treatment for Human TSEs

Many therapeutic agents used to treat human TSEs have produced disappointing results (Brown, 2002). Treatment barriers include the inability to diagnose TSEs in early preclinical stages, the appearance of clinical signs only after advanced CNS infection, the failure of many agents to cross the blood–brain barrier, intrinsic toxicity associated with the therapeutic agents, and difficulties in translating promising therapeutic results from the research laboratory to the clinic. Studies in cell-free systems, in cell cultures, and even in mice have shown reduction or even clearance of prions following treatment, yet these results do not guarantee a cure for humans. To date, no drug or other agent has demonstrated consistent or prolonged success in treating human TSEs.

Some sophisticated, cunning experiments in animals and animal cells have demonstrated that certain modifications to the *Prnp* gene can delay or prevent the development of TSE infectivity in a host challenged with a TSE agent (Holscher et al., 1998; Meier et al., 2003; Perrier et al., 2002). Although the array of effects of altered genes on molecular functions is not completely understood and can lead to unintended consequences, these studies help uncover important linkages between genomics and

proteomics, building a stronger foundation for future endeavors to develop TSE therapeutics.

The committee believes the most promising candidates for near-term therapeutics are synthetic products such as peptides, antibodies, or antibody fragments (scFvs) that bind to specific epitopes on PrP^{C} or PrP^{Sc} and disrupt further PrP conversion (Enari et al., 2001; Heppner et al., 2001; Peretz et al., 2001; Sigurdsson et al., 2003; White et al., 2003). To realize the promise of these synthetic products, researchers will need to uncover and target specific structural sites on PrP^{C}, PrP^{Sc}, or intermediate molecules involved in PrP conversion.

Recommendation 7.7: Fund research to develop new therapeutic agents, including antibodies, that either block the conversion of PrP^{C} to PrP^{Sc} or disrupt the molecular mechanisms of pathogenesis of transmissible spongiform encephalopathies after this conversion has taken place. The most promising approach appears to be rational drug design, which begins with knowledge of the tertiary structure of the protein or molecule that the therapeutic agent will target.

The clues that lead to success in diagnostics will help uncover new therapeutic agents. Likewise, having preclinical diagnostic tests for TSEs will make early treatment—and a better prognosis—possible.

RESEARCH INFRASTRUCTURE

The committee determined that prion science would advance more rapidly in the United States if more investigators worked in this small research community and if more funds were consistently available. The U.S. infrastructure for TSE research, though of high quality, is small compared with its European counterpart. At present fewer than 20 principal investigators conduct prion research funded by the National Institutes of Health (NIH), the largest sponsor of TSE research in the United States. In fiscal year 2002, NIH spent $27.2 million of its total budget of $23.2 billion on TSE research (personal communication, R. Zalusky, National Institute of Neurological Disorders and Stroke, May 27, 2003). Furthermore, 75 percent of the NIH funds awarded for TSE research go to only two laboratories (personal communication, R. T. Johnson, special consultant to NIH on TSEs, 2002).

Investigators and Facilities

It has been difficult to attract new investigators to prion research for several reasons. First is the lack of available laboratory space and the high start-up costs associated with setting up a TSE research laboratory. Such

laboratories have special containment requirements and rely on costly laboratory animals and dedicated equipment that cannot be shared with other researchers because of concerns about cross-contamination. Second, confusion exists within the TSE research community about biosafety-level (BSL) requirements. Formal standards should replace the presently informal and inconsistent guidance in this area. Third, it often takes years to reach experimental end points because prion diseases have relatively long incubation periods. This makes it difficult to attract doctoral and postdoctoral fellows, whose academic programs last for a relatively short time.

Recommendation 8.1: Provide funds to attract and train more investigators in prion disease research. In addition, for investigators conducting prion bioassay research, provide grants for 5- to 7-year periods.

Recommendation 8.2: Provide funds to boost the capacity of the U.S. infrastructure for research on transmissible spongiform encephalopathies by expanding or upgrading existing laboratories, animal facilities, and containment laboratories (biological safety levels 2 and 3), and by building new ones.

Recommendation 8.3: Provide funds to develop scientifically based biological safety level standards for laboratories conducting research that involves infectious agents known to cause transmissible spongiform encephalopathies.

If embraced by the community of organizations that fund TSE research, nationwide BSL standards could serve as a common, consistent framework for regulations governing laboratory biosafety for the full spectrum of TSE research.

Reagents

Because prion biology is a relatively new science, many of the reagents and other materials used by investigators are not commercially available, so each laboratory has needed to produce its own. Consequently, standardization of these reagents and materials across different laboratories or even within the same laboratory is lacking. As a result, the experimental conclusions reported by investigators can be difficult to replicate and easy to challenge. The committee views this as an area that would benefit from attention and funding.

Recommendation 8.4: Provide funds to support new or established transmissible spongiform encephalopathy (TSE) repositories that

contain a collection of reference materials and genetically engineered animals (including transgenic mice), as well as reagents useful for developing TSE diagnostics and for other TSE research. All registered investigators involved in prion research should have access to these collections.

Recommendation 8.5: Provide funds to support the U.S. Food and Drug Administration's development of panels of reference reagents needed to evaluate the performance characteristics of tests designed to detect the prion protein and TSE infectivity. These panels would be used to confirm the performance characteristics of test kits before they are approved for public use, as well as to perform quality control on test kit lots before their release to the market.

International Collaboration

An additional strategy to improve TSE research capacity is to find more opportunities for U.S. researchers to work with investigators in Europe and elsewhere. The committee believes that exploiting opportunities for U.S. investigators to conduct TSE research on site in a European laboratory or to work in a collaborative fashion with a European investigator on a joint research project is not only feasible but also highly desirable.

Recommendation 8.6: Provide funds to enable U.S.-based investigators of transmissible spongiform encephalopathies (TSEs) to collaborate or train with TSE investigators internationally and to use TSE research facilities abroad. Exploiting such opportunities will expand the range of TSE research that U.S. scientists can conduct.

THE RISK OF TSEs TO THE U.S. MILITARY

The committee deliberated upon the risk of prion infection to members of the U.S. military and their families who are or have been deployed to Europe. Discussions focused mainly on the possibilities for consumption of beef products or transfusion of blood products contaminated by prions. The food and blood supplies of deployed U.S. troops are closely controlled and generally originate at U.S. sources. However, some beef products sold in U.S. military commissaries and post exchanges (commonly known as PXs) in Europe were procured from suppliers in the United Kingdom and continental Europe that later reported cases of BSE among their livestock. In exceptional circumstances, some therapeutic blood is also obtained from a host nation's medical facilities.

The committee determined that the U.S. military is at an increased risk for acquiring vCJD as a result of its deployment to Europe. However, that risk was judged to be small and certainly less than that of the local population in the United Kingdom and other European countries reporting BSE.

Recommendation 9.1: Use existing passive surveillance systems to monitor the incidence of Creutzfeldt-Jakob disease and variant Creutzfeldt-Jakob disease among individuals receiving medical care from the health systems of the U.S. Department of Defense and the Department of Veterans Affairs.

CONCLUSION

This final report of the IOM Committee on Transmissible Spongiform Encephalopathies: Assessment of Relevant Science provides an unprecedented overview of the ways in which TSEs impact human and animal health—from donated blood to deer hunting. The committee's recommendations are intended to provide guidance to the NPRP on the most pressing TSE research needs. This report should also serve as a useful reference for those interested in TSEs worldwide. Educated laypeople, physicians, scientists, and TSE experts should all find herein relevant and readable information about deadly diseases that appear to be caused by a new and mysterious biological mechanism.

TABLE S-1 Committee Recommendations by Functional Area and Priority for the National Prion Research Program

Recommendation	Priority (1 = highest)
Basic Research	
3.1 Fund basic research to elucidate: **(1) the structural features of prions** **(2) the molecular mechanisms of prion replication** **(3) the mechanisms of pathogenesis of transmissible spongiform encephalopathies** **(4) the physiological function of PrP^{C}**	**1**
Improving Diagnostics	
Fund research to:	
4.1 Develop new assays most likely to achieve quantum leaps in the quality of prion detection tools, rather than incremental improvements to existing tests. Any efforts to improve existing tests should aim to increase their sensitivities by several orders of magnitude (at least 10^3). The optimal test should detect less than 1 infectious unit (IU) of PrP^{Sc} per unit of ultimate product used (e.g., 1 liter of blood or 100 grams of beef).	**1**
4.2 Improve in vitro techniques that amplify small amounts of PrP^{Sc} to enhance the sensitivities of diagnostic tests.	2
4.3 Develop novel methods and reagents that detect or bind to prions, including new antibodies, peptides, nucleic acids, synthetic derivatives, and chimeric molecules. This research could lead not only to better diagnostics, but also to better therapeutic and prophylactic strategies.	**1**
4.4 Identify surrogate markers or signatures for the detection of prions or prion diseases.	3
4.5 Improve techniques for propagating prions in cultured cells and develop new in vitro cell systems as a means to assay and study prions.	2
4.6 Develop functional imaging for the presence of PrP^{Sc} in brain tissue, leading to an early diagnostic test similar to the imaging diagnostics being developed for Alzheimer's disease.	3
Testing Blood for Evidence of TSEs	
5.1 Fund research (1) to determine the amount of sporadic Creutzfeldt-Jakob disease (sCJD) prions and variant Creutzfeldt-Jakob disease (vCJD) prions in human blood and (2) to estimate the amount of PrP^{Sc} corresponding to one infectious unit of sCJD and vCJD prions in human blood.	**1**

TABLE S-1 Continued

Recommendation	Priority (1 = highest)
U.S. Surveillance for TSEs	
Provide funds to:	
6.1 Promote an increase in the proportion of cases of human neurodegenerative disease, especially suspected cases of transmissible spongiform encephalopathy, that are recognized and autopsied.	2
6.2 Increase the number and diversity of epidemiological studies on human transmissible spongiform encephalopathies (TSEs) in the United States. In particular, support research to identify potential cases of variant Creutzfeldt-Jakob disease and new human TSEs possibly caused by the agent of chronic wasting disease.	2
6.3 Support the development of a nationwide surveillance system for chronic wasting disease in the United States.	2
6.4 Expand research on the natural history, prevalence, distribution, exposure and transmission characteristics, host susceptibility, and host range of transmissible spongiform encephalopathies, especially chronic wasting disease.	**1**
Assessment of Strategies to Prevent and Treat TSEs	
7.1 Fund research to improve rapid, accurate, and affordable screening assays for central nervous system (CNS) tissue such that the assays can specifically identify CNS material from cattle in processed meat products.	2
7.2 Fund risk assessments that characterize the exposure of hunters, cervid processing establishments, and consumers to the infectious agent of chronic wasting disease.	3
Fund research to:	
7.3 Develop novel methods for removing prions from or inactivating prions in blood products and tissues in vitro, using physical, chemical, or immune mechanisms alone or in combination.	2
7.4 Develop standard assays for the detection of PrP^{Sc} or TSE infectivity on the surfaces of reusable medical instruments and materials, as well as research to develop better methods to disinfect such instruments and materials.	2
7.5 Develop standard test methods for detecting prion contamination in environmental samples.	3

Continued

TABLE S-1 Continued

Recommendation		Priority (1 = highest)
7.6	Identify safe, cost-effective disposal mechanisms for animals and rendered waste infected with agents of transmissible spongiform encephalopathies. This research would best be conducted with a multidisciplinary approach involving experts in such fields as prion biology, biochemistry, environmental engineering, and commercial disposal technology.	2
7.7	**Develop new therapeutic agents, including antibodies, that either block the conversion of PrP^{C} to PrP^{Sc} or disrupt the molecular mechanisms of pathogenesis of transmissible spongiform encephalopathies after this conversion has taken place. The most promising approach appears to be rational drug design, which begins with knowledge of the tertiary structure of the protein or molecule that the therapeutic agent will target.**	**1**
Prion Research Infrastructure		
	Provide funds to:	
8.1	**Attract and train more investigators in prion disease research. In addition, for investigators conducting prion bioassay research, provide grants for 5- to 7-year periods.**	**1**
8.2	**Boost the capacity of the U.S. infrastructure for research on transmissible spongiform encephalopathies by expanding or upgrading existing laboratories, animal facilities, and containment laboratories (biological safety levels 2 and 3), and by building new ones.**	**1**
	Provide funds to:	
8.3	Develop scientifically based biological safety level standards for laboratories conducting research that involves infectious agents known to cause transmissible spongiform encephalopathies.	2
8.4	**Support new or established transmissible spongiform encephalopathy (TSE) repositories that contain a collection of reference materials and genetically engineered animals (including transgenic mice), as well as reagents useful for developing TSE diagnostics and for other TSE research. All registered investigators involved in prion research should have access to these collections.**	**1**
8.5	Support the U.S. Food and Drug Administration's development of panels of reference reagents needed to evaluate the performance characteristics of tests designed to detect the prion protein and TSE	3

TABLE S-1 Continued

Recommendation	Priority (1 = highest)
infectivity. These panels would be used to confirm the performance characteristics of test kits before they are approved for public use, as well as to perform quality control on test kit lots before their release to the market.	
8.6 Enable U.S.-based investigators of transmissible spongiform encephalopathies (TSEs) to collaborate or train with TSE investigators internationally and to use TSE research facilities abroad. Exploiting such opportunities will expand the range of TSE research that U.S. scientists can conduct.	3
Risks to the U.S. Military	
9.1 Use existing passive surveillance systems to monitor the incidence of Creutzfeldt-Jakob disease and variant Creutzfeldt-Jakob disease among individuals receiving medical care from the health systems of the U.S. Department of Defense and the Department of Veterans Affairs.	3

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1 Introduction

In June 2002, the Institute of Medicine (IOM) formed the Committee on Transmissible Spongiform Encephalopathies: Assessment of Relevant Science to assist the U.S. Department of Defense (DOD) in its administration of the National Prion Research Project (NPRP). Congress had established NPRP and had directed the U.S. Army Medical Research and Materiel Command (MRMC) to administer the program, allocating $50 million to fund it (U.S. Senate Committee on Appropriations, 2001). Congress ultimately reduced the allocation to $42.5 million. MRMC manages NPRP through the preexisting Congressionally Directed Medical Research Program.

NPRP issued its first call for proposals in August 2002 (DOD, 2002). To evaluate the proposals submitted, the program uses the two-tiered approach recommended by the Institute of Medicine (IOM, 1993). Proposals first undergo peer review for scientific merit. Those passing review undergo another level of review that involves evaluating how well the proposed research would support NPRP's objectives. Subject-matter experts, clinicians, and consumers chosen by DOD conduct the programmatic review (DOD, 2002).

To support this proposal evaluation process, DOD asked the IOM to provide independent advice on the state of prion science and the field's most pressing research needs (see Box 1-1). In June 2002, the IOM formed an 11-member committee supplemented by six consultants who are internationally recognized experts in prion research; a member of the Board of the Medical Follow-up Agency was added to the committee as a liaison in early 2003. The committee members were selected for their expertise in

BOX 1-1
Statement of Task
Committee on Transmissible Spongiform Encephalopathies: Assessment of Relevant Science

The Committee will assess the state of science regarding transmissible spongiform encephalopathies (TSEs) and advise the Medical Research and Materiel Command and its Congressionally Directed Medical Research Program Office.

Specifically, the Committee will:

- Recommend research that will best lead to sensitive, reproducible and inexpensive methods for detecting prions and in diagnosing prion diseases/TSE. This will be based on an assessment of critical technologies (current and novel) needed to detect prions.
- Assess the status of currently available assays and their detection limits based on strains and biological system employed.
- Assess the availability of standardized and reference reagents as well as physical facilities required to validate assays.
- Recommend key opportunities for collaboration with foreign investigators that would facilitate the development of effective prion detection methods.
- Assess the availability of trained investigators and specialized facilities dedicated to prion research and identify any critical gaps.
- Assess the role that prion diseases pose for the military force including but not limited to the military's food and blood supply.
- Recommend relevant surveillance efforts and public health policies at home and abroad regarding TSEs in humans, livestock, and wildlife that impact on military health or that urgently require further research.
- Provide recommendations for future TSE research.

infectious disease, prion molecular biology, microbiology, neurology, epidemiology, blood banking, veterinary medicine, and food safety. The consultants provided essential technical insight. The committee evaluated information from the sponsor, peer-reviewed journal articles provided by committee staff, and presentations by invited guests with expertise relevant to prion science (see Appendix A).

CHARGE TO THE COMMITTEE

The committee was tasked to produce both an interim and a final report. The interim report, published in January 2003, provided guidance to the panel that conducted the programmatic review for NPRP's fiscal year 2002 grants. The report concentrated on diagnostics for transmissible spongiform encephalopathies (TSEs), the focus of both the law establishing NPRP and the program announcement. The law states: "The priority goal of the Project's first phase is to rapidly develop a diagnostic test to detect the presence of prion disease." The investment strategy and guidance published in the NPRP program announcement reflect this imperative (DOD, 2002). Nearly 50 percent of the funds allocated in fiscal year 2002—$20 million—was to support investigator-initiated research designed to do the following:

- Develop a rapid, sensitive, and reproducible test for the detection of prions suitable for use as an antemortem diagnostic test.
- Develop a rapid, sensitive, and reproducible test for the detection of prions suitable for use as a screening assay.
- Study the prevention, transmission, inactivation, or pathogenesis of TSEs, including chronic wasting disease (CWD) (see Table 1-1).

This, the committee's final report, recommends the highest-priority research in TSE surveillance, prevention, and therapy. It also contains an updated version of the material from the first report, with an additional chapter on the unique challenges of detecting TSE infectivity in blood.

This report could help shape the objectives of NPRP should the program receive future funding. The report is also intended to perform a public service by providing the most comprehensive overview known to the committee and consultants of the many ways in which TSEs impact human and animal health. Educated laypeople, physicians, scientists, and TSE experts should find relevant information herein about a class of diseases that appear to be caused by a new and mysterious biological mechanism.

ORGANIZATION OF THE REPORT

Chapter 2 provides background information about prion diseases, while Chapter 3 reviews the basic research requirements to advance prion science. Chapters 4, 5, and 6 address, respectively, diagnostics for TSEs, testing of blood for evidence of TSE agents, and measures taken by the United States to conduct surveillance for the occurrence of TSEs in people and animals. Chapter 7 provides an assessment of strategies to prevent and treat TSEs.

TABLE 1-1 NPRP Award Mechanisms

Award Mechanism	Experience of Principal Investigator	Key Mechanism Elements	Dollars Available
Idea Awards	All levels of experience	• Reward innovative ideas and technology • No preliminary data required	$375K for direct costs over a 3-year performance period, plus indirect costs as appropriate
Investigator-Initiated Research Awards *(with optional Nested Postdoctoral Traineeship[s])	Independent investigators at any level **Nested Postdoctoral Trainees:* Recent doctoral graduates with 3 years or less of postdoctoral experience	• Sponsor basic and clinically oriented TSE research • Preliminary data required • Encourage development of partnerships between academic and industry researchers or between an established TSE researcher and a researcher from another discipline to leverage diverse expertise and resources toward development of antemortem diagnostics	Maximum of $2.5M, inclusive of direct and indirect costs, for a performance period of up to 5 years **Nested Postdoctoral Traineeships:* Maximum of $60K per year inclusive of direct and indirect costs for a maximum of $180K per trainee over 3 years

Career Transition Awards	Postdoctoral fellows	Encourage scientists or clinicians currently in postdoctoral and/or fellowship training positions to pursue a TSE-related research career	*Postdoctoral fellow (years 1–2):* Average of $60K/year, inclusive of direct and indirect costs, for a maximum of $120K *Junior faculty (years 3–5):* Average of $100K/year in direct costs, for a maximum of $300K, plus indirect costs as appropriate
Prion Techniques Fellowship Awards	• Postdoctoral trainees, medical residents, or clinical fellows; or • Researchers with independent program of prion research; or • Researchers with established independent program of research with limited or no experience in prion field	Offer investigators the opportunity to work in the laboratory of established TSE researchers in order to acquire critical skills or learn new methods relevant to TSE research	Up to $125K for up to 1 year, inclusive of direct and indirect costs

NOTE: K = thousand, M = million.
SOURCE: Adapted from Department of Defense Fiscal Year 2002 National Prion Research Program, Program Announcement, Part 1. http://cdmrp.army.mil/funding/archive/02nprp.pdf.

The U.S. infrastructure for TSE research is reviewed in Chapter 8, while Chapter 9 presents an assessment of the risks of TSEs to the U.S. military.

Throughout the text, the committee presents its recommendations in bold print, numbered by chapter for ease of reference. (Table S-1, presented in the Summary, lists all of the committee's recommendations.) Clearly, we believe all the recommendations in this report are important. Given that NPRP has a limited amount of resources, however, it can act on only a limited number of recommendations each fiscal year. Therefore, we have prioritized the recommendations by placing them in one of three ranks, 1 being the highest. We assigned approximately a third of the recommendations to each rank. The rankings are based upon the following criteria:

- Impact on public health
- Impact on protecting animal health (mainly cattle)
- Impact on protecting the U.S. economy (bovine spongiform encephalopathy [BSE]/CWD)
- Impact on advancing scientific understanding of prions (potential for breakthroughs)
- The need to accomplish first rather than second (stepwise progression of the science)
- Return on investment
- Likelihood of success (How feasible is it?)

In addition to prioritizing all of our recommendations, we indicate the most urgent and critical areas of research related to recommendations 3.1 and 6.4 (see Boxes 3-1 through 3-4 and Box 6-3 in the respective chapters).

The report ends with two appendices: Appendix A contains the agendas for the five meetings held by the committee during the course of the study, while Appendix B presents biographical sketches of the committee members and consultants.

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2 Prion Diseases: An Overview

This chapter provides the historical backdrop for today's research into prion diseases, also called transmissible spongiform encephalopathies (TSEs). Most of this history occurred during the past century. In fact, the outbreaks of bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD)—catalysts for much of the present research on TSEs—occurred within the past two decades.

Prions, also called PrP^{Sc}, and their normal, cellular isoform, PrP^{C}, are encoded by the *PRNP* gene on chromosome 20 in humans. Like all proteins, PrP^{C} has a characteristic conformation. Under certain conditions, however, it folds into an abnormal shape that is associated with fatal neurodegeneration after a long incubation period. This report uses the terms prion and PrP^{Sc} interchangeably in reference to the abnormally folded, protease-resistant protein associated with TSEs. However, the committee is sensitive to the fact that such usage is controversial. We want to explain our choice of words in the context of this controversy.

If prion aggregates represent the infectious agent that causes TSEs and if prions also are the misfolded protein known as PrP^{Sc}, then by parallel reasoning, aggregates of PrP^{Sc} represent the infectious agent of TSEs. This view, known as the protein-only theory, has many proponents. Yet some reputable TSE experts believe that PrP^{Sc} alone may be insufficient to cause a TSE infection, although the protein may be associated with and even necessary for such an infection. A number of these investigators hypothesize that the infectious particle may contain hidden nucleic acid, additional proteins, or some other additional, essential material. This camp uses the term prion to refer to TSE infectivity, but does not equate prion with PrP^{Sc}. To respect

and recognize this alternative point of view, the report often uses the phrase "the infectious agent of [TSE under discussion]" instead of the term prion or PrP^{Sc}.

The committee believes that the preponderance of scientific evidence favors the hypothesis that prions, consisting of PrP^{Sc}, are associated with infection in TSEs. Nevertheless, the purpose of this report is not to resolve the controversy or to proffer the committee's opinion regarding the prion hypothesis. Rather, the purpose of this report is to call for additional research—especially studies of the fundamental molecular structures and mechanisms related to TSEs—so that disparate views may converge and advance prion science.

ORIGINS AND DEVELOPMENT OF PRION SCIENCE

The identification of a previously unknown malady in the Fore Tribe of Papua New Guinea drew international attention to the group of brain-wasting diseases called TSEs. Physicians Vincent Zigas and Daniel Carleton Gajdusek in 1957 described an epidemic among the Fore people characterized by loss of balance, dementia, and death (Gajdusek and Zigas, 1957). The tribe called the illness *kuru*, meaning to tremble or to shiver. Studies of the brains of deceased patients revealed widespread neurodegeneration marked by vacuoles in the cytoplasms of nerve cells (Klatzo et al., 1959). The vacuoles gave the victims' brains a spongelike appearance at the microscopic level—hence the term spongiform encephalopathy.

Veterinary neuropathologist William Hadlow was the first to recognize similarities between kuru and scrapie, a TSE of sheep and goats that had been known since the 1700s. He pointed out in a 1959 letter to *The Lancet* that the brains of mammals with both conditions had a unique form of widespread neuronal degeneration. "Large single or multilocular 'soap-bubble' vacuoles in the cytoplasm of nerve-cells have long been regarded as a characteristic finding in scrapie," he wrote; "this extremely unusual change, apparently seldom seen in human neuropathological material, also occurs in kuru, and first aroused my curiosity about the possible similarity of the two diseases" (Hadlow, 1959:290).

Scrapie and kuru both were endemic to specific populations in which the usual incidence was low, he added. Clinical symptoms could appear months after a victim had been separated from the source community, and both diseases were found in previously healthy communities after the introduction of an individual from a known source community. Hadlow also noted that data suggested a genetic predisposition toward both diseases, which did not appear to be infectious in the traditional sense. Victims exhibited increasingly severe ataxia, tremors, and behavioral changes, yet no consistent abnormalities appeared in their blood or cerebrospinal fluid. Both

diseases began insidiously, he wrote, "and usually end fatally . . .; only rarely have remissions and recoveries been observed"[1] (Hadlow, 1959:290).

On the basis of these observations, Hadlow suggested that experimental transmission of kuru into nonhuman primates might prove fruitful, since veterinary scientists were successfully investigating scrapie by inoculating healthy sheep and goats with brain tissue from animals with the disease. After extensive work, Gajdusek and colleagues did transmit a "kuru-like syndrome" with an incubation period of 18 to 21 months to chimpanzees by inoculating them with brain suspensions from kuru patients (Gajdusek et al., 1966), indicating that a noninflammatory neurodegenerative disease could be transmissible.

Ethnological and epidemiological studies indicated that kuru was transmitted during an endocannibalistic[2] funeral ritual (Alpers, 1968; Gajdusek, 1977; Glasse, 1967). Women would remove the brain of a deceased relative, eat it along with other tissues, and smear it over their bodies and those of young children of both sexes (Gajdusek, 1977; Glasse, 1967). Women who fell victim to kuru outnumbered men who fell victim to the disease by more than 14 to 1 (Gajdusek and Zigas, 1957). After a 1957 ban on cannibalism in Papua New Guinea, the number of kuru cases gradually declined over decades, reaching single figures in recent years (Huillard d'Aignaux et al., 2002; Klitzman et al., 1984).

Similarities in the neuropathology of kuru and of a rare, fatal condition called Creutzfeldt-Jakob disease (CJD) led investigators to attempt experimental transmission of CJD to nonhuman primates. The disease was transmitted successfully to a chimpanzee, which first displayed clinical signs after a 13-month incubation period, providing more evidence that spongiform encephalopathies are transmissible (Gibbs et al., 1968).

These studies and observations generated a groundswell of interest in discovering the nature of the infectious agent or agents that caused scrapie and kuru. Although many scientific articles referred to the scrapie agent as a slow virus, a number of new hypotheses on the nature of the kuru and scrapie agents surfaced between 1962 and 1981, ranging from a small DNA virus to a replicating polysaccharide to naked nucleic acid similar to plant viroids (Prusiner, 1982). None of these explanations gained widespread acceptance, however, and the cause of scrapie remained an enigma.

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[1]It was later discovered that the apparent cases of kuru reported to be in remission or recovery were not, in fact, kuru. In other words, kuru is uniformly fatal.

[2]Endocannibalism: humans eating the tissue of other humans who belong to their tribe.

The Birth of Molecular Prion Science

A rapid succession of discoveries about scrapie during the early to mid-1980s marked the birth of molecular prion science. In 1981, scientists first recognized the rod-shaped structures named scrapie-associated fibrils in concentrated suspensions from scrapie brain (Mertz et al., 1981). Investigators identified in partially purified samples of such suspensions the predominant protein band that proved to be the prion protein (PrP) (Prusiner et al., 1981).

In 1982, Prusiner asserted that the infectious agent of scrapie was either a protein or a small nucleic acid surrounded by a tightly packed protein (Prusiner, 1982, 1999). He called this infectious agent a prion, which stands for "small, proteinaceous infectious particles that are resistant to inactivation by most procedures that modify nucleic acids" (Prusiner, 1982:141). At the time, the theory that replication of microorganisms and viruses requires nucleic acids was well established. Yet several investigators had iconoclastically proposed that the infectious agent of scrapie might not require nucleic acids and could be a replicating protein (Alper et al., 1967; Griffith 1967; Lewin, 1972; Pattison and Jones, 1967). Until Prusiner's entry into the field, however, no investigator had provided compelling data to support this hypothesis.

Applying advanced biochemical techniques, Prusiner demonstrated that the scrapie agent resisted six different procedures known to attack nucleic acids and was susceptible to six methods of protein inactivation (Prusiner et al., 1981; Prusiner, 1982). It was later established that prions and PrP^{Sc} were resistant to limited digestion by one of those methods, exposure to proteinase K (McKinley et al., 1983). Like some investigators whose theoretical work preceded him, Prusiner (1982:139) correctly suggested that a prion might act as "an inducer or template for its own synthesis." The following year, Diringer and colleagues demonstrated TSE infectivity in fibril-containing suspensions from scrapie-infected brain tissue (Diringer et al., 1983).

The determination of a partial amino acid sequence for PrP (Prusiner et al., 1984) facilitated the production of corresponding oligonucleotides, which were used independently by three groups—those of Robakis, Chesebro, and Weissmann—to isolate cDNA clones corresponding to the PrP mRNA in scrapie brain tissue (Chesebro et al., 1985; Locht et al., 1986; Oesch et al., 1985; Robakis et al., 1986). The cDNA clones corresponding to the full-length PrP protein sequence for mouse and hamster were found by the Chesebro and Robakis groups, respectively (Locht et al., 1986; Robakis et al., 1986). All three groups found PrP mRNA in both scrapie-infected and uninfected brain, indicating that *Prnp* was a normal host gene and did not come from the genome of an exogenous infectious agent.

In addition, Oesch and colleages (1985) reported that PrP from scrapie brain is partially resistant to digestion by proteases, whereas PrP from normal brain is not resistant. This pivotal finding is the basis for the Western blot diagnostic test for the presence of PrP^{Sc} in brain and other tissues in virtually all types of human and nonhuman TSEs, as discussed in Chapter 4.

After two decades of research, many—but not all—TSE experts accept the protein-only hypothesis. Prusiner won the Nobel Prize in physiology or medicine in 1997 for his groundbreaking work. But because Koch's postulates[3] have not been demonstrated for prions, some scientists believe that prions alone do not explain all aspects of the etiology of TSEs (Chesebro, 1998, 1999; Rohwer, 1991). An overview of all known TSEs appears in Table 2-1.

THE NATURE OF PRIONS AND PRION PROTEIN

The normal, cellular prion protein, PrP^{C}, resides on the membranes of many avian and mammalian cells. Its physiologic function is poorly understood, although its function or loss of function could possibly contribute to some aspects of the disease state in TSEs.

Primary Structure[4] of Human PrP^{C}

Human PrP^{C} is a string of 253 amino acids (see Figure 2-1). At one end, called the C-terminus, the molecule is attached to the cell membrane by a glycosyl phosphatidylinositol (GPI) moiety known as the GPI anchor. This anchor is added to the molecule when amino acids 231–253, known as the GPI signal sequence, are removed during a process called transamidation. Two cystine amino acid residues form a tight bond in the C-terminus region. In addition, various complex carbohydrates attach to asparagine amino acids in this region. Some important determinants of the protein's tertiary structure lie in the central portion of the molecule, where the protease-resistant segment resides. Gene mutations or polymorphisms on the PrP gene cause amino acid substitutions on PrP^{C} that can either predispose a person to or protect one from TSEs.

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[3]Koch's postulates are "criteria for proving that a specific type of microorganism causes a specific disease. 1) The organism should be constantly present in the animal suffering from the disease and should not be present in healthy individuals. 2) The organism must be cultivated in pure culture away from the animal body. 3) Such a culture, when inoculated into susceptible animals, should initiate the characteristic disease symptoms. 4) The organism should be reisolated from these experimental animals and cultured again in the laboratory, after which it should still be the same as the original organism" (Brock et al., 1994:19).

[4]Primary structure denotes the order of amino acids in a polypeptide.

TABLE 2-1 Classification of TSEs

Type of TSE	Affected Mammals	Modes of Natural Transmission	Date First Recognized
In humans			
Sporadic Creutzfeldt-Jakob disease (sCJD)		Unknown	1920
Sporadic fatal insomnia (sFI)		Unknown	1997[a]
Familial Creutzfeldt-Jakob disease		Genetic	1924[b]
Fatal familial insomnia (FFI)		Genetic	1986[c]
Gerstmann-Sträussler-Scheinker disease		Genetic	1936[d]
Kuru		Exposure to contaminated human tissues during endocannibalistic rituals	1957
Iatrogenic Creutzfeldt-Jakob disease		CJD-infected surgical equipment or tissue transplants	1974[e]
Variant Creutzfeldt-Jakob disease (vCJD)		Food-borne exposure to BSE-infected tissue; other modes[f]	1996
In animals			
Scrapie	Sheep, goats	Contact with infected sheep, placenta, or contaminated environment; possible oral exposure	18th century
Transmissible mink encephalopathy	Mink	Food-borne exposure to infected tissue	1947
Chronic wasting disease (CWD)	Deer, elk	Unknown; likely oral	1967
Bovine spongiform encephalopathy (BSE)	Cattle	Food-borne exposure to TSE-infected tissue	1986

TABLE 2-1 Continued

Type of TSE	Affected Mammals	Modes of Natural Transmission	Date First Recognized
In animals (continued)			
BSE (continued)	Nyala, gemsbok, Arabian oryx, eland, kudu, scimtar-horned oryx, puma, cheetah, ocelot, tiger, African lion, Asiatic golden cat[g]	Food-borne exposure to BSE-infected tissue	1986
Feline spongiform encephalopathy	Domestic cat	Food-borne exposure to BSE-infected tissue[h]	1990

[a]Mastrianni et al. (1997).
[b]Gambetti et al. (1999). This chapter cites Kirschbaum (1924) as reporting the first authentic case of familial Creutzfeldt-Jakob disease.
[c]Lugaresi et al. (1986).
[d]Kretzschmar et al. (1991).
[e]Duffy et al. (1974).
[f]It is unknown whether the disease is transmissible by transfusion or transplantation.
[g]The TSE affecting these zoo animals was called "exotic ungulate encephalopathy" until transmission studies demonstrated that some of the animals had BSE. It is believed that these animals became infected by eating feed—or, in the case of exotic feline species, fresh bovine tissues—contaminated with the BSE agent.
[h]Domestic cats develop feline spongiform encephalopathy in nature by eating feed contaminated with the BSE agent.
SOURCES: Godon and Honstead (1998), Johnson and Gibbs (1998), Haywood (1997); personal communication, E. Williams, University of Wyoming, December 2002; Prusiner (1995), and Young and Slocombe (2003).

At the opposite end of the protein, called the N-terminus, lie five repeating sequences of eight amino acids. It is thought that these "octapeptide repeats" have a strong affinity to copper, giving the protein an antioxidant function (Brown and Sassoon, 2002). The 22 amino acids at the N-terminus are removed as the protein is synthesized in the endoplasmic reticulum. Then the carbohydrates and the GPI anchor are added to the protein, and it migrates to the cell surface membrane.

Conversion of PrP^C to PrP^{Sc}

Mature human PrP^C polypeptide consists of amino acids 23–230. The three-dimensional structure of this protein is depicted in Plate 2-1 (Zahn et

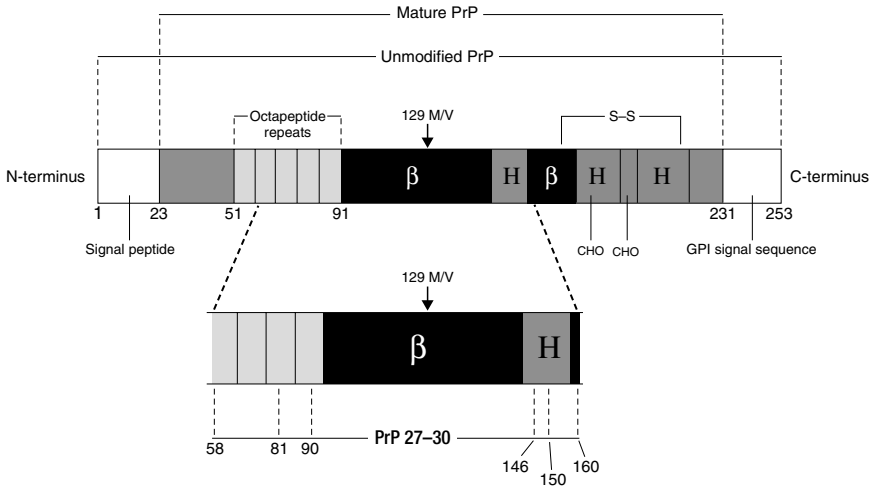

FIGURE 2-1 Diagram of the primary structure of normal human prion protein, PrP^{C}. The complete, unmodified polypeptide has 253 amino acid residues, while the mature polypeptide extends from residues 23 to 230. An arrow indicates the approximate position of the methionine-valine (M/V) polymorphism at codon 129. Other important features include the two β-sheet regions (β), three α-helical regions (H), internal disulfide bond (S-S), two glycosylation sites (CHO), and sequence of eight residues repeated five times in a row (octapeptide repeats). The lower, enlarged image depicts PrP 27–30, a PrP region of variable length that resists proteinase-K digestion more than any other part of the protein. Vertical dotted lines labeled with the number of an amino acid residue indicate the points at which proteinase K is believed to cleave PrP to produce PrP 27–30.
NOTE: GPI = glycosyl phosphatidylinositol.
SOURCE: Adapted from Gambetti et al. (1999:512).

al., 2000); notice the three α-helices and the two flat regions, called β-sheets. By a poorly understood mechanism, prions convert PrP^{C} into the abnormally folded conformation (see Plate 2-2), which contains more β-sheets than the normal isoform. In a demonstration of the pivotal role of normal prion proteins in the progression of TSEs, mice in which PrP^{C} expression was knocked out or ablated remained healthy after infection with prions (Bueler et al., 1993). It is widely hypothesized that one or more so-called chaperone molecules may play a role in the conversion of PrP^{C} to PrP^{Sc} (Chernoff et al., 1995; Telling et al., 1995). Figure 2-2 depicts the presumed processes of PrP production, conversion, and degradation (Caughey, 2002).

Unlike PrP^{C}, the aberrantly folded PrP^{Sc} can aggregate and become in-

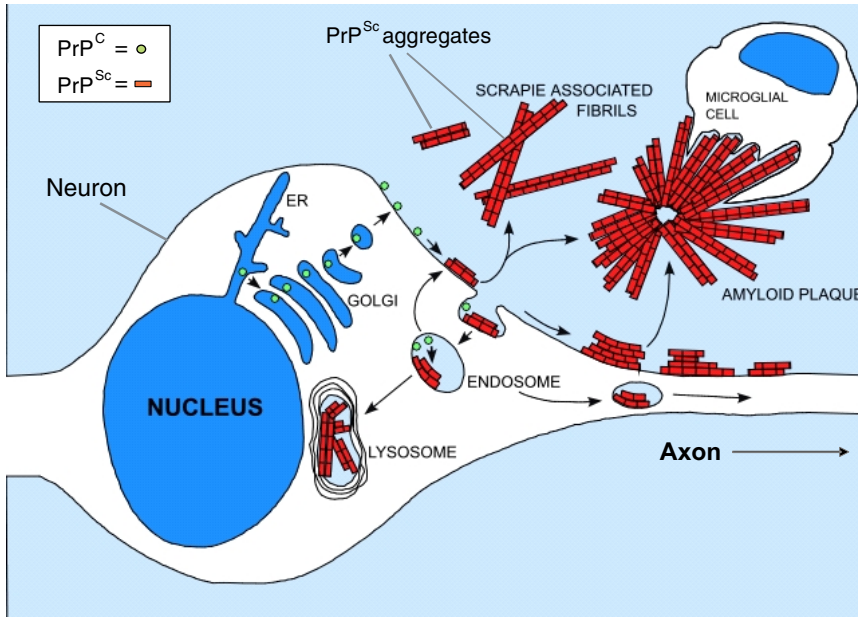

FIGURE 2-2 Model of PrP^{Sc} formation and deposition in a neuron infected with the agent of TSE. Normal cellular prion protein, PrP^{C} (grey circles), is produced in the endoplasmic reticulum (ER), processed in the Golgi apparatus, and transported to the cell surface. There, PrP^{C} normally has a short half-life as a result of endocytosis followed by proteolytic degradation within lysosomes. In cells infected with the TSE agent, PrP^{C} is converted to PrP^{Sc} (dark rectangles) on the cell surface or in endosomes. The conversion of PrP^{C} occurs upon contact with PrP^{Sc} clusters and, perhaps, accessory molecules. Unlike PrP^{C}, PrP^{Sc} is resistant to proteolytic degradation and can accumulate within lysosomes, on the cell surface, or in extracellular deposits, such as rod-shaped fibrils or amyloid plaques. These abnormal accumulations are ultimately neurotoxic. However, it is unclear whether the accumulations cause neurotoxicity directly or whether they cause it indirectly through changes induced in accessory cells, such as microglia (which often surround amyloid plaques) or astrocytes (not shown). Reprinted from Caughey and Chesebro (2002) with permission from ASM Press. Copyright 2002 by ASM Press.

soluble, in which case it resists complete digestion by proteinase K (Prusiner, 2001).[5] To date it appears that the immune system does not recognize and

[5] PrP^{Sc} demonstrates a gradient of resistance to proteinase K (PK) and is associated with infectious potential and TSEs even in circumstances when it is sensitive to PK digestion. By contrast, the term PrP^{res} stands for abnormally folded prion protein that is highly resistant to PK digestion and is strongly associated with TSEs. PrP^{res} is sometimes used synonymously with PrP^{Sc}, creating confusion about the differences between the two proteins.

destroy PrP^{Sc} despite its distinct conformation, presumably because it has the same primary structure as PrP^{C}.

Pathogenesis of TSEs

In experiments in which animals received PrP^{Sc} by peritoneal inoculation or through the gastrointestinal tract, the proteins migrated to the lymphoreticular system and propagated there (Brown et al., 1999; Weissmann et al., 2001). The propagation of prions in the lymphoreticular system is not essential for neuroinvasion, however. PrP^{Sc} migrates to the brain along peripheral nerves (Beekes et al., 1998; Kimberlin et al., 1983; Oldstone et al., 2002; Race et al., 2000). The misfolded proteins aggregate into rod-shaped fibrils, and it has been hypothesized that prions at the ends of the rods continue converting normal PrP into PrP^{Sc} (Caughey, 2002). By an unknown mechanism, the aggregated prions appear to destroy nerve cells and create microscopic vacuoles in the brain. Clinically, this destruction manifests itself differently in different species and in different TSEs within the same species, but the process appears to lead inevitably to death.

Prion Strains

Both the incubation period of a TSE and the length of time between the onset of clinical symptoms and death vary widely depending on the host species and the strain of PrP^{Sc}. Investigators initially differentiated among prion strains through clinical observation of goats that displayed either drowsy or hyperactive behaviors (Pattison and Millson, 1961). Later work with mice revealed that genetic factors play a role in determining strain differences. Dickinson and colleagues identified in inbred mice two different alleles, called sinc (strain incubation) genes, that consistently resulted in a long or short incubation period prior to the onset of disease (1968). The investigators later published additional findings that strains could be differentiated by the distribution of microscopic lesions (vacuoles) in the brain (Fraser and Dickinson, 1973). Studies using the agent of transmissible mink encephalopathy in hamsters showed differences in clinical presentation and PrP^{Sc} glycoform patterns by prion strain (Bessen and Marsh, 1992).

Recently, the use of selected inbred and transgenic mice to characterize prion strains has led to important insights, including the idea that a similar strain causes both BSE and vCJD (Bruce et al., 1997; Scott et al., 1999). Even more recently, physicochemical methods have been employed to characterize and differentiate strains. These include the Western blot to demonstrate different glycoform patterns of PrP^{Sc} (Collinge et al., 1996) and the immunoassay to identify different molecular conformations of PrP^{Sc} (Safar et al., 1998).

Despite these advances, much about prion strains remains a mystery. Researchers must be able to differentiate one strain from another and to determine the scope of strain diversity, the special characteristics of a strain's conformation that make the strain unique, a host's susceptibility to various strains, and how incubation periods and patterns of disease expression vary by strain and host.

THE EPIDEMIC OF BSE AND THE EMERGENCE OF vCJD

Prion diseases remained obscure outside the circles of infectious disease specialists and neurologists until the mad cow epidemic struck in the United Kingdom. The illness was first recognized in 1985 when a handful of cattle from disparate locations in the United Kingdom began dying of a strange illness marked by insidious onset, progressive behavioral and locomotive signs, and death (Wells et al., 1987; Wilesmith et al., 1988). Neuropathological examination of the sick cattle's brains revealed abnormal, microscopic vacuoles and fibrils, much like the spongiform characteristics of scrapie and kuru. A team of veterinary scientists at the United Kingdom's Ministry of Agriculture reported in 1987 that the new disease strongly resembled the so-called unconventional viral-agent encephalopathies previously observed in sheep and the Fore people, so the scientists named the disease bovine spongiform encephalopathy (Wells et al., 1987). The outbreak appeared mainly in dairy cows rather than beef cattle (Anderson et al., 1996) because it was much more common to feed meat-and-bone meal, the attributed source for BSE transmission, to dairy cows than to beef cattle.

BSE quickly ballooned into an epidemic that peaked at more than 37,000 annual cases in the United Kingdom in 1992 (see Figure 2-3) (Department for Environment, Food and Rural Affairs, 2002). It has been estimated that 840,000 to 1.25 million infected animals entered the human food chain from 1974 through 1995 (Anderson et al., 1996; Wilesmith et al., 1992).

Conscious of the fact that the transmission of BSE to humans was a possibility, the United Kingdom in 1990 increased epidemiological surveillance of CJD, a rare human spongiform encephalopathy. It was thought that changes in the pattern of CJD could signify a link to BSE. The neuropathological profiles and age distribution of 10 of the 207 patients with CJD examined between 1990 and 1997 differed markedly from those typical for CJD (Will et al., 1996). This discovery led to the conclusion that a new variant of CJD had arisen in the United Kingdom. Biochemical studies revealed that the new variant, vCJD, involved the same prion strain implicated in BSE (Collinge et al., 1996), and transmission studies with inbred mice confirmed this finding (Bruce et al., 1997; Collinge et al., 1996).

There is evidence of genetic susceptibility to vCJD. A 1997 study found

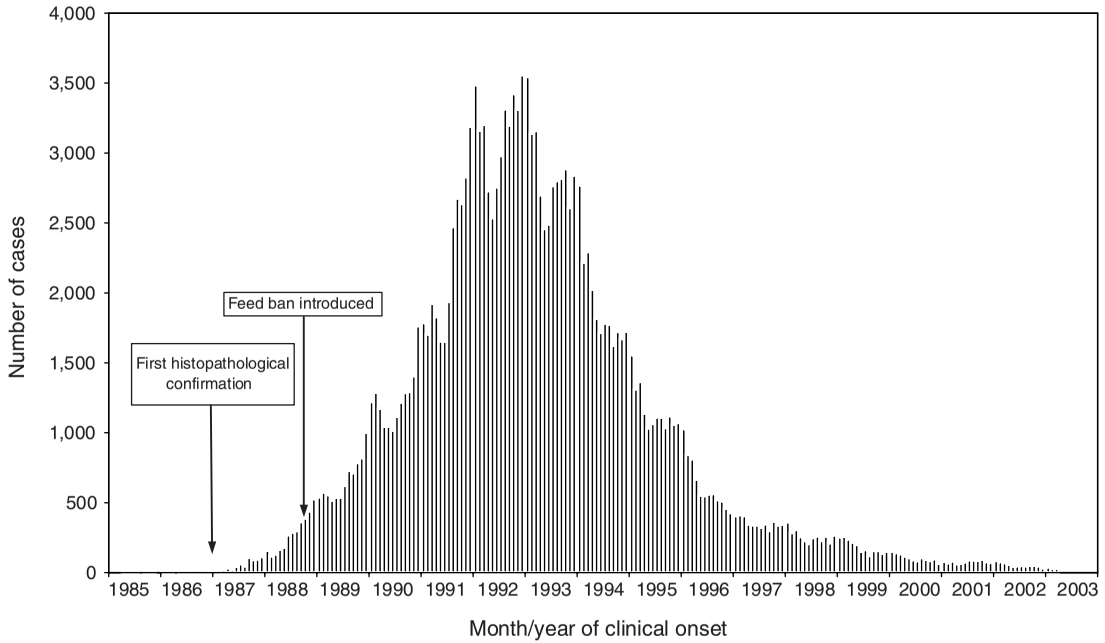

FIGURE 2-3 Confirmed cases of BSE by month and year of clinical onset. Reprinted with permission from the Department for Environment, Food and Rural Affairs (DEFRA), United Kingdom (2003). Copyright 2003 by DEFRA.

that a specific genotype of codon 129 in *PRNP* was correlated with vCJD in all 26 patients tested (Zeidler et al., 1997). This codon normally codes for methionine on one allele and valine on the other, or the alleles may be homozygous, but the *PRNP* genes from all patients tested were consistently homozygous for methionine (Collinge et al., 1996). It is possible, however, that individuals with the other genotypes are susceptible, but that their incubation periods may be longer. It is well known that substantial variation in the incubation periods of strains of a mouse-adapted scrapie agent results from their passage through mice with different PrP genotypes.

As of September 3, 2003, 136 residents of the United Kingdom have died of definite or probable vCJD since 1990 (The UK Creutzfeldt-Jakob Disease Surveillance Unit, 2003). Until recently, graphs of the quarterly incidence of vCJD onsets and deaths took an exponential trajectory, suggesting the epidemic would grow for the foreseeable future. Then in early 2003, scientists reported for the first time that the quarterly incidence of vCJD onsets and deaths in the United Kingdom no longer appeared to be increasing exponentially (see Figure 2-4) (Andrews et al., 2003). This finding suggested that the primary epidemic,[6] which includes exclusively individuals who are homozygous for methionine at *PRNP* codon 129, might ultimately be less extensive than expected.

Additional evidence for a smaller-than-expected primary epidemic comes from Ghani and colleagues (2003). After analyzing the United Kingdom's vCJD statistics through 2002, the group forecast that the worst-case number of *PRNP* 129 methionine-homozygous vCJD cases will be lower than the group had previously projected.

According to Andrews and colleagues (2003:751) it would be premature to conclude that the overall vCJD epidemic is in permanent decline. They refrain from predicting the epidemic's size or end point because the new statistical trend—a quadratic model—is appropriate only for short-term forecasts (2003). Moreover, they note, the epidemic's future is clouded by many unknowns: the uncertain incubation period of vCJD in people who are heterozygous at *PRNP* codon 129 or are homozygous for valine; the possibility that subgroups within the methionine-homozygous population have different incubation periods; the possibility that there are unidentified strains of BSE that incubate longer in humans than does the known strain; and the possibility of human-to-human transmission through blood products, surgical instruments, or tissue transplants.

[6]Scientists speculate that a secondary vCJD epidemic may occur in the future in individuals who are homozygous for valine (V/V) or are heterozygous (M/V) at *PRNP* codon 129 because they may incubate the disease longer than the methionine-homozygous population.

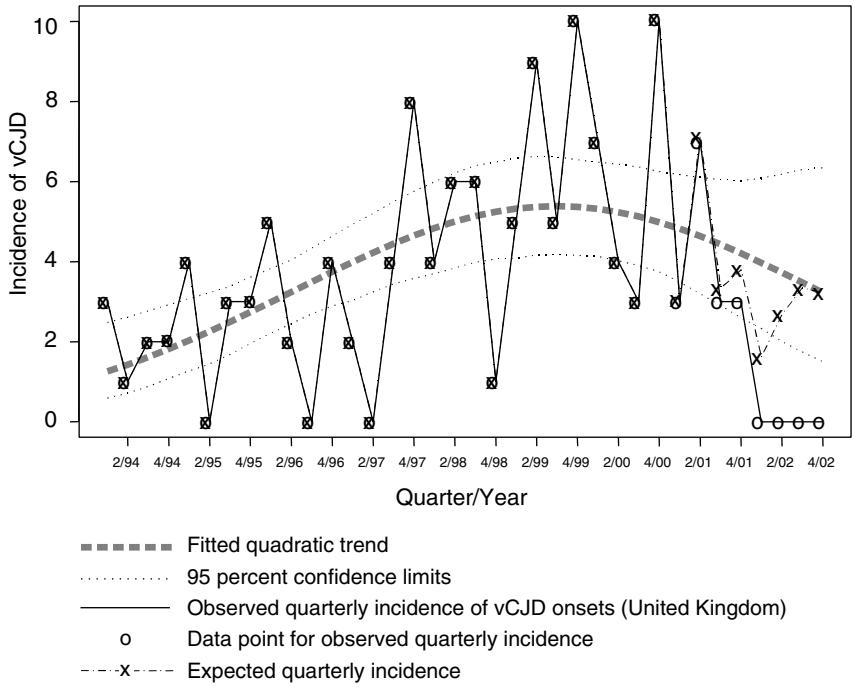

FIGURE 2-4 The best-fit curve for the observed quarterly incidence of vCJD onsets in the United Kingdom through December 2002 is quadratic. The fitted quadratic trend appears within its 95 percent confidence limits and has been adjusted for the time delay between onset and diagnosis.
SOURCE: Will (2003).

GLOBAL IMPACT OF BSE AND vCJD

The BSE outbreak and fear of vCJD, coupled with the outbreak of another animal illness, foot-and-mouth disease, devastated the United Kingdom's beef industry. Hundreds of thousands of cattle have been slaughtered as suspect cases or culled because of BSE. Many other countries, especially in Europe, unknowingly imported BSE-positive cattle, beef products, meat-and-bone meal, and ruminant feed from the United Kingdom before the BSE epidemic and its cause had become apparent. Consequently, these countries also have suffered outbreaks of BSE, culling of herds, public panic, financial losses, and political repercussions. In addition, the United Kingdom's trading partners have banned the import of cattle, beef products, meat-and-bone meal, and ruminant feed from that country. Similar import bans have been imposed on other countries—most

recently Canada[7]—that have reported or are at risk for BSE, as discussed in Chapter 7.

Two recent reports (GAO, 2002; Harvard Center for Risk Analysis and Tuskegee University Center for Computational Epidemiology, 2001) suggest that the U.S. government should strengthen its policies designed to avert BSE and vCJD, although the first of these studies concludes that the risks of a BSE outbreak in the United States are minimal. Chapters 6 and 7 examine these reports in the context of U.S. surveillance for and prevention of TSEs. Chapter 7 also reviews an analysis of the potential impact of a single case of BSE in the United States (Matthews and Perry, 2003) and examines the repercussions of the BSE-positive cow discovered in Canada in May 2003.

THE SPREAD OF CHRONIC WASTING DISEASE IN THE UNITED STATES

As noted earlier, although the BSE epidemic that struck Europe has spared the United States thus far, chronic wasting disease (CWD) is affecting free-ranging and captive deer and elk in several midwestern and western states; it has also occurred in Canada. The unusual, insidious, and fatal illness began appearing in a captive herd of mule deer in the late 1960s at a research facility in Fort Collins, Colorado (Williams and Young, 1980). The disease affected young adult deer that had been captive for approximately 2.5 to 4 years. Sick animals became listless, depressed, and anorexic; they died of emaciation, secondary complications, or euthanasia within 2 weeks to 8 months after the onset of clinical signs. The nature of these signs led biologists to name the illness chronic wasting disease.

The most striking and consistent pathological features observed by the early CWD researchers were nerve cell degeneration and widespread microscopic vacuoles in the neurons of the brain and spinal cord—trademarks of the spongiform encephalopathies previously described in sheep, goats, and humans. This commonality led scientists Elizabeth Williams and Stuart Young to conclude in 1978 that CWD was a new spongiform encephalopathy. Captive Rocky Mountain elk living in the same Colorado and Wyoming facilities as the affected deer were diagnosed with the disease a few years later (Williams and Young, 1982).

Unlike BSE, CWD can spread efficiently from an infected animal to an uninfected animal of the same species, as well as to related species, either directly after exposure or indirectly from the pasture occupied by an in-

[7]EDITORS' NOTE: After this report was completed, the first U.S. case of BSE was identified in Washington State and was announced to the public on December 23, 2003.

fected animal (Gross and Miller, 2001; Williams and Miller, 2002). It is clear that the disease can be transmitted among mule deer, white-tailed deer, and elk (Williams and Miller, 2002). The lack of understanding of how CWD spreads and whether it can cause disease in humans and cattle justifies research on the disease, as well as the development of tools for detecting the infectious agent in humans, animals, and the environment. We discuss such tools and research in Chapters 4 and 6, respectively.

Major media outlets reported on the growing number of CWD-infected deer and elk in North America during 2002 (Blakeslee, 2002; Regalado, 2002). Although the disease appears to be spreading, the larger numbers and wider geographic distribution of CWD cases also may reflect more active surveillance during the past several years. This surveillance has resulted in the identification of foci of CWD that have existed for a decade or two in the wild and on game farms—the news is their discovery (Miller et al., 2000). Most of the current CWD epidemics in free-ranging and farmed cervids appear to be independent of each other, although they may have a common origin dating back several decades (Williams and Miller, 2002). It is unknown how the disease initially arose.

The spread of CWD among free-ranging cervids will likely follow the animals' predictable, natural movements. Some researchers speculate that CWD in farmed animals has spread more widely and unpredictably as a result of market forces (Williams and Miller, 2002).

UNIQUE CHALLENGES IN CONDUCTING TSE RESEARCH

Much about TSEs remains unclear: how prions replicate, why they target neurons, and whether prions or some other entity kill neurons are but a few examples. TSE research has progressed slowly because of a number of challenges unique to the field. First and foremost, prions are an entirely new type of infectious entity, precluding the use of many tools designed for studying infectious diseases. Moreover, since PrP^{Sc} and PrP^{C} have identical amino acid sequences, the existence of a prion-specific antibody has not been confirmed to date, and infected individuals do not exhibit a prion-specific immune response. In addition, prions replicate sluggishly in existing cell culture systems and incubate for several months to several years in animal models, limiting the pace of research.

TSE investigators face not only scientific challenges but logistical ones as well. Their work often must take place in laboratories designed for research with biohazardous materials; these laboratories are expensive to construct, and the United States has few such facilities for prion research. In addition, standardized reagents are difficult to come by. There is only one U.S.-based repository for vCJD tissue, and U.S. scientists need repositories in this country for other reagents and transgenic animals. Until recently, the

federal government's limited interest in prion diseases meant that it was relatively difficult to win research grants to study them, and this apparent lack of financial stability has discouraged young scientists from entering the field. Hence, the community of TSE researchers in the United States is small.

Sensitive, specific TSE diagnostics would help protect people and animals from fatal prion infections in the absence of prophylactics and treatments. Given that there are few tools to inactivate prions, the ability to test blood and other tissues for prions would help prevent the inadvertent transmission of vCJD by blood transfusion or organ transplantation, provided that there is actually any infectious agent to be detected in blood or the organs used for transplantation (see Chapter 5). Despite many attempts in Europe and the United States, no one has developed a reliable antemortem diagnostic test for TSEs. The next chapter describes the technologies that offer the greatest promise for achieving this important goal.

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3 Basic Biomedical Research on Transmissible Spongiform Encephalopathies

Research focused directly on the development of antemortem tests for the diagnosis of transmissible spongiform encephalopathies (TSEs) may prove fruitful, and certain promising approaches will be discussed in the next chapter. However, the committee strongly believes that the fastest way to develop rapid, noninvasive, early-stage diagnostic tools for TSEs is through basic biomedical research that can fill gaps in the fundamental knowledge of prions and their disease-causing properties.

The European experience provides evidence that applied research alone is insufficient. The European Union has spent approximately 30 million euros ($30.7 million) over the past 10 years in an attempt to develop satisfactory postmortem diagnostic tests for BSE, yet the Western blot test, which has been in use for three decades, is still the most commonly employed method (personal communication, A. Aguzzi, University Hospital of Zurich, July 15, 2002).

The history of medicine also provides numerous examples of basic discoveries that were essential precursors to the development of diagnostics. For instance, before inexpensive and rapid diagnostics were available for non-A, non-B hepatitis, the hepatitis C virus had to be identified and cloned. Likewise, before an effective blood test became available for screening for HIV, the virus first had to be identified and isolated.

Many brilliant and dedicated scientists have been working for more than 20 years to solve the mysteries related to disease-causing prions. Collectively they have made great progress, yet many fundamental questions remain. To answer these questions, scientists must solve the structure of

PrP^{Sc} and relate that structure to prion strain differences (see Box 3-1); determine endogenous and exogenous mechanisms of prion replication (see Box 3-2); clarify the pathways and pathogenic mechanisms used by prions (see Box 3-3); and elucidate the physiological function of PrP^{C} (see Box 3-4). Each of these priority areas for basic research on prions is discussed, in turn, below.

STRUCTURAL FEATURES OF PRIONS

Current models of prion conformation and tertiary structure are neither complete nor conclusive. Defining the critical structural differences between infectious and noninfectious forms of the prion protein could provide the basis for TSE diagnostics and elucidate the correlations between PrP structures and strains. The committee believes that research to these ends should be high priority for support by the National Prion Research Program (NPRP).

Defining the structural differences between PrP isoforms might enable scientists to synthesize a PrP^{Sc}-specific antibody probe or aptamer, opening the door to the development of a TSE diagnostic tool. Antibody probes are increasingly being used to detect infectious agents in tissue, but their application to prion detection is limited because no independently validated antibody binds exclusively to PrP^{Sc} without prior digestion of PrP^{C}. Of note, two different groups of investigators have reported separate antibodies specific to PrP^{Sc} (Korth et al., 1997; Paramithiotis et al., 2003).

Many of the tests for detection of PrP^{Sc} use antibody probes, as described in Chapter 4. Most tests, however, must use proteinase K to distin-

BOX 3-1
Priority Research on the Structural Features of Prions

- Define the tertiary structure of PrP^{Sc}.
- ➤ Define the structure and composition of the infectious particle if it is more than PrP^{Sc}.
- Identify the subtypes of PrP strains and their properties.
- ➤ Identify the bases of strain variations.
- Determine whether strain differences have a structural correlate.
- Identify new reagents, such as aptamers, that can be used to detect and define structural differences.

NOTE: ➤ indicates the most critical, urgently needed research.

guish PrP^C from PrP^{Sc}. Proteinase K digestion reduces the already miniscule amount of material usually available for prion detection. The committee looks forward to the development of new test methods that exclude proteinase K.

Defining the prion structure could also reveal structure-based phenotypic differences among prion strains. This information would be important because it is thought that PrP^C binds to PrP^{Sc} before it is converted into PrP^{Sc} (Caughey, 2002). If the structure of the prion strain differs too greatly from the host's PrP^C, binding may occur, but conversion will not (Bessen et al., 1995). Defining the structures of PrP^C and PrP^{Sc} at the sites where they interact during binding and conformational change would support the development of molecules to block those interactions.

Since prions are largely insoluble, it is particularly difficult to study their structures with standard proteomic tools. A newer technique called solid-state nuclear magnetic resonance (NMR) overcomes the solubility problem and is in the early stages of application to research on the three-dimensional structure of prions (Laws et al., 2001; Wemmer, 2002). In addition, electron crystallography is being used to probe the two-dimensional structures of PrP^{Sc} crystals (Wille, 2002). Nobel Laureate Kurt Wüthrich produced three-dimensional models of PrP^C using liquid-phase NMR experiments (Zahn et al., 2000). Nevertheless, the research community must do much more to obtain a comprehensive understanding of the structural differences between infectious and noninfectious PrP.

MOLECULAR MECHANISMS OF PRION REPLICATION

It is believed that both the conversion of cellular PrP to PrP^{Sc} and the accumulation of prions require the assistance of one or more molecules

BOX 3-2
Priority Research on Molecular Mechanisms of Prion Replication

- ➤ Identify exogenous cofactors, such as chaperones, membranes, and scaffolding.
- ➤ Identify endogenous modifiers of prion replication.
- • Develop in vitro model systems.
- • Develop alternative model systems.
- ➤ Identify the structural features of the conversion event.
- • Determine why some proteins convert and others do not.
- • Identify the intermediate states in the prion conversion process.

NOTE: ➤ indicates the most critical, urgently needed research.

(Caughey, 2001). These ancillary or chaperoning factors could serve as surrogate markers for prion detection and as drug targets for TSE therapeutics and prophylaxes. Therefore, it is critical that the NPRP fund research designed to identify the molecules that facilitate PrP^{Sc} formation and accumulation in vivo.

Experiments have demonstrated that the chaperone proteins GroEL and hsp104 can stimulate the cell-free conversion reaction, as can sulfated glycans (Wong et al., 2001) and partial denaturants (DebBurman et al., 1997). Other chaperone proteins thought to modulate PrP conversion include hsp73 (Tatzelt et al., 1995), members of the hsp60 class (DebBurman et al., 1997; Edenhofer et al., 1996; Stockel and Hartl, 2001), and BiP (Jin et al., 2000). There are at least a half-dozen apparently natural PrP ligands or conversion modulators: copper (II) (Hornshaw et al., 1995), the laminin receptor (Weiss and Randour, 2002), laminin (Graner et al., 2000), stress-induced protein 1 (Zanata et al., 2002), and nucleic acids (Cordeiro et al., 2001; Gabus et al., 2001; Nandi and Leclerc, 1999).

The molecule or molecules associated with prion conversion may be easier to detect than prions themselves. For instance, there may be a known antibody that binds specifically to a chaperone protein involved with PrP formation or accumulation. Ancillary or chaperoning factors could potentially amplify PrP^{Sc}, helping to overcome current limits to prion detection.

A related goal of NPRP should be to fund research aimed at isolating the multiprotein complexes that contain prions. Such studies might identify new cofactors that are important in the formation and stabilization of PrP^{Sc} and infectivity. For instance, molecules such as sulfated glycosaminoglycans appear to be associated with PrP^{Sc} deposits in vivo (Snow et al., 1990) and may play a role in their formation in vivo, as can be the case with a variety of other amyloid protein deposits. Further understanding of the identities and roles of PrP^{Sc}-associated molecules might suggest new therapeutic and diagnostic approaches.

MECHANISMS OF TSE PATHOGENESIS

If better diagnostics are to be developed, much more understanding of the nature and dynamics of prion infection must be gained. Research on the pathogenesis of TSEs holds the keys to such understanding. Researchers, clinicians, and public health officials must know which tissues are infectious and when, what mechanisms are involved when the infectious agent enters and disseminates in the body and then invades the brain, what causes cellular toxicity (prions, prions plus another molecular species, or a totally different molecular entity), what the mechanisms are by which the toxic events lead to cellular dysfunction and clinical symptoms, how the infectious agent spreads from host to host, and what features of the host deter-

BOX 3-3
Priority Research on Mechanisms of TSE Pathogenesis

- Identify all possible routes of TSE transmission.
- ➤ Determine individual host susceptibility and resistance, including genetic factors.
- Examine the determinants and nature of host immune response to endogenous and exogenous prions.
- ➤ Characterize the early events of TSE infection.
- ➤ Determine the anatomic location of conversion events, the distribution of the infectious agent throughout the course of infection, and the role of local host mediators.
- Examine the intracellular trafficking of PrP in a variety of cell types.
- ➤ Determine the mechanism of neuroinvasion.
- ➤ Define the specific molecular species and mechanisms that cause cellular toxicity.
- Determine the mechanism of nerve-cell dysfunction.
- Determine the biological bases of neurological and psychiatric symptoms.
- ➤ Determine whether different prion subtypes and strains exhibit distinct pathogenic mechanisms.
- Use alternative model systems (e.g., yeast or *Drosophila*) to study pathogenic mechanisms.

NOTE: ➤ indicates the most critical, urgently needed research.

mine susceptibility to infection. Understanding of these matters will lead to improved characterization of diagnostic targets, better diagnostic strategies, greater target discrimination, and improved diagnostic sensitivity and specificity.

The pathogenesis of TSE agents may vary among strains and hosts, and the same host may be infected by more than one strain. For example, we cannot assume that known TSE strains are pure or uniform. In mouse assays, a given strain (phenotype) has characteristic histopathological features and incubation periods. Yet in a recent study, transgenic mice expressing human PrP and inoculated with the BSE agent manifested not only the expected vCJD phenotype, but also, surprisingly, the sCJD phenotype (Asante et al., 2002). The researchers suggest, in general, that TSE infections may involve multiple strains resulting in variable host responses. More specifically, they postulate that some of the sCJD occurring in the United Kingdom and elsewhere may be due to BSE exposure. A better understanding of prion strains and subtypes and their differential pathogenesis, there-

fore, is crucial. In addition, the conditions under which a TSE agent may exist in an asymptomatic carrier state within the host should be explored.

The general factors affecting host resistance are known and include genetic, environmental, and agent-specific characteristics. Their interrelationships are poorly understood, however. A recent experiment by Jean Manson and colleagues demonstrates this point. Manson's group showed that mice expressing PrP with the Leu101 mutation, corresponding to human GSS, did not spontaneously develop clinical or subclinical prion disease (Manson et al., 1999). However, their experiment also showed that this disease-associated mutation differentially increased susceptibility to infection with human GSS-derived infectivity and simultaneously decreased susceptibility to several mouse scrapie strains. Thus, the study demonstrated that mutant PrP expression was an important genetic susceptibility factor, although it was unable to generate spontaneous infectivity in vivo alone. Continued research in this area will lead to a firmer understanding of how genetic factors interact with the TSE agent and environmental factors.

All pathogenetic studies must be interpreted in their specific contexts, a requirement that makes the development of diagnostics more difficult. At the same time, what investigators learn about the pathogenesis of one prion disease will yield information relevant to the understanding of other prion diseases.

Studies raising questions about presumed pathogenic mechanics are important in stimulating new theories and new scientific inquiry. For example, a new team of investigators studying transmissible mink encephalopathy (TME) in hamsters showed that the agent proceeded along cranial nerves in the tongue directly to the brain, bypassing the intestinal route (Bartz et al., 2003). This finding suggests that prions could enter the body through oral lesions on the tongue, then migrate to the central nervous system through cranial nerves. Bartz and colleagues also showed that the TME agent, if injected into the brain of an uninfected hamster, would travel down to the tongue in a retrograde fashion. This finding generated some concern that BSE-infected cattle or CWD-infected cervids may harbor the infectious agent in their tongues. However, similar studies must be done to determine whether the agents of BSE and CWD appear in the tongues of infected bovines and cervids, since the aforementioned work involved a different agent (that of TME) as well as a different host animal (hamster).

The possibility that cranial nerves may serve as fast tracks for the TSE agent to enter the central nervous system was recently heightened by the discovery of PrP^{Sc} in the olfactory tracts of nine patients with sCJD, suggesting that the olfactory pathway might serve as a portal of entry for the natural transmission of sCJD (Zanusso et al., 2003). This could have implications for iatrogenic transmission of the disease.

Understanding TSE pathogenesis at the cellular level—essential for the

development of effective diagnostic tools and therapeutic agents—requires knowledge of the mechanisms of toxicity. TSE researchers do not know precisely how and where cellular toxicity occurs. Although most experts believe toxicity occurs at or near the cell membrane, new research in this area is challenging current theories. For example, studies by Ma and colleagues seem to indicate that cellular toxicity may occur in the cytosol due to an aggregation of misfolded proteins that accumulates by retrograde transport through the endoplasmic reticulum (Ma and Lindquist, 2002; Ma et al., 2002). Although these studies need validation, and have even been disputed (Drisaldi et al., 2003), they open up new research possibilities.

The study of TSEs should not be limited to mammalian species. Much can be learned from the study of prions found in other, nonmammalian organisms. For example, the prions found in fungi have been studied extensively, including two prions of the yeast *Saccharomyces cerevisiae*. In 1994, those two prions, [URE3] and [PSI+], were discovered to be infectious forms of their normal proteins, Ure2P and Sup35p, respectively (Wickner, 1994). The first prion-inducing domain was defined, and the protease resistance of the Ure2P in [URE3] prion strains gave the first hint of the mechanism involved (Masison and Wickner, 1995). Soon after, it was discovered that, as in mammals, multiple strains of yeast prions can exist (Derkatch et al., 1996). Ter-Avanesyan's group was the first to show that the [PSI+] prion is a self-propagating aggregation of Sup35p in vivo and in vitro (Paushkin et al., 1997).

Later that year, King and colleagues showed that the prion domain of Sup35p could form amyloid in vitro (King et al., 1997). Then Glover and colleaguesshowed that the full length of Sup35p could form filaments in vitro having characteristics of amyloid that were stimulated by extracts of [PSI+] cells, but not the extracts of [psi-] cells (Glover et al., 1997). Also in 1997, a prion was found in the filamentous fungus *Podospora anserine* (Coustou et al., 1997). Another prion, [PIN+], of the Rnq1p protein of *S. cerevisiae* was reported in 2001 (Derkatch et al., 2001).

Work with these prions has been highly rewarding. The experiments with *S. cerevisiae* described above provided the first evidence that chaperone proteins are involved in prion propagation (Chernoff et al., 1995). Additionally, the Mks1 protein was shown to be necessary for generation of the [URE3] protein (Edskes and Wickner, 2000). Also, the Ras-cyclic AMP pathway was found to negatively regulate generation of the [URE3] prion protein (Edskes and Wickner, 2000).

The presence of one prion in a cell can promote the generation of another (Derkatch et al., 2001). Amyloid of the HET-s protein formed in vitro was shown to be infectious for fungal colonies, whereas nonspecific aggregates or the soluble form of the protein had no effect (Maddelein et al., 2002). Efforts to replicate this process in mammals have not yet been suc-

cessful. In addition, investigators have shown that artificial prions can be constructed by using a prion domain of one protein and a reporter domain of another (Li and Lindquist, 2000). Another landmark discovery from a study of *S. cerevisiae* that used [Het-s] was that prions can be advantageous to the prion host (Coustou et al., 1997).

In view of the close parallels between yeast and mammalian prions, scientists could potentially use yeast to screen for prion-curing agents that might aid in the development of a TSE treatment. For example, yeast prions can be cured by growth on low concentrations of guanidine, an inhibitor of the chaperone Hsp104 (Bach et al., 2003; Jung et al., 2002; Tuite et al., 1981). Also, fragments of yeast prion proteins cure the respective prion (Edskes et al., 1999).

Drosophila and *Caenorhabditis elegans* have also proved to be superb models for the study of a variety of cellular and molecular processes (Hariharan and Haber, 2003) and should be exploited to study prion diseases. These organisms have recently been used to model several human neurodegenerative conditions, including Parkinson's disease, tauopathies, and polyglutamine disorders. These model systems may clarify the basis for neurodegeneration in prion diseases, as they have in other neurodegenerative processes.

PHYSIOLOGICAL FUNCTION OF PrP^C

Because the primary structure of PrP^{Sc} is virtually identical to that of normal PrP, understanding as much as possible about PrP^C would be very

BOX 3-4
Priority Research on the Physiological Function of PrP^C

- ➤ Determine the normal functions of PrP (oxidative stress, copper binding, etc.).
- • Study models for the loss or gain of PrP function, both internal and external.
- ➤ Determine the relationship between prion disease and the loss or gain of normal PrP function.
- • Determine the variability of PrP^C expression in healthy hosts.
- • Determine how the normal variability of PrP^C expression affects an organism's health or disease.
- ➤ Determine how polymorphisms in the prion protein gene in humans (*PRNP*) affect prion disease phenotypes.

NOTE: ➤ indicates the most critical, urgently needed research.

helpful in the development of TSE diagnostic tests. A successful test must discriminate between these two closely related molecules. Moreover, understanding the normal role of PrP^{C} may reveal associated molecules and pathways that are appropriate detection targets. It remains unclear whether the basis for nerve cell dysfunction and death in prion disease is related to the toxicity of PrP^{Sc}, to the loss of function of PrP^{C} as a result of its conversion to PrP^{Sc} and its aggregation during a prion infection, or to other factors.

Recommendation 3.1: Fund basic research to elucidate (1) the structural features of prions, (2) the molecular mechanisms of prion replication, (3) the mechanisms of pathogenesis of transmissible spongiform encephalopathies, and (4) the physiological function of PrP^{C}. [Priority 1][1]

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[1]The committee denotes each recommendation as priority level 1, 2, or 3 based on the criteria and process described in the Introduction.

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4 Diagnostics for Transmissible Spongiform Encephalopathies

Diagnostic tests lie at the forward edge of all efforts to control human and animal diseases, for it is the diagnostic test that clarifies the specific clinical problem. A reliable and speedy diagnosis leads to appropriate clinical management and intervention. Yet very few diagnostic tests are available to detect the infectious agents associated with transmissible spongiform encephalopathies (TSEs).

A number of tests have been developed for the rapid detection of PrP specific to bovine spongiform encephalopathy (BSE) in the central nervous system (CNS) tissue of cattle. At present, the European Community has approved five such tests to screen slaughtered cattle for BSE. Similarly, two rapid postmortem tests are available in the United States to diagnose chronic wasting disease (CWD) in deer and elk. However, there is no test approved by the U.S. Department of Agriculture (USDA) or the Food and Drug Administration (FDA) to detect TSEs in live animals or people.

This chapter first addresses the unique challenges involved in developing antemortem clinical diagnostics for TSEs. This is followed by a discussion of the diagnostic tools used at present and some of the newer techniques being explored. The final section presents the committee's recommendations for research that is likely to yield sensitive, reliable, and cost-effective antemortem TSE diagnostics in the future.

In the case of animals, the ability to diagnose or detect an infection drives food safety interventions, which can prevent the introduction of contaminated food into the food chain and offset economic damage to the food production industry. In the case of people, the detection of infection can avert the introduction of potentially infectious blood into the blood supply

system and be used to direct appropriate treatment. In addition, the use of diagnostic tests for mass screening has epidemiological utility since it can reveal the extent and distribution of a prion-related disease within a herd or population, and can be used to monitor the effects of animal and public health intervention strategies.

Most infectious diseases, such as malaria, tuberculosis, hepatitis, and AIDS, can be diagnosed by established methods. This is not the case with TSE. A prion cannot be identified by direct visualization under a microscope, cultivation in a laboratory, detection of specific antibodies or antigens by standard immunology methods, or detection of its nucleic acid by molecular methods such as polymerase chain reaction (PCR). It consists of host protein with an altered conformation such that the body does not recognize it as foreign and does not produce antibodies against it. It also lacks identifiable DNA or RNA, so it cannot be identified by PCR or other nucleic acid–based tests. These factors make detecting the agent very difficult.

TSE agents have other peculiarities that confound detection. They are largely insoluble, distributed unevenly in body tissues, and found in a limited set of tissues by currently available tests. PrP^{Sc} (the protease-resistant protein associated with prion disease; see Chapter 2) is neurotropic, so it ultimately affects cells of the nervous system tissues. Where and how PrP^{Sc} progresses through the body before its final assault on the nervous system is largely unclear, however, complicating the ability to locate and detect it.

The similarities between host PrP^{C} (the protease-sensitive cellular protein) and PrP^{Sc} pose a fundamental problem. Since it is normal to find PrP^{C} in healthy individuals, detection tests must differentiate between the two proteins. The strategy thus far has been to mix the test material with the proteinase K (PK) enzyme, which digests normal prion protein but only a portion of the abnormal protein. Then various techniques, described below, detect the residual PrP^{Sc} after digestion. Since this process incidentally reduces the small amount of original PrP^{Sc} captured, it is inherently less sensitive than methods that do not rely on PK digestion.[1]

Thus two major factors challenge the designer of any useful antemortem diagnostic test for a TSE agent. First, only small amounts of prions may be available for detection in accessible living tissues, such as blood, urine, and cerebrospinal fluid (CSF), necessitating an exquisitely sensitive test. Second, since hosts incubating a TSE lack overt evidence of illness during most of the period between infection and death, both infected and uninfected animals and people would need to undergo antemortem diagnostic testing.

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[1]The degree to which PrP^{Sc} resists PK digestion depends on its strain. The limit of resistance to PK digestion may relate to the conformation of each strain (Safar et al., 1998). Some loss of PrP^{Sc} is also due to the test process itself.

Therefore, this test must be of sufficient specificity to differentiate accurately between normal and misfolded PrP. For some purposes, the test would also need to discriminate among one or more prion strains—a challenge heightened by basic deficiencies in our understanding of prion strain diversity and the nature of strain variation.

The ultimate objective of a TSE diagnostic test is to detect a single infectious unit (IU) while avoiding a false-positive result. In this report, we define an IU as the smallest amount of infectious agent leading to an infection in a single person or animal exposed. Achieving the ideal TSE diagnostic test will be complex because a single IU may not be equivalent to a single infectious particle. In other words, a single IU of a TSE agent likely consists of many aggregates of PrP^{Sc}, not just one aggregate. For further explanation, please refer to the detailed definition of the term infectious unit in the glossary at the beginning of this volume.

The quest for antemortem diagnostics will play a fundamental role in controlling the spread of TSEs, yet current tests are largely unvalidated and not readily available. Although the ultimate objective is far from being achieved, scientists continue to invest much worthwhile effort into improving TSE detection methods.

CLINICAL DIAGNOSTICS

In general, diagnoses of prion diseases by clinical description or ancillary clinical tests are not specific enough to confirm a specific prion disease. In important circumstances, however, they give the clinician some clues that may help support or question the diagnosis of a prion disease.

Differentiation of prion disease from other neurodegenerative diseases and differentiation among different prion strains on clinical grounds are problematic because affected individuals exhibit similar symptoms. Clinical diagnostic criteria have nevertheless been established for sporadic Creutzfeldt-Jakob disease (sCJD) and variant Creutzfeldt-Jakob disease (vCJD) (Will et al., 2000). Some general clinical differences distinguish sCJD from vCJD (see Table 4-1). For example, vCJD, unlike Creutzfeldt-Jakob disease (CJD), occurs in patients generally younger than 40 years old; often presents with early psychiatric and sensory neurological symptoms; and has a longer duration of illness prior to death, usually more than a year (WHO, 2001a). Spencer and colleagues (2002) reviewed the early psychiatric manifestations of vCJD. They describe the clinical characteristics of the first 100 vCJD patients identified and conclude that "the combination of a psychiatric disorder with affective or psychotic features and persistent pain, dysarthria, gait ataxia, or sensory symptoms should at least raise the suspicion of vCJD, particularly if this is combined with any suggestion of cognitive impairment" (Spencer et al., 2002:1482). Despite these differences between vCJD and CJD, they are not sufficient to establish a definitive diagnosis.

TABLE 4-1 Clinical Differentiation of sCJD and vCJD

Clinical Feature or Supporting Clinical Procedures	Classical sCJD (M/M or M/V 1)	vCJD
Average age at clinical onset	63 yr	29 yr
Length of survival from date of clinical onset	4 mo	14 mo
Early psychiatric symptoms	Unusual	Common
Electroencephalography (EEG)	Bi- or triphasic periodic complexes	Nonspecific, slow
Magnetic resonance imaging (MRI)	Increased signal in basal ganglia, caudate nucleus, and putamen	Hyperintense signal in pulvinar region of the thalamus
Cerebrospinal fluid (CSF)	14-3-3 protein levels usually elevated	14-3-3 protein levels not usually elevated
Histopathology of brain tissue	No amyloid plaques	100% florid plaques
PrP immunohistochemical staining pattern of brain tissue	Punctate pattern	Widespread plaque staining pattern
Immunohistochemical staining of tonsil or appendix tissue	Negative	PrP present in tissue, especially toward late stage of disease
PrP^{Sc} isotype by Western blot	Type 1A	Type 2B

NOTE: M/M = methionine homozygous at codon 129; M/V 1 = heterozygous at codon 129, subtype 1 (see Table 4-2).

Ancillary clinical testing typically supplements the medical work-up for vCJD or CJD. The most helpful noninvasive tests have been electroencephalography (EEG); neuroimaging; examination of CSF; and, more recently, tonsillar biopsy and prion strain identification by immunoblotting. Evaluation of tissue obtained by brain biopsy establishes or excludes the diagnosis of TSE in almost all cases, but brain biopsy is highly invasive and is limited to cases in which a treatable condition must be excluded.

Electroencephalography

In typical cases of sCJD (see Table 4-2), the EEGs of more than 80 percent of patients show distinctive changes (Parchi et al., 1999). The trac-

TABLE 4-2 Classification of Sporadic Prion Diseases

Subtype[a]	Previous nomenclature	Percentage of cases	Onset (avg. age)	Duration (months)	Distinctive features
Sporadic CJD (sCJD)					
M/M 1 or M/V 1	Myoclonic or Heidenhain	70	65	3.9	Typical CJD clinically and pathologically. Typical EEG in 83 percent of cases. Synaptic pattern of immunostain.
V/V 1	Not established	1	39	15.3	Early onset. No typical EEG. Cerebellum spared. Weak synaptic immunostain.
M/M 2	Not established	2	64	15.7	No typical EEG. Cerebellum spared. Coarse spongiosis and immunostain.
M/V 2	Cerebellar or ataxic	9	59	17.1	Ataxia at onset. Rarely typical EEG. Kuru plaques. No cerebellar atrophy.
V/V 2	Cerebellar or ataxic	16	61	6.5	Ataxia at onset. Rarely typical EEG. No kuru plaques. Cerebellar atrophy.
Sporadic Fatal Insomnia (sFI)					
M/M 2	Thalamic or fatal familial insomnia (FFI)	2	52	15.6	Clinically and pathologically indistinguishable from fatal familial insomnia.

[a] Each subtype is defined by the type of PrP^{Sc}—1 or 2—and the genotype at codon 129: homozygous for the amino acid methionine (M/M) or valine (V/V), or heterozygous (M/V).
SOURCE: Personal communication, P. Gambetti, Case Western Reserve University (November 2002).

ing shows biphasic and triphasic periodic complexes in the clinical course (Parchi et al., 1999), which are evident more than 90 percent of the time with repeated tracings (Chiofalo et al., 1980). These periodic complexes are observed less frequently in patients with the other subtypes of sCJD and in familial CJD (Gambetti et al., 1999; Parchi et al., 1999) and have never been found in patients with vCJD, although nonspecific slow-wave abnormalities can be seen.

Neuroimaging

Neuroimaging by computed tomography (CT) and magnetic resonance imaging (MRI) can be useful, especially to rule out non–prion-related neurological diseases. The CT result is usually normal, although the scan may show atrophy in patients with a protracted clinical course (WHO, 1998). This finding may be absent and is nonspecific. The MRI scan may also show atrophic changes in patients with late-course disease. When patients are evaluated by T2 MRI, proton-density-weighted MRI, or fluid-attenuated-inversion-recovery MRI, there is an increased signal in the basal ganglia about 80 percent of the time (WHO, 1998), as well as gray matter hyperintensities noted in diffusion-weighted imaging sequences in patients with CJD (Mendez et al., 2003). MRI also can be used to help differentiate vCJD from sCJD because the posterior pulvinar region of the thalamus shows a hyperintense signal in patients with vCJD. This pulvinar sign is present in 90 percent of patients with vCJD and is more than 95 percent specific in selected cases, making it the best available in vivo test for the diagnosis of vCJD (WHO, 2001b).

CSF Protein

A neuronal protein called 14-3-3 can be detected in CSF, particularly in individuals who have diseases, such as sCJD, involving rapid neuronal destruction (Zerr et al., 2000). This protein may help differentiate TSE from Alzheimer's disease and other dementias, where it is usually not detectable. In necrotizing diseases such as stroke, viral encephalitis, and transverse myelitis, the test for this protein may also be positive (Johnson and Gibbs, 1998; WHO, 1998). In more slowly progressing forms of CJD such as familial cases of human TSEs and vCJD, this CSF protein test is often negative. A WHO study (2001a) found the 14-3-3 test to be only 53 percent sensitive when used to diagnose vCJD.

Tonsil Biopsy

More recently, tonsil biopsy has been used for the presumptive identification of vCJD. Immunohistochemical testing for the prion protein in

these tissues has demonstrated that the protein is present in patients with vCJD but not in those with sCJD (Hill et al., 1999; WHO, 2001a). The postulated reasons for this difference include a strain effect, a species-barrier effect, or the oral route of exposure in vCJD (Hill et al., 1999). There have been too few case series to determine the sensitivity or specificity of this ancillary test.

Although controversial, tests of tonsil and appendix lymphoid tissues are being used to screen large, asymptomatic populations for TSEs. The largest study to date was conducted in the United Kingdom (Hilton et al., 2002). Between 1995 and 1999, Hilton and colleagues tested 8,318 tonsil and appendix tissue samples from 10- to 50-year-old individuals and found one appendix tissue sample that tested positive for PrP^{Sc}. From that result, they estimated the prevalence of vCJD to be 120 per million in the United Kingdom, with a 95 percent confidence limit of 0.5 to 900 cases (Hilton et al., 2002).

It remains unknown how long before the onset of symptoms PrP^{Sc} accumulates in human tonsils or the human appendix and whether all positive individuals will inevitably progress to the fatal CNS disease. Nevertheless, studies of sheep naturally infected with the scrapie agent and of mice experimentally infected with that same agent have demonstrated that PrP^{Sc} is detectable in lymphoid tissues long before clinical signs of neurological disease appear.

Brain Biopsy

In atypical cases of CJD, brain biopsy with histological examination for spongiform changes, immunocytochemical staining, and Western blotting for PrP^{Sc}, as well as analysis of the PrP gene, is diagnostic in virtually all cases. However, this approach is seldom needed to diagnose patients with a typical clinical course and consistent findings of classical sCJD by EEG, MRI, and CSF analysis. Histological examination of brain tissue should be performed for all patients with possible and probable cases of TSE, as well as for all individuals with questionable neurodegenerative diseases at autopsy, to ensure that a new phenotype of prion disease is not missed.

In both sCJD and vCJD, histology typically reveals the spongiform appearance of the CNS tissues. However, amyloid plaque formations with the characteristic morphology known as florid plaques are seen in all patients with vCJD, whereas kuru plaques (without the characteristics of the florid plaques) are observed only in patients with the sCJD subtype methionine/valine 2 (M/V 2), which accounts for about 10 percent of all cases of sCJD (Johnson and Gibbs, 1998; Parchi et al., 1999).

Isotype by Western Blotting

Additional diagnostic precision has been made possible by the introduction of PrP^{Sc} isotypes on the basis of the mobility of the PrP^{Sc} fragment, which is PK-resistant, after gel electrophoresis and Western blotting (Collinge et al., 1996; Monari et al., 1994; Parchi et al., 1997). According to a widely used typing method, there are two major types (or strains) of PrP^{Sc} in all forms of CJD and fatal insomnia, including sCJD, iatrogenic CJD, vCJD, fatal familial insomnia (FFI), and sporadic fatal insomnia (sFI) (Parchi et al., 1997).

PrP^{Sc} type 1 migrates to 21 kilodaltons (kDa) on gels after treatment with PK and deglycosylation. PrP^{Sc} type 2 migrates to 19 kDa under the same conditions. The different gel mobilities of the two PrP^{Sc} types are due to the different sites of PrP^{Sc} cleavage by PK, resulting in PK-resistant fragments of differing sizes. These two types codistribute with distinct disease phenotypes and are conserved upon transmission to receptive animals. Therefore, they fulfill the definition of prion strains, and this strongly indicates that they have distinct conformations. Additional subtypes of PrP^{Sc} have been distinguished on the basis of both the ratios of the three PrP^{Sc} glycoforms (Parchi et al., 1997) and the profile generated by two-dimensional gel electrophoresis (Pan et al., 2001).

In addition to the PrP^{Sc} type, the phenotype of human prion diseases is influenced by the genotype at codon 129 of the *PRNP*, the site of a common M/V polymorphism. A classification of sporadic prion diseases has been generated on the basis of the combination of the genotype at codon 129 and the PrP^{Sc} type (Parchi et al., 1996, 1999). This classification includes five subtypes of sCJD and sFI (see Table 4-2). Each of these subtypes has distinct clinical and pathological features.

Despite these advances, clinical methods remain supportive rather than diagnostic. As in virtually all other disease conditions, the diagnosis is most reliable when obtained by combining information from the clinical examination, ancillary clinical tests, and laboratory tests. Yet even when all this information is combined, present diagnostic tools lack sufficient sensitivity and specificity. The development of tests to improve the early diagnosis of human prion disease and to detect presymptomatic infections in humans and animals more reliably is a major priority.

CURRENT LABORATORY DIAGNOSTICS

Histopathology and Immunohistochemistry

The first method used to confirm the diagnosis of a TSE is postmortem neuropathogical examination of brain tissue from an animal or a human,

and this method remains the gold standard. WHO's position is that "a definitive diagnosis of CJD including nvCJD [new variant CJD] is established only by neuropathological examination" (WHO, 1998:13). Tissue is collected; preserved in formalin; sectioned; stained; and then examined with a light microscope, which is used to look for the characteristic pathological abnormalities on histological examination. This procedure is generally augmented with immunohistochemical staining of the tissue, which uses a PrP antibody-tagged stain that affixes onto PrP. The stain will be abnormally dark or dense in areas where an abnormal amount of PrP is present (see Plate 4-1). Electron microscopy can also be used to observe fibrils, called scrapie-associated fibrils, in fresh postmortem tissue (Merz et al., 1983) as well as in autolytic tissue (see Figure 4-1).

Immunochemical Detection Methods

Five standardized commercial screening tests (two by Prionics AG and one each by Enfer Scientific Ltd., Bio-Rad Laboratories Inc., and InPro Biotechnology Inc.) have been approved by the European Commission (EC) for use in the direct and rapid detection of PrP^{Sc} (Moynagh and Schimmel, 1999; EC, 2003). The test by San Francisco-based InPro was developed in the United States, while the other four tests were developed in Europe. Those tests now on the market are used primarily in Europe.

In the United States, no rapid postmortem or antemortem diagnostic test for human TSEs had been submitted to FDA for approval as of August 2003 (personal communication, D. Asher, FDA, 2003). Furthermore, there are no USDA-approved rapid postmortem or antemortem tests to diagnose TSEs in sheep or cattle. USDA has approved three rapid postmortem diagnostic tests for CWD: the Bio-Rad enzyme-linked immunosorbent assay (ELISA) by Bio-Rad of Hercules, California; the Dot Blot ELISA by Veterinary Medical Research and Development (VMRD) Inc. of Pullman, Washington; and the IDEXX HerdChek® CWD Antigen Test Kit from IDEXX Laboratories Inc. of Westbrook, Maine (IDEXX, 2003; personal communication, Rick Hill, USDA APHIS Center for Veterinary Biologics, November 25, 2003; USDA APHIS, 2002; VMRD Inc., 2003a). All three tests are designed to detect the infectious agent of CWD in peripheral lymphoid tissues of select cervids.[2]

[2]The Bio-Rad test is approved for use on tissue samples from mule deer, white-tailed deer, and elk (personal communication, Rick Hill, USDA APHIS Center for Veterinary Biologics, November 25, 2003; USDA APHIS, 2002). The VMRD test is approved for use on tissue samples from white-tailed deer and mule deer (VMRD Inc., 2003a). The IDEXX test is approved for use on white-tailed deer tissue. All tests are approved for use exclusively in APHIS-

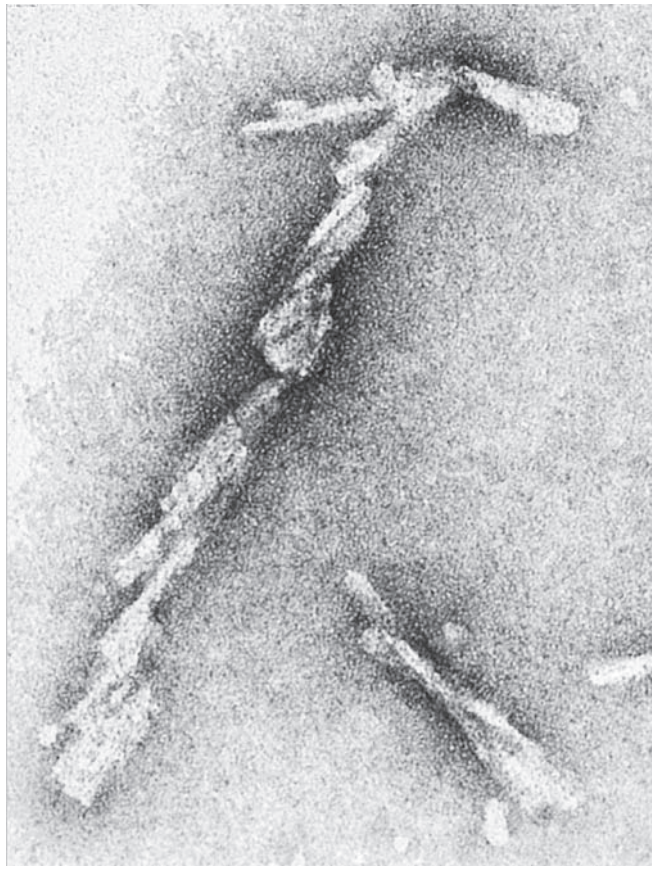

FIGURE 4-1 Electron micrograph of negatively stained fibrils composed of PrP 27–30 from scrapie-infected Syrian hamster brains.
SOURCE: H. Wille, Institute for Neurodegenerative Diseases, University of California San Francisco, September 9, 2003.

approved veterinary laboratories and for surveillance purposes only. The sensitivities and specificities are as follows:

	Sensitivity (%)	Specificity (%)	No. Specimens
Bio-Rad	99.6	99.9	2,892
VMRD	91.5	100.0	298
IDEXX	N/A	N/A	N/A

NOTE: N/A = not available.
SOURCE: Bio-Rad Laboratories Inc. (2003); VMRD Inc. (2003b).

TABLE 4-3 Estimated Detection Limits of the First Three EC-Approved Postmortem Tests for BSE

Dilution of Homogenate[a]	Number of BSE-Infected Brain-Homogenate Samples Scoring Positive		
	Prionics Check Western	Test by Enfer Scientific	Test by Bio-Rad
0	6/6	6/6	6/6
10^{-1}	15/20 (+2?)[b]	20/20	20/20
$10^{-1.5}$	0/20	20/20	20/20
$10^{-2.0}$		0/20	20/20
$10^{-2.5}$			18/20
$10^{-3.0}$			1/20
$10^{-3.5}$			0/20

NOTE: The data represent the number of samples testing positive/total number of samples tested.

[a] ". . . positive brain homogenate of known infectivity titer was tested at dilutions in negative brain" (Moynagh and Schimmel, 1999:105).

[b] Two samples rated inconclusive at this dilution.

SOURCE: Adapted from Moynagh and Schimmel (1999).

Tests Produced by Prionics, Enfer, and Bio-Rad

The rapid test most widely used to screen for BSE in Europe is the Western blot test called Prionics Check Western, produced by Schlieren, Switzerland-based Prionics. Test kits are available for the diagnosis of scrapie in sheep and BSE in cattle. The test uses gel electrophoresis with a specific antibody against PrP after the PrP^{C} in homogenized brain tissue has been digested by PK.

The Enfer and Bio-Rad tests use slightly different mechanisms for detection. The test by Cashel, Ireland-based Enfer is an ELISA. After digestion by PK, residual PrP^{Sc} binds to a capture antibody at the bottom of the ELISA plate wells. After the wells have been washed, a detection antibody complexed with an activating enzyme is added. This second antibody binds to PrP^{Sc} and colors the substrate if PrP^{Sc} is present. The Bio-Rad test, created in France, is also an ELISA, but in the initial step after digestion, it uses two different antibodies to bind to different epitopes of PrP^{Sc}. This is the most sensitive of these first three approved tests (see Table 4-3).

Prionics-Check Luminescence Immunoassay (LIA) and InPro Automated Conformationally Dependent Immunoassay (aCDI)

In April 2003, the EC approved two more rapid tests for the postmortem detection of BSE—Prionics-Check LIA and InPro aCDI (EC, 2003).

The EC protocol used the three tests approved earlier (discussed above) to evaluate the specificity and sensitivity of LIA and aCDI (see Table 4-4), thus ensuring that any newly approved test would be at least as sensitive and specific as the authorized ones. No attempt was made to determine which of the five tests is most sensitive or specific.

LIA can detect the PK-resistant fragment of PrP^{Sc} at levels as low as approximately 30 pg/ml (Biffiger et al., 2002). On a microplate that simultaneously screens 200 samples in duplicate, LIA employs two monoclonal "sandwich" antibodies: one to capture the PK-resistant fragment, PrP 27–30, and another to detect that fragment. The detection antibody is bound to horseradish peroxidase, which emits light upon exposure to a chemiluminescent substrate. The amount of emitted light correlates with the amount of PrP 27–30 in the sample and, by extension, with the amount of prions in the sample.

The aCDI test putatively can detect concentrations of PrP as low as 1 ng/ml (Safar et al., 2002). The test employs two recombinant, radiolabeled antibody fragments to capture and detect PrP. Unlike most other assays for prions, the aCDI test does not involve treating with PK, thereby increasing the number of prions that can potentially be detected.

This test is based on the difference in binding affinity between prions and detection-antibody fragments when the prions are in their native state compared with when they are denatured. In its native, disease-associated conformation, a prion's target binding sites are less accessible to the antibody fragment employed in aCDI than when the prion is chemically denatured by guanidine hydrochloride (Safar et al., 1998). To characterize the presence and amount of PrP^{Sc}, aCDI measures the amount of antibody fragments that binds to the sample before and after the application of guanidine hydrochloride. Then the ratio of binding after denaturation to binding before denaturation is calculated. A ratio above a specified number indicates that the sample contains PrP^{Sc}.

Safar and colleagues (1998) used a conformation-dependent immunoassay not only to detect PrP^{Sc} with a notable degree of sensitivity, but also to characterize eight different strains of PrP^{Sc}. The quantitative ratios appear to be strain specific.

The main limitation of all five EC-approved tests is that they cannot detect PrP^{Sc} in infected cattle until BSE has incubated long enough for PrP^{Sc} to accumulate significantly in brain tissue. (The tests all use postmortem brain tissue samples.) Consequently, these approved tests are sufficiently sensitive for the detection of BSE only in clinically sick cattle or in apparently healthy cattle that are near the clinical onset of prion disease. By contrast, it is possible to detect prions fairly early in the incubation period of CWD in deer by testing CNS and peripheral lymphoid tissues with immunohistochemical and ELISA diagnostics (Sigurdson et al., 1999). The limitations of the EC-approved techniques for BSE detection have helped stimu-

TABLE 4-4 Results of Field Trial Evaluations of Two New Rapid Postmortem Tests for BSE

Test Evaluated	Characteristic Evaluated	Reference Tests	Total Number of Samples	Percent Identified Correctly
LIA (Prionics-Check Luminescence Immunoassay)	Sensitivity	Bio-Rad Platelia, Prionics Check Western	230	100
	Specificity	Bio-Rad Platelia, Prionics Check Western	13,882	100
aCDI (InPro Automated Conformation Dependent Immunoassay)	Sensitivity	Bio-Rad Platelia, Prionics Check Western	218	100
	Specificity	Enfer, Bio-Rad Platelia	10,219	100

SOURCE: Adapted from European Commission (2003).

late attempts to develop more sensitive TSE diagnostics. The following sections review the progress of those efforts.

Animal Bioassays

Animal bioassays have been used extensively in TSE research and diagnostic testing. Like all tests, animal bioassays have limitations. Two striking limitations are the length of time it takes to obtain results and the species barrier effect. Since the end-point measurement is neurodegenerative disease and death of the test animal, and since the incubation period from the time of infection to the time of death is measured in months and years, this method is very time consuming. Yet animal bioassays remain the most sensitive assays available for the detection of TSE infectivity, even though they do not detect PrP^{Sc} directly.

The animals first used to demonstrate infectivity successfully were goats infected with sheep scrapie (Cuille and Chelle, 1939). Goats were used in experiments to study sheep scrapie because they became infected more consistently than did sheep (Pattison, 1966). Sheep were also used to demonstrate how resistant the scrapie agent was to formalin inactivation (Pattison and Millson, 1961).

A breakthrough in the pace of TSE research occurred when investigators successfully infected mice with the scrapie agent by intracranial inoculation (Chandler, 1961). Mice incubated the scrapie agent for only 4 or 5 months before clinical signs of the disease became apparent (Chandler, 1961)—many months less than the amount of time required for the appearance of clinical signs in sheep and goats. Later, the successful use of Syrian hamsters reduced the incubation period to illness even further to 70 days (Marsh and Kimberlin, 1975). Further enhancements to the mouse model produced inbred strains that helped elucidate the role of the mouse *Prnp* gene in susceptibility, incubation times, and prion transmissibility. Understanding of the effect of *Prnp* on the molecular and biochemical mechanisms of PrP improved with the introduction of mutant, transgenic, and PrP-deficient (knockout) strains of mice (Asante and Collinge, 2001). These engineered murine models helped "define the biochemical and genetic basis of the 'species barrier,' demonstrated the inverse relationship between the level of PrP^{C} expression and the incubation time, established the de novo synthesis of prion infectivity from mutant PrP, and revealed the molecular basis of prion strains" (Prusiner et al., 1999:116).

Transgenic knockout mice possessing the *Prnp* of a different species are more sensitive to prion infectivity than are bioassays that involve either wild-type mice or the species that is the source of the transgene. For example, transgenic mice with bovine *Prnp* were 10 times more sensitive than

cattle to the infectious agent of BSE, and were more than 10,000 times more sensitive to the agent than wild-type mice (Safar et al., 2002).

Although mice are the predominant animal model used in bioassays for TSE research, nonhuman primates have been used in the past and continue to be important. The reason for this is that the species-barrier effect is reduced when the prion being tested is more similar in composition to the host animal's prion protein. Because the gene that produces PrP in nonhuman primates is more similar to the human *PRNP* gene than *Prnp* is, nonhuman primates are excellent candidates for the study of human prion disease and represent a more authentic surrogate than rodents for such study. Yet the cost and scarcity of nonhuman primates, the complexity of their PrP genotype, and the long incubation period involved when they are infected with a TSE agent limit their use to selected studies. When the use of nonhuman primates is not feasible, transgenic mice that express human *PRNP* may be the best available assays for the study of human TSE.

Cell Culture Assay Systems

No cell culture assay system for the identification of PrP^{Sc} has been approved although a number of investigators have used in vitro cell culture systems to learn more about the biology of prions. The obvious advantage of using a cell culture system for studying PrP^{Sc} would be to shorten significantly the time to detection of an observed end-point effect, such as cell death following infection with PrP^{Sc}. In addition, cell cultures are simpler models with fewer biological interactions than whole-body animal systems. This greater simplicity makes it easier to interpret the molecular and biological effects due to any specific variable being studied. Significantly less space and fewer personnel are needed to maintain a cell culture system than to maintain an animal colony for laboratory studies.

Scientific investigators have successfully used some cell culture systems in prion research. One cell type that has been employed rather extensively is the N2a mouse neuroblastoma cell. Both sheep and human prions have been propagated in this cell system after the agent was first passaged through mice (Kingsbury et al., 1984; Race et al., 1987). Other cells reported to have been used in cell culture systems include the GT-1 cell line, which is derived from mouse hypothalamic neurons and has been used successfully to study the scrapie agent (Schatzl et al., 1997); and the PC 12 cell line, derived from rat pheochromocytoma cells and used to study mouse prions (Prusiner et al., 1999; Rubenstein et al., 1984).

Rabbits are extremely resistant to infection by TSE agents, and some TSE investigators have capitalized on this by conducting experiments in the RK13 rabbit kidney epithelial cell line (personal communication, S. Priola. National Institutes of Health, Rocky Mountain Laboratories, August 2003). A French research group demonstrated that the overexpression of sheep

PrP^C in RK13 cells made them vulnerable to infection by the agent of sheep scrapie (Vilette et al., 2001). Another group showed that mouse neuroblastoma cells expressing rabbit PrP^C did not become infected when challenged with a mouse-adapted scrapie agent (Vorberg et al., 2003).

A recent study demonstrated that RK13 cells with the polymorphic allele VRQ, which renders sheep susceptible to natural scrapie infection, could be infected when challenged with the sheep scrapie agent. By contrast, when the RK13 cells carried the scrapie-resistant polymorphic allele ARR, the cells could not be infected with sheep scrapie agent. The authors claim this is the first study to show that genetic polymorphisms can affect susceptibility to prion infection at the cellular level (Sabuncu et al., 2003).

Another cell line that has shown research utility is scrapie mouse brain (SMB), known for its reliability in remaining chronically infected with the same Chandler scrapie agent over multiple generations. In one study, investigators used pentosan sulfate to cure a scrapie infection in SMB cells, then reinfected the cell culture with two different strains of scrapie agents. The strains' characteristics were based on their neuropathological profile in the mice. The scientists successfully infected mice with both strains from the cultured cells, leading to the conclusion that PrP^{Sc}, not PrP^C, enciphered prion strain characteristics (Birkett et al., 2001).

The main shortcomings of existing cell culture systems are that they do not replicate large amounts of PrP^{Sc}, the efficiency of infection is low, and the factors that influence susceptibility to infection are poorly understood. These problems diminish the usefulness of present cell culture systems for the detection of PrP^{Sc}. However, the existing systems are safe, cost-effective, and efficient assays for basic research on PrP^C and PrP^{Sc}, as well as for the screening of drugs for their potential therapeutic value in reducing the amount of PrP^{Sc} in cells or clearing PrP^{Sc} from cells.

Cell-Free Conversion Assays

Cell-free conversion assays provide considerable knowledge about the mechanisms and dynamics involved in the conversion of PrP^C to PrP^{Sc} under a variety of conditions. Cell-free studies provided the first direct evidence that PrP^{Sc} has at least limited self-propagating activity and that it must be an infectious agent (Bessen et al., 1995; Kocisko et al., 1994).

PrP^{Sc}-induced conversion reactions occur under a variety of conditions. The simplest, most biochemically defined reactions contain mixtures of largely purified PrP^C and PrP^{Sc} preparations and can be stimulated by chaotropes,[3] detergents, and chaperone proteins (DebBurman et al., 1997;

[3]Chaotrope: a substance that can denature proteins by disrupting the structure of water, thereby making nonpolar substances soluble in water. Chaotropes are used to study protein folding and the interactions of proteins with other molecules.

Horiuchi and Caughey, 1999; Kocisko et al., 1994). Conversion reactions between purified PrP isoforms also have been stimulated by sulfated glycans and by elevated temperature in the absence of denaturants (Wong et al., 2001). Cell-free systems of greater complexity that use membrane-bound PrP^{C} more closely simulate the cell-to-cell interactions regarding conversion to PrP^{Sc} (Baron and Caughey, 2003).

To date no one has been able to demonstrate infectivity of cell-free converted PrP^{Sc}. This has been a major barrier to proving with certainty that pure PrP^{Sc} is the infectious agent in TSEs.

In cell-free conversion assays, the amount of PrP^{Sc} after conversion is generally a smaller amount than that used to initiate the conversion. In an effort to substantially amplify PrP^{Sc} in an in vitro conversion reaction, the protein misfolding cyclic amplification (PMCA) system was developed (Saborio et al., 2001). The system is not a diagnostic method in and of itself, but rather a novel ancillary technique to enhance the detection capabilities of existing and new tests. In this system, detergent extracts of TSE-infected brain homogenate are mixed with vast excesses of similar extracts of PrPC-containing normal brain tissue and subjected to repeated cycles of sonication and incubation. Saborio and colleagues reported more than 30-fold increases in the amount of PrP^{Sc} over that provided in the infected brain extract. This new conversion system should improve the chances of detecting any new infectivity associated with conversion and may also be exploited to enhance the detection of PrP^{Sc} in TSE diagnostic tests.

Cell-free conversion assays have been used to gauge the relative susceptibilities of various hosts to TSE agents from different source species or genotypes (Bossers et al., 2000; Raymond et al., 1997, 2000) and to explore the molecular interactions controlling TSE species barriers. These assays can also be used to show that TSE strain–associated conformations are maintained or "templated" through cell-free conversion, thereby providing evidence for a protein conformation–based mechanism of TSE strain propagation.

Additionally, cell-free conversion assays have been used to study mechanisms of PrP^{Sc} accumulation. These mechanistic studies have shown that the PrP conversion is induced by PrP^{Sc} aggregates/polymers and not soluble, monomeric forms of PrP (Caughey et al., 1995) and that newly converted PrP molecules become bound to the polymers (Bessen et al., 1997; Callahan et al., 2001). Also, conversion involves a conformational change in addition to the binding of PrP^{C} to PrP^{Sc}. A review by Caughey and colleagues (2001) reveals that these and various other observations, such as the formation of amyloid fibrils by PrP^{Sc}, are consistent with an autocatalytic, templated, or seeded polymerization mechanism.

A major strategy for the development of prophylactic and therapeutic treatments for TSEs is the inhibition of PrP^{Sc} formation. Therefore, in vitro

assays of PrP conversion can be used to identify direct inhibitors of PrP^{Sc} formation and evaluate their mechanisms of action (Caughey et al., 1998).

Finally, a cell-free conversion assay that uses recombinant PrP^{C} derived from bacteria rather than from traditional mammalian tissue-culture cells was validated in early 2003 (Kirby et al., 2003). The advantage of this technique is that bacteria produce more PrP^{C} in less time than mammalian cells do.

NEWER, EXPERIMENTAL DIAGNOSTICS FOR LABORATORY USE

Various strategies have been adopted to increase the sensitivity of tests used to detect PrP^{Sc}. These strategies include concentrating PrP^{Sc} within a given test sample, amplifying the initial amount of prions present in a sample, developing antibody tags that bind preferentially to various conformations of prion protein, applying electrophoretic separation techniques, and employing special spectroscopic methods (see Table 4-5). In most cases, the test protocols combine many of these strategies.

Physical techniques such, as centrifugation, and chemical techniques, such as those that use sodium phosphotungstate (Na PTA), can concentrate PrP^{Sc} in a test sample. Safar and colleagues (1998) report that the use of Na PTA resulted in selective precipitation of the oligomers and polymers of PrP^{Sc} and PrP 27-30, the PK-resistant fragment of PrP^{Sc}, but not PrP^{C}. Other agents, including plasminogen (Fischer et al., 2000), procadherin-2, immobilized metal ion affinity chromatography (IMAC), wheat germ agglutinin, heparin, and various antibodies, have been used to bind selectively to PrP^{Sc} and thus concentrate the abnormal protein for further characterization (Harris, 2002).

Protein Misfolding Cyclic Amplification (PMCA)

A novel in vitro approach, introduced by Saborio, Soto and colleagues, involves the cyclic amplification of PrP^{Sc} by sonication (Saborio et al., 2001; Soto et al., 2002). PrP^{Sc} in a test sample is incubated with an excess of normal prion protein such that PrP^{C} converts to PrP^{Sc} and aggregates into complexes. These complexes are subjected periodically to sonication, which breaks them up and turns them into several new templates for the further conversion of PrP^{C} to PrP^{Sc}. In the laboratory of Saborio and colleagues, the amount of PrP^{Sc} in the original sample was found to represent only 3 percent of the ultimate amount generated. Therefore, the test generated an approximately 30-fold increase in the amount of PrP^{Sc}. Like most new techniques described here, PMCA will need further validation; however, it appears to be a rational method for increasing the yield of PrP^{Sc}.

TABLE 4-5 Diagnostic Tests for TSEs

Method	Key Characteristics	Protease Digestion	Detection Limit[a]
Established			
Histopathology	Staining of tissue section	No	Nonquantitative
Immunohistochemistry	Staining of tissue section; anti-PrP antibody	No	Nonquantitative
Western blotting	Gel electrophoresis; anti-PrP antibody; anti-IgG enzyme-linked antibody; chemiluminescence	Yes	10–20 pM
ELISA	PrP^{Sc} absorption; anti-PrP antibody; anti-IgG enzyme-linked antibody; chemiluminescence	Yes	2 pM
aCDI	Radiolabeled recombinant antibody fragments; denaturization with guanidine hydrochloride; differential binding to native and denatured PrP	No	28 pM[b]
LIA	Two monoclonal antibodies for capture and detection; chemiluminescent substrate	Yes	~1 pM[c]

Capillary (Immuno)Electrophoresis (CIE)

At least one group of investigators has reported the use of capillary electrophoresis to detect PrP^{Sc} in sheep and elk blood (Schmerr and Jenny, 1997, 1998; Schmerr et al., 1999). This method has been used to study other proteins in the past (Tsuji, 1994) and was adapted for the detection of PrP^{Sc}.

The technique involves competitive binding by an antibody generated from rabbits immunized with a synthetic peptide. Following protease digestion, the fluorescent-labeled peptide is mixed with buffy coat from a blood sample and with the antipeptide antibody. The mix is then subjected to electrophoresis. In normal animals, much of the antibody binds to the peptide. In TSE-affected animals, the antibody preferentially binds to PrP^{Sc}. Deferential binding is measured by special instrumentation that distinguishes specimens containing PrP^{Sc} from normal specimens.

TABLE 4-5 Continued

Method	Key Characteristics	Protease Digestion	Detection Limit[a]
Unvalidated[d]			
PMCA	Incubation with substrate PrP^{C}; ultrasound sonication	Yes	10- to 100-fold more sensitive than Western blotting[e]
CIE	Gel electrophoresis; Beckman capillary device	Yes	100-fold more sensitive than Western blotting[f]
FCS	Two fluorescent antibodies; confocal microscopy	No	2 pM
MUFS	Ultraviolet light; fluorescence; multivariate analysis	Yes	In the pM range
FTIR	FTIR spectroscopy; artificial neural networks	No	Not specified

NOTE: aCDI = automated conformationally dependent immunoassay; ELISA = enzyme-linked immunosorbent assay; LIA = luminescence immunoassay; PMCA = protein misfolding cyclic amplification; CIE = capillary immunoelectrophoresis; FCS = fluorescent correlation spectroscopy; FTIR = Fourier transform infrared spectroscopy; MUFS = multispectral ultraviolet fluorescence spectroscopy.

[a]In the brains of strain 263K of scrapie agent–infected hamsters, one 50 percent lethal dose is equivalent to ~0.02 to 0.2 picomoles (pM) of PrP^{Sc}, or 6×10^5 to 6×10^6 molecules. One picomole (pM) equals 10^{-12} moles (M).

[b]Safar et al. (2002). The reported concentration of 1 ng/ml was converted to picomoles using 35,000 = molecular weight of PrP (personal communication, D. Harris, University of Washington–St. Louis, October 2, 2003).

[c]Biffiger et al. (2002). The reported concentration of 30 pg/ml was converted to picomoles using 35,000 = molecular weight of PrP (personal communication, D. Harris, University of Washington–St. Louis, October 2, 2003).

[d]Not replicated by independent investigators as of October 2002.

[e]Harris (2002).

[f]Schmerr et al. (1997).

SOURCE: Adapted from Ingrosso et al. (2002).

In 1997 Schmerr and colleagues reported that capillary electrophoresis was 100 times more sensitive than Western blot for the detection of PrP^{Sc}. They found further improvements during the next 2 years. In 1999, the group reported a 25-fold increase in sensitivity over their 1998 results.

Recently, a team of investigators experimented with a similar immunocompetitive capillary electrophoresis assay test in humans and chim-

panzees (Cervenakova et al., 2003). The test could not differentiate between controls and CJD-infected chimpanzees and humans. Cervenakova and colleagues (2003) concluded that immunocompetitive capillary electrophoresis is unsuitable for screening human blood for prions.

Fluorescent Correlation Spectroscopy (FCS)

Another approach to improving the sensitivity and specificity of TSE diagnostics is to use newer, more advanced biotechnology tools, such as fluorescent correlation spectroscopy. One group of investigators tagged PrP-specific antibodies with fluorescent dyes designed to bind to any PrP complexes within CSF (Bieschke et al., 2000). They measured the bound complexes using FCS, which was further modified by using a dual-colored fluorescence intensity distribution analysis system and confocal microscopy with a scanner. This method incorporates a technology involving the scanning of intensely fluorescent targets, which improves both the sensitivity and the specificity of a test. The sensitivity alone is 20 times better than that of the Western blotting test (Bieschke et al., 2000).

Multispectral Ultraviolet Fluorescence Spectroscopy (MUFS)

Other spectroscopic devices and techniques have been developed to improve PrP detection. An example is multispectral ultraviolet fluorescence spectroscopy (MUFS) (Rubenstein et al., 1998). This technique excites a test sample by exposing it to monochromatic light at specific wavelengths. The resulting ultraviolet fluorescence from that exposure is then captured and plotted. Rubenstein and colleagues successfully applied this method to PrP. They showed that PK-treated hamster brain had spectral signatures different from those of untreated hamster brain. They also demonstrated that the spectral signals from PK-treated PrP^{Sc} proteins of two different species, the mouse and the hamster, were sufficiently intense and distinctive that the two proteins could be differentiated by least-squares analysis, which quantifies the orthogonal difference in the signals. They concluded that MUFS has great promise as a rapid, sensitive, and specific tool for the direct detection of PrP^{Sc}, as well as for the differentiation of disparate prion strains (Rubenstein et al., 1998).

Fourier Transform Infrared (FTIR) Spectroscopy

A recently reported spectroscopic approach to the identification of prion-infected hosts involves using Fourier transform infrared (FTIR) spectroscopy, in combination with a highly sophisticated automated computer-assisted pattern recognition program referred to as artificial neural net-

works, to detect disease-associated differences in patterns of small molecules in serum (Schmitt et al., 2002). Using this approach, the investigators correctly differentiated between blood from Syrian hamsters with terminal infections and blood from healthy control hamsters. They reported a sensitivity of 97 percent and a specificity of 100 percent; the predictive value was 100 percent for a positive test result and 98 percent for a negative test result. The investigators suggest that the test needs to be assessed with species other than hamsters, and caution that the differences observed between the scrapie-infected animals and the controls may not be specific for detection of the scrapie agent. It is noteworthy that FTIR spectroscopy does not involve PK digestion.

Summary of Newer Experimental Diagnostics

Despite recent improvements in the sensitivity of diagnostic tests for TSE, the tests still are not sensitive enough for antemortem screening of asymptomatic animals and humans, nor are they adequately specific. False-negative and false-positive results still occur too frequently.

False-positive tests for the detection of TSE in human populations would result in individuals being erroneously informed that they have an incurable, fatal disease. The impact of a false-positive test result for livestock in a country reporting BSE[4] would be the disposal of perfectly good meat. In countries where a false-positive test result would represent a sentinel BSE case; however, the economic, political, and societal consequences of that incorrect result would be monumental. On the other hand, the impact of a false-negative test result might be to allow a contaminated beef product to enter the food chain. And a false-negative test result to diagnose scrapie or CWD not only might allow an animal to escape detection but also might allow horizontal transmission of the infectious agent.

Even if they were adequate, many of these newer tests for TSEs are not available for general diagnostic use or for screening purposes. Rather these tests are being used exclusively in research laboratories. Their utility for commercial applications still requires validation and scaling for high-throughput testing.

The larger issue here is that investigators have focused on relatively few strategies for prion detection. They have relied heavily on PK digestion of PrP^C, on a small number of antibodies, and on a few model systems. The result of this narrow focus is today's limited set of experimental approaches

[4]EDITORS' NOTE: After this report was completed, the first U.S. case of BSE was identified in Washington State and was announced to the public on December 23, 2003.

and reagents. Circumstances call urgently for fresh ideas that exploit a broader array of new technologies. However, the ability to leverage novel technologies to develop better diagnostics and the chance of success in doing so will improve significantly by first advancing fundamental knowledge of prion biology through basic research.

RESEARCH RECOMMENDATIONS FOR TSE DIAGNOSTICS

Exploitation of New Technology

New antemortem laboratory tests for the detection of TSE agents are imperative. Research to develop those tests should proceed with full recognition that major breakthroughs are needed to achieve the levels of sensitivity and specificity required to test live animal and human tissues.

The committee believes an ideal test would detect less than 1 IU of prions in the relevant organism or sample. Prusiner and colleagues (1982) estimated that 1 ID_{50} contained approximately 10^5 PrP^{Sc} molecules in a purified prion preparation of scrapie agent from hamster brain. It is reasonable to think, however, that 1 IU contains fewer molecules than 1 ID_{50} (see the definition of IU in the glossary at the beginning of this report). It is also possible that the 1 IU differs in size depending on the host species and the species of origin of the TSE agent. Laboratory tests designed to detect prions directly are unable to identify less than 1 IU.

Infectivity studies with animal bioassay models are among the most sensitive methods for demonstrating the presence of the infectious agent of TSEs, albeit indirectly. Yet these animal tests, such as the murine bioassay, are hampered by the species barrier. For example, conventional mice provide 1,000 times less sensitivity than cattle for detecting BSE infectivity (Wells et al., 1998). The sensitivity of murine bioassays also is limited by the small size of the inoculum that can be administered intracerebrally (Wadsworth et al., 2001).

Recommendation 4.1: Fund research to develop new assays most likely to achieve quantum leaps in the quality of prion detection tools, rather than incremental improvements to existing tests. Any efforts to improve existing tests should aim to increase their sensitivities by several orders of magnitude (at least 10^3). The optimal test should detect less than 1 infectious unit (IU) of PrP^{Sc} per unit of ultimate product used (e.g., 1 liter of blood or 100 grams of beef). [Priority 1][5]

[5]The committee denotes each recommendation as priority level 1, 2, or 3 based on the criteria and process described in the Introduction.

Recommendation 4.2: Fund research to improve in vitro techniques that amplify small amounts of PrP^{Sc} to enhance the sensitivities of diagnostic tests. [Priority 2]

New Reagents and Detection Methods

Novel Recognition Molecules

Current technology does not allow detection of small enough numbers of prion proteins, ready detection of the conformation of the infectious form of the prion protein, or detection of and distinction among different allelic and strain variants of the prion protein. Such distinctions could be made, in principle, through the use of antibodies or other molecular affinity reagents, such as peptide or nucleic acid aptamers, with high specificities for target recognition. When coupled with sensitive methods for detection of a reagent bound to a target, such as those that rely on upconversion of phosphors with negligible natural background fluorescence or those discussed below, a number of approaches offer significant potential. In general, researchers need to exploit novel and fast-breaking developments in biotechnology—for example, rapid advances in proteomics and mass spectrometry that enable high-throughput, precise characterization of proteins—if significant breakthroughs in prion detection are to be achieved.

Practical detection schemes for the near term are likely to involve the use of molecules that recognize specific epitopes on prions, such as epitopes that are specific for the disease conformation or for different alleles. In principle, these molecules could be monoclonal antibodies, such as monoclonal mouse antiprion antibodies, which are made by immunizing mice with a preparation of a protein containing the desired epitopes and isolating hybridomas after cell fusion, as described by Köhler and Milstein (1975). Due to the difficulties of producing monoclonal antibodies to PrP, however, prion detection methods presently are dependent on relatively few antibodies produced in vivo. Despite early disappointments with monoclonal antibodies, research in this area is still promising.

Antibodies can also be selected in vitro, for example, after display on the surface of a filamentous phage. The advent of recombinant DNA techniques has made it possible to construct useful antibody derivatives, including single-chain antibodies that contain the binding regions for the heavy and light chains on a single polypeptide, referred to as single-chain antibody variable region fragments (scFvs), and derivatives that contain well-behaved constant regions (e.g., from mouse immunoglobulin G fetal calf serum) that can be recognized by secondary reagents such as staphylococcal proteins A and G.

Recognition molecules could also be nucleic acid (RNA or DNA)

aptamers that bind to the target epitope. Aptamers are selected from large pools of nucleotides with different sequences. The aptamers' affinities are typically increased after rounds of mutagenesis and selection for those that bind to epitopes more tightly (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Protein aptamers are molecules that display conformationally constrained regions with variable sequences from a protein scaffold (Colas et al., 1996). Pools of nucleotides with random sequences encode the regions with variable sequences. Selection for binding targets is performed in vitro (for example, by selection of phages that display aptamers that bind to the desired target) or in vivo by yeast two-hybrid methods. RNA aptamers can readily be synthesized from DNA templates by transcription in vitro. In addition, aptamers can easily be synthesized by expression in bacteria, yeast, or other cell-based systems.

The development of new antibodies to PrP^{Sc} using the methods described above could significantly improve the sensitivity of current assay methods. For example, the radioimmunoassay was developed in the 1950s (Yalow and Berson, 1959). Alternative assays, such as ELISAs, use the activity of a lytic enzyme (such as alkaline phosphatase) on a fluorogenic or chromogenic substrate in lieu of radioactivity and have a lower detection limit of millions to billions of epitopes (Engvall and Perlman, 1971).

Recently, Paramithiotis and colleagues (2003) reported the development of a PrP^{Sc}-specific antibody. Korth et al. (1997) reported a similar development in 1997, but their respective antibodies need further validation. Paramithiotis's group developed their antibody based on their earlier observation that three repeating tyrosine–tyrosine epitopes were exposed on PrP^{Sc} but not PrP^{C} in several different mammals. The most conserved and dominant epitope of the three was a tyrosine–tyrosine–arginine combination on β-strand 2 of PrP^{Sc}. The investigators produced monoclonal antibodies in mice that selectively recognized the tyrosine–tyrosine–arginine epitope. The antibodies were highly sensitive and specific when tested against several different prion agents, including those affecting humans. Since PrP^{Sc} can be found in follicular dendritic cells within peripheral lymph nodes, Paramithiotis and colleagues postulated that tyrosine–tyrosine–arginine monoclonal antibodies may have a benefit in targeting this PrP^{Sc} and blocking neural invasion, if administered during the incubation period of a prion infection. They also believe these antibodies could lead to a better understanding of the structural peculiarities of PrP^{Sc}, which in turn, could result in the development of new diagnostics and therapeutic approaches.

Physics-Based Methods

Within the past two decades, numerous detection methods based on physical phenomena have been devised. They include evanescent wave meth-

ods (such as those based on surface plasmon resonance), methods that detect resonances in the microwave range, methods that detect changes in the frequency of surface acoustic waves, methods that detect changes in the frequency of piezoelectric cantilevers, microcalorimetric methods, methods based on the field effect in transistors and capacitors, and methods that use evanescent wave–dependent changes in Raman scattering on metallic nanoparticles. With the exception of the latter, however, none of these methods is as sensitive as radioimmunoassays and ELISAs; nonetheless they offer advantages, as they can be used with underivatized recognition and target molecules and can be coupled directly to optical or electrical readouts. Evanescent wave devices, which are widely used, solve the issue of coupling wet and dry elements by making the part of the apparatus that comes into contact with the biological sample disposable. Modern Fourier transform ion cyclotron resonance methods are capable of detecting about 1,000 molecules with a given mass:charge ratio and with unambiguous identification.

Wet Methods

More recently, wet methods have been developed that may have even greater sensitivity for the detection of prions. One of these is the protein-fragment complementation assay (Remy and Michnick, 1999). Another couples recognition proteins with PCR-amplifiable DNA tails (protein PCR). The resulting chimeric molecules can be used with existing real-time PCR techniques and may allow extension of PCR to protein detection at a level of 1 to 10 arbitrarily designated epitopes (I. Burbulis, R. Carlson, and R. Brent, unpublished results, 2002).

Use of any of these methods for the reliable detection of prions in clinical and environmental samples requires that the prions be purified and concentrated. This requirement can be addressed by a variety of approaches.

Conclusions Regarding Reagents and Detection Methods

In broad terms, the present limitations to prion detection lie not in the lack of methods but in the paucity of antibody and other recognition molecules specific for prion species, strain, and allelic variants and for the infectious conformation. Efforts to select antibodies specific for the conformation of prions have been hampered by the lack of immunogenicity of the revealed epitopes, the tolerance of the mammalian immune system to these epitopes, the lack of an industrial-scale effort, and perhaps other factors as well.

Whatever the reason for the failure of past efforts, the reasonable response to the problem is to select more modern kinds of recognition molecules in vitro, bypassing the vertebrate immune system completely. Some

of these problems are common to many areas of application in the biological sciences and have received high-level scientific and national attention (Desai et al., 2002).

A daunting number of people and organizations own the rights to the intellectual property needed to generate modern molecular reagents with affinities for prion proteins and to use those molecules in detection schemes. This situation may make the commercial application of such reagents difficult until patent-sharing schemes can be devised. Nevertheless, neither technical nor legal barriers block government or philanthropic groups from funding the production of these reagents for use in detecting prion particles.

In summary, a wealth of natural and engineered molecules, along with a variety of detection methods, have been developed for recognition of biological targets. Prion investigators must now apply these molecules and methods to the development of selective, sensitive tools that can target PrP^{Sc}. Once bound by specific reagents, prions become detectable and susceptible to attack. That attack might employ catalytically active binding reagents, such as ribozymes, that offer the potential for target inactivation.

Recommendation 4.3: Fund research to develop novel methods and reagents that detect or bind to prions, including new antibodies, peptides, nucleic acids, synthetic derivatives, and chimeric molecules. This research could lead not only to better diagnostics, but also to better therapeutic and prophylactic strategies. [Priority 1]

Surrogate Markers and Signatures of Prion Disease

Diagnostic approaches based on detection of indirect disease markers have a long and inconsistent history. In general, these approaches have been hindered by their lack of specificity (e.g., tests for the erythrocyte sedimentation rate and C-reactive protein) or by their dependence on the generation of a specific antibody that is delayed in all disease processes and absent, altogether in some, including TSEs. Today, powerful methods for detection of robust surrogate markers of disease create new opportunities for diagnosis and force reconsideration of these approaches. These methods are based on genomic or proteomic techniques, focus on complex biological patterns, and depend on pattern recognition algorithms.

All forms of mammalian pathophysiology and pathology are accompanied by stereotyped and highly choreographed intra- and extracellular changes in the diversity, abundance, and spatial distribution of biomolecules. Mammalian biological systems are particularly sophisticated and sensitive in their recognition of and response to perturbations. These responses can be defined by complex changes in many classes of molecules,

including changes in DNA structure, RNA transcript abundance, protein abundance and modification, and protein localization. Modern genomic techniques have greatly facilitated comprehensive measurement of these various changes in parallel. For example, changes in the abundance of RNA transcripts for nearly all genes expressed in humans can be measured simultaneously and repeatedly over short periods of time by using DNA microarrays. Similarly, changes in the abundance of oligosaccharides or proteins among a massive number of species can be measured either by a method that uses a solid-state format or by mass spectrometry.

Diagnostic and prognostic signatures can be identified by examining complex patterns of biomolecules that occur in various disease conditions. Pattern recognition methods fall into two categories: those that discover possible signatures (class discovery methods) based on the association of specific patterns with an outcome of interest, and those that test and validate these signatures (class prediction methods). Both of these methods have been used successfully to classify cancer subtypes, to predict survival, and to evaluate response to therapy. Specific patterns of RNA transcript abundance predict the outcomes for patients with various malignancies, such as breast and lung cancer, lymphoma, and leukemia (Alizadeh et al., 2000). Stereotyped, discriminant patterns of transcript abundance may also be characteristic of the mammalian response to infection (Boldrick et al., 2002).

In a recent study using mass spectrometry, investigators were able to identify a group of surrogate proteins in 50 of 50 patients with ovarian cancer, including 18 patients with early-stage disease. This pattern was absent from 60 of 63 patients with a noncancer diagnosis (Petricoin et al., 2002a). The same technique was used to diagnose prostate cancer in 36 of 38 patients whose diagnosis was blind to the investigators to identify correctly 177 of 228 patients without prostate cancer (Petricoin et al., 2002b).

In diagnosing prion infections, it appears reasonable to postulate that there are patterns of altered transcript abundance or protein expression in, for example, blood, lymph nodes, or cerebrospinal fluid that are characteristic of infection. One approach is to conduct a blind search for such protein patterns by means of protein mass spectrometry (Petricoin et al., 2002b) followed by isolation of recognition molecules directed against the proteins that have been identified. The components that make up the diagnostic pattern need not necessarily be directly involved in pathogenesis, nor must they have a known function. This kind of approach, however, must rely on rigorous evaluation with well-chosen control samples and on predictions obtained from the results of tests with sets of test samples.

Recommendation 4.4: Fund research to identify surrogate markers or signatures for the detection of prions or prion diseases. [Priority 3]

Cell Culture Systems

Research gains leading to better diagnostics would be accelerated if better cell culture systems were in place. These systems have significant advantages over animal bioassay systems, the most important being that they can greatly shorten the length of time required to complete the test.

At present, only a few lines of cultured cells can be infected with prions. The efficiency of infection is low, the rate of PrP^{Sc} accumulation is slow, and the yield of PrP^{Sc} is limited. In addition, the factors that determine susceptibility to infection are poorly understood. Therefore, investigators must find new cell cultures or model systems with bona fide CNS properties that are susceptible to prions in vitro, as well as new ways to enhance the efficiency of the initiation and propagation of infection (e.g., molecules that enhance the conversion of PrP^{C} to PrP^{Sc}). This work would not only enhance the potential for the use of cultured cells to assay prions, it would also shed light on the cellular mechanisms underlying prion replication and nerve-cell death.

Recommendation 4.5: Fund research to improve techniques for propagating prions in cultured cells and develop new in vitro cell systems as a means to assay and study prions. [Priority 2]

Clinical Neuroimaging

Recent improvements in clinical neuroimaging have shown increasing utility in clinical diagnostics for TSEs. MRI is able to visualize the brain lesions of patients with CJD and can even help in differentiating vCJD from sCJD (as noted in Chapter 3). Newer scanning devices and tissue uptake reagents will further increase the utility of this clinical tool.

MRI of vCJD patients has been helpful from a diagnostic point of view because of the frequent and specific pulvinar sign, an abnormality described by Zeidler and colleagues (2000). Symmetric hyperintense signals have been reported in the basal ganglia of patients with sCJD; however, this finding is frequently absent and lacks specificity, making it less useful. For this reason, investigators have examined new imaging methods that can enhance the capabilities of present methods or provide very new technical approaches, such as multiphoton microscopy.

Multiphoton microscopy uses near-infrared light, which penetrates more deeply than visible or ultraviolet light and permits imaging of microscopic structures within the cortex of the living animal at an extraordinarily high resolution with no apparent deleterious effects. To visualize β-amyloid deposits in living transgenic mice with lesions like Alzheimer's disease, researchers have used multiphoton microscopy with locally applied fluorescently labeled antibody against β-amyloid or systemically adminis-

tered fluorescent derivatives of chemicals that bind to β-amyloid, such as thioflavine A and Congo red (Bacskai et al., 2001; Christie et al., 2001; Klunk et al., 2002). This in vivo imaging approach has allowed characterization of the natural history of senile plaques and evaluation of antiplaque therapy in mouse models of the disease. One could envision the application of similar studies to transgenic mouse models of prion disease, especially since thioflavine A and Congo red bind to PrP^{Sc}. The technique would enable characterization of the progression of PrP^{Sc} accumulation and localization in animals or patients with disease by repeatedly imaging the same diseased region of the brain over time.

Although multiphoton microscopy requires a portion of the skull to be thinned or removed for the passage of light, modifications to this technique may obviate this need. In addition, advances in detection sensitivity and improved means of entry of β-amyloid–binding probes into the central nervous system may allow the use of similar kinds of β-amyloid-imaging by MRI and positron emission tomography for studies with humans (Bacskai et al., 2002; Mathis et al., 2002; Shoghi-Jadid et al., 2002). These methods may be valuable in the diagnosis of humans with prion disease, especially individuals who are at risk for inherited or iatrogenic prion disease, and the evaluation of TSE therapies.

Recommendation 4.6: Fund research to develop functional imaging for the presence of PrP^{Sc} in brain tissue, leading to an early diagnostic test similar to the imaging diagnostics being developed for Alzheimer's disease. [Priority 3]

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5 Testing Blood for Evidence of the Agents of Transmissible Spongiform Encephalopathies

Cellular degeneration of the central nervous system leads to the symptoms and demise of patients with transmissible spongiform encephalopathies (TSEs). After the infectious agent of a TSE enters the host though the alimentary tract or by a parenteral route, however, it travels along extraneural pathways prior to neuroinvasion. During this extraneural phase, prions invade the lymphoreticular system. Since lymphoreticular cells, such as macrophages, follicular dendritic cells, and lymphocytes, can circulate in the blood as well as in the lymphatic system, there is a theoretical risk of blood-borne transmission of TSE agents.

This theoretical risk has several implications and justifies an intense search to develop laboratory tests that can detect prions in blood. One implication is that a blood test might allow earlier diagnosis and treatment for persons infected with a TSE agent. Another implication relates to ensuring the safety of the blood supply. And a third implication is that individuals now deferred from donating blood could possibly be returned to the donor pool. In the absence of such blood tests, an ultraconservative TSE-related policy for the deferral (prohibition) from blood donation will remain in effect, influenced by unfortunate past incidents involving the transmission of human immunodeficiency virus (HIV) and hepatitis C virus in blood products that led the U.S. Food and Drug Administration (FDA) to require tighter, more effective safeguards for the collection, processing, and testing of blood (Hoots et al., 2001).

At present, all persons who have traveled to countries reporting bovine spongiform encephalopathy (BSE) for specified time periods are deferred from donating blood. This policy reduces the available pool of donors by 3

to 9 percent (Dodd, 2002). Studies in 2001 showed that actual donations of blood units had decreased by approximately 1 percent as a result of the BSE deferral guidelines (Dodd, 2002). This deferral has a greater impact on the military than on the civilian blood supply (a comparison of the military and civilian blood deferral policies appears in Table 9-2 in Chapter 9).

ANIMAL STUDIES TO ASSESS TSE INFECTIVITY OF BLOOD

Most research on the detection of prions in blood is based on experimental studies in animals. Excellent reviews of these studies have been published (Brown, 2001; Brown et al., 1999, 2001; Evatt, 1998). Several different animal species and different TSE agents have been used in these studies. Most experiments used fewer than 20 animals; nearly all donor animals were experimentally infected with the TSE agent; and in most cases, different blood components were inoculated into the recipient animal by the intracerebral route, the most sensitive form of in vivo assay. In 20 studies, involving five different donor-animal species, at least one assay animal receiving blood from experimentally infected donor animals was infected (Brown, 2001). In no case, however, did blood or blood components from naturally infected donor animals transmit disease to recipient animals. In four separate studies, blood elements of sheep, goats, and cows infected with scrapie and BSE agent, respectively, failed to transmit the agent to recipient mice by the intracerebral or intraperitoneal route (Brown, 2001).

Animal studies investigating the transmissibility of blood-borne human TSE agents have demonstrated transmission most successfully when the animals were innoculated intracerebrally. For instance, the agent associated with Gerstmann-Sträussler-Scheinker disease (GSS) from the blood of donor mice was transmitted to recipient mice intravenously, intraperitoneally, and intracerebrally. The agent was successfully transmitted from 11 of 14 donor mice to recipient mice (Brown et al., 1999; Kuroda et al., 1983). In addition, blood (buffy coat) of 10 of 28 donor guinea pigs experimentally infected with the agent of Creutzfeldt-Jakob disease (CJD) infected recipient guinea pigs by the intracerebral, subcutaneous, intramuscular, and intraperatoneal routes (Manuelidis, 1978). And the BSE agent, an acknowledged TSE agent that can infect humans, was shown to transmit infectivity to 4 of 48 mouse recipients when pooled plasma, obtained from blood collected by heart puncture of 55 TSE-affected donor mice, was injected intracerebrally into these recipients (Taylor et a.l, 2000).

Despite evidence in these animal studies that blood can transmit prions experimentally, the majority of exposed animals were not affected even by intracerebral inoculation. Furthermore, studies in which inoculation was by the intravenous route demonstrated zero to low levels of transmissibility (see Table 5-1). These results demonstrate that prion titers in blood are low.

TABLE 5-1 Studies of the Infectivity of Blood Components Transmitted Intravenously

Type of TSE	Donor Host	Recipient Animal	Blood Component	TSE-Positive Recipients/ Total Recipients	Study
Animal donors					
Scrapie	hamster	hamster	whole blood	3/108	Rohwer (2000)
Scrapie	sheep	sheep	whole blood	1/19	Houston et al. (2000)
Scrapie	sheep	sheep	whole blood, buffy coat[a]	4/21	Hunter et al. (2002)
BSE	sheep	sheep	whole blood, buffy coat	2/24	Hunter et al. (2002)
GSS[b]	mouse	mouse	buffy coat, plasma	2/2[c]	Brown et al. (1999)
Human donors					
sCJD	3 humans	chimpanzee	whole blood	0/3	Brown et al. (1994)
sCJD	1 human	spider monkey	whole blood (also administered intracerebrally [IC] and intraperitoneally)	0/3	Brown et al. (1994)
sCJD	4 humans	squirrel monkey	buffy coat (also administered IC)	0/4	Brown et al. (1994)

[a]Buffy coat is the layer of white blood cells that lies at the top of the solid portion of the centrifuged whole blood.

[b]GSS = Gerstmann-Sträussler-Scheinker disease. This study used the mouse-adapted Fakuoka-1 strain of GSS.

[c]The numbers in this fraction represent two groups of donor mice (405 rodents altogether). The investigators collected and pooled blood from each donor group, all of whose members had been infected with the mouse-adapted Fakuoka-1 strain of GSS. After the pooled blood components had been transfused into recipient mice, several recipients became infected. This result indicated that the pooled blood from each donor group contained the infectious agent of mouse-adapted Fakuoka-1 GSS. Thus, two out of two pools of donor blood (2/2) were infectious.

SOURCE: Adapted from Brown (2001).

However, recent work by Hunter and colleagues (2002) demonstrated the ability to pass both the scrapie agent and the BSE agent from asymptomatic affected sheep to normal sheep via blood transfusion. That study expanded upon the single transfusion case reported by Houston and colleagues (2000) 2 years previously. Hunter's team reported that 2 of 24 recipient sheep transfused with blood from BSE-infected donor sheep and 4 of 21 recipient sheep transfused with blood from scrapie-infected donor sheep succumbed to the respective TSE agent. This number of transmissions could increase as the animals are followed for longer time periods. Transmission was demonstrated using either whole blood or buffy coat. This study has significant implications for assessing whether variant Creutzfeldt-Jakob disease (vCJD), a human BSE-induced prion disease, can be transmitted by a blood transfusion. The study findings also have been used to provide further justification for the precautionary donor deferral policy currently in place. Though it is true that published studies to date have failed to demonstrate blood-borne transmission of the infectious agent of sporadic Creutzfeldt-Jakob disease (sCJD) to nonhuman primates by any route (Brown et al., 1994), concerns remain about possible transmission of the infectious agent of sCJD—and especially vCJD—from blood and its products.

Other related research is ongoing. In a study funded by the European Union, scientists at the German Primate Center in Göttingen are performing transmission studies with rhesus monkeys to elucidate the pathogenesis of TSE in lymphoid tissue (personal communication, A. Aguzzi, University Hospital of Zurich, October 12, 2002). Baxter International Inc., a Deerfield, Illinois–based pharmaceutical company, is conducting transmission studies with monkeys in an effort to understand the potential for prion infection from blood products (personal communication, A. Aguzzi, University Hospital of Zurich, October 12, 2002). The Commissariat à l'Energie Atomique in Paris, France, plans to build a large new facility to house 60 macaques for TSE-related studies, including the infectivity of different prion strains, such as those that cause vCJD (Deslys, 2002). Yet more studies are needed to determine whether the blood of donors infected with an agent of CJD–particularly vCJD– can infect nonhuman primates.

A number of studies have increased our understanding of the distribution of TSE infectivity in the blood of infected animals. For instance, an investigation into the concentration of TSE infectivity in various blood compartments of a mouse model showed a 4-fold higher concentration in the buffy coat than in the plasma. The plasma had a 10-fold higher concentration of infectivity than the Cohn fractions, and the red blood cells had no infectivity (Brown et al., 1998). In a follow-on study, the investigators showed that during the early preclinical incubation period, the infectivity compartmentalized in the same manner as in sick animals, but that the infectivity was at much lower levels and was present in only trace amounts

in the plasma and the plasma fractions (Brown et al., 1999). Brown and colleagues (1999) also demonstrated that infection by i.v. inoculation required seven times as much plasma and five times as much buffy coat as infection by the i.c. route.

RISK OF HUMAN-TO-HUMAN TRANSMISSION OF TSE AGENTS BY TRANSFUSION AND TRANSPLANT

Concern that human-to-human transmission of prions could occur through blood products has been based, in part, on the knowledge that human TSEs have been documented to result from the administration of other human tissues or by contaminated instrumentation. A recent article summarizes the 267 known cases of iatrogenic transmission of CJD (Brown et al., 2000). They include transmission by corneal transplantation (3 cases); stereotactic electroencephalography (EEG) (2 cases); neurosurgery (5 cases); dura mater grafts (114 cases); pituitary-derived hormones (139 cases); and gonadotropin (4 cases) (Brown et al., 2000). To date, not a single case report of human CJD resulting from transmission by blood or blood products has been validated.[1] However, single case reports are difficult to prove or disprove. Some case reports have suggested a possible association of CJD with transfusions but those reports remain questionable (Collins and Masters, 1996; Klein and Dumble, 1993; Ricketts et al., 1997), necessitating more appropriate epidemiological studies.

Many epidemiological studies have been conducted to assess the risk of transmitting CJD among humans through blood products (see Table 5-2). The least complex is epidemiologic surveillance. Surveillance systems have not shown a concordant increase in CJD cases, as one would expect during the past several decades, despite the increased frequency of using blood and blood products (Evatt, 1998). A more complex approach, a case-control study, is designed to determine whether exposure to blood is higher in CJD patients than in a comparable group that does not have CJD. Several case-control studies have failed to show such a difference (Davanipour et al., 1985; Harries-Jones et al., 1988; Kondo and Kuroiwa, 1982; van Duijn et al., 1998; Will, 1991).

[1]On December 17, 2003, the United Kingdom's Secretary of State for Health announced a case of vCJD in a 69-year-old man who had received a transfusion of packed red blood cells in 1996 from an individual who later developed vCJD (Department of Health [UK], 2003). This single case does not prove that the blood transfusion transmitted the vCJD agent from the donor to the recipient, but it does suggest such causality. The probability that the 69-year-old developed vCJD independent of the blood transfusion is between 1:20,000 and 1:40,000 (personal communication, R. Will, The UK Creutzfeldt-Jakob Disease Surveillance Unit, December 17, 2003).

TABLE 5-2 Risk of Transmitting Human TSE Agents Through Blood, Transplanted Tissues, or Surgical Instruments

Type of Study	Study Question	Transmission Demonstrated? Yes	No
Clinical case reports	Can the infectious agent of a TSE be transmitted from infected human tissues by injection or transplantation?		
dura mater transplants		X	
corneal transplants		X	
human pituitary hormone		X	
gonadotropin		X	
reuse of surgical instruments contaminated by prions		X	
blood products[a]			X
Epidemiological studies			
surveillance[b]	Is there an increase in the number of CJD cases commensurate with the increased use of blood transfusions?		X
case-control[c]	Are people who contract CJD more likely to have received blood products than people who do not have CJD?		X
look-back[d]	Has the blood of donors with CJD caused recipients of that blood to develop CJD?		X
high-risk groups (e.g., hemophiliacs)[e]	Do subpopulations that receive multiple transfusions exhibit a higher-than-average rate of CJD?		X

[a]Ricketts et al. (1997).
[b]Belay and Schonberger (2002).
[c]Esmonde et al. (1993, 1994); Davanipour et al. (1985); Will (1991); Harries-Jones et al. (1988); Kondo and Kuroiwa (1982); van Duijn et al. (1998).
[d]Heye et al. (1994); Satcher (1997); Dodd (2002).
[e]Evatt et al. (1998); Epstein (2003).

Another study design involves evaluating cohorts of recipients of blood known to have been donated by a person who subsequently developed CJD. These retrospective, look-back studies compare the occurrence of CJD in this recipient population against the norm for the population. Two such studies—one of 27 recipients of blood from CJD donors and the other of 178 such recipients—failed to show any cases of CJD in the recipients thus far (Dodd, 2002; Heye et al., 1994; Satcher, 1997). Another look-back study in the United Kingdom examined 114 patients diagnosed with vCJD, 17 of whom had donated blood in the past. The investigators were able to trace the blood products from 8 of these donor-patients; these consisted of 48 blood products, 22 of which had been transfused. None of those recipients were on the CJD registry. Of these original 114 patients with vCJD, 8 had received blood transfusions in the past. Four of these patients were traceable and had received 117 blood components from 111 different donors; 105 of those donors being traced, and none were on the CJD registry (Dodd, 2002).

Yet another approach is to study special high-risk populations, such as hemophiliacs (Evatt et al., 1998), who receive many more blood products than does the general population to determine whether they show an increased prevalence of CJD. The majority of the blood-clotting factors they receive is collected from multiple donors and pooled prior to use. The exposure of these populations, therefore, is perhaps the highest of all possible study populations. The U.S. Centers for Disease Control and Prevention (CDC) has followed more than 12,000 hemophiliacs, and no CJD cases have emerged (Epstein, 2003). Another study reviewed pathological brain tissue among 24 decedent hemophiliac patients from 144 hemophiliac centers who had died between 1983 and 1997; in no case was CJD diagnosed (Evatt et al., 1998). These studies provide some assurances for the lack of blood transmission of TSE agents, but the inherent deficiencies of epidemiological approaches, the rarity of the conditions, the difficulty of correctly diagnosing true cases, and the long incubation period prior to case expression make these assurances both tentative and infirm. This is particularly true for assessing the risk of transmitting the vCJD agent through the transfusion of blood or one of its derivatives since this is such a new TSE.

BLOOD TESTS FOR TSE AGENTS

Sensitivity and Specificity

Given the theoretical risks for transmissibility of prions in blood or blood products, the perceived need for a reliable screening blood test is apparent. Absent such a test to clear persons exposed to the agent associated with vCJD, donor deferral, based on geographic history, will remain in

effect, thereby shrinking the available donor pool. The lack to date of an approved test to detect prions in human blood has a great deal to do with the technical challenges of developing a test with sufficient sensitivity to detect a single infectious unit (IU). The titers of prions circulating in the blood of patients with sCJD or vCJD are not known at present, nor is the quanity of prions sufficient to constitute an infectious unit in human blood. Also unknown is whether the titer of the sCJD or vCJD agent in blood might change and even revert to zero during the incubation period. This information is particularly relevant to the agent of vCJD because it is acquired from outside the body and because it travels a circuitous route through peripheral systems on its way to the CNS. The dynamic nature of the vCJD agent increases the complexity of designing antemortem diagnostics for the disease.

In addition, the size and number of prion aggregates in a sample affect the detection and removal of PrP^{Sc} from blood, blood products, and blood derivatives. For example, if a blood or plasma sample contained an IU that was a single prion aggregate containing 10^5 PrP^{Sc} molecules, the IU would be relatively easy to filter out but difficult to detect, due to the low probability that a random sample would contain the aggregate. By contrast, if a blood or plasma sample contained 1,000 PrP^{Sc} aggregates, each comprised of 100 molecules, the aggregates would be much harder to filter out but theoretically easier to detect as a result of the higher probability that a random sample would contain an aggregate—assuming the detection tool were sensitive enough to detect a 100-molecule aggregate.

Recently, information gained from compartmentalized infectivity studies in a mouse model and a complex series of mathematical calculations helped an investigator determine that 100 IU of infectivity (in buffy coat) was equivalent to 10 picograms/mL of PrP^{Sc} (Brown, 2001). Brown used this figure as an estimate target level that a future successful diagnostic test would need to achieve, although he gave caveats that might alter this estimate. Other models and methods need to be applied to reach more precise estimates.

Recommendation 5.1: Fund research (1) to determine the amount of sporadic Creutzfeldt-Jakob disease (sCJD) prions and variant Creutzfeldt-Jakob disease (vCJD) prions in human blood and (2) to estimate the amount of PrP^{Sc} corresponding to one infectious unit of sCJD and vCJD prions in human blood. [Priority 1][2]

Until one IU is determined for sCJD and vCJD in human blood admin-

[2]The committee denotes each recommendation as priority level 1, 2, or 3 based on the criteria and process described in the Introduction.

istered to other humans, animal assays will need to be at least sensitive enough to detect one mouse IU, within a specified transgenic strain, given a specified volume and dilution of human blood, administered by the intracerebral route. This would improve the consistency and reliability of an assay test.

Once the technical problem of developing a sufficiently sensitive test has been solved, the other technical challenge is to develop a test so specific that it can correctly identify a negative subject with a negative test result. Failure to achieve this high level of specificity will result in false-positive tests. This problem is especially acute in the case of CJD, which is uniformly fatal, is associated with a prolonged asymptomatic incubation period and has no effective prophylaxis or treatment. The psychological and social damage to persons told mistakenly that they have CJD would be staggering.

This concern regarding false-positive test results is based on the statistical fact that the predictive value (correctness) of a positive test decreases as the prevalence of the disease decreases in the population. For a rare disease such as CJD, which occurs in 1 in 1 million persons, this is a thorny dilemma (see Table 5-3). If one had an excellent screening test for CJD whose sensitivity and specificity were both an exceptional 99.9 percent and used that test to screen 1 million persons, the percent correctness of a positive test would vary with disease prevalence. If the disease being screened occurred in 1 of every 100 persons, a positive test would be correct 91 percent of the time. If the disease were rare, on the other hand, affecting 1 of every 1 million persons, the positive test would be correct less than 1 percent of the time. In this case, with 1 million persons being screened, the true positive case would be correctly identified, but 1,000 persons would be incorrectly identified as positive. Thus only 1 of 1001 (0.1 percent) would be correctly identified as positive, and virtually all the positive test results would be false-positives.

The practical solution would be to perform a second- or third-level confirmatory test that would be highly specific. That is how a similar dilemma with HIV screening is being approached. The HIV screening test, despite having a specificity of 99.8 percent, has a predictive value of only 8 percent for a correct positive test (Dodd and Stramer, 2000). Follow-on confirmatory tests are then used to verify to the initial screening test. Unfortunately, such confirmatory tests for CJD or other TSEs are not available at present.

Reporting Results and Counseling Donors Who Test Positive for TSE

There are additional concerns related to proper counseling and reporting of TSE screening tests. Most of these concerns focus on management of consent for use of the test and notification of the test result. It is standard

TABLE 5-3 The Predictive Value of a Positive Test Relative to the Prevalence of a Disease in a Population

Prevalence of a Disease in a Population	Number of True Positives	Number of Positive Test Results	Number of False-Positive Test Results	Predictive Value of a Positive Test (percentage)
1 case per 100 people	10,000	9,990	990	91
1 case per 1,000	1,000	999	999	50
1 case per 10,000	100	100	1,000	9
1 case per 100,000	10	10	1,000	1
1 case per 1,000,000	1	1	1,000	0.1

NOTE: Characteristics of the hypothetical test: sensitivity = 99.9 percent; specificity = 99.9 percent. It is assumed that 1 million tests are performed at each level of prevalence.

practice to advise blood donors about the tests to be performed on their blood and to indicate that they will be told about any significant results. In addition, at least in the United States, donors are notified of any deferral; that is, any prohibition of further donation. Effective application of these policies implies effective knowledge of the significance of the test and its results. It is is unclear, however, whether accurate information about the prognostic significance of a given test result will be available when a test first becomes available.

It is appropriate that donors be provided with information that is consistent with the current norms for informed consent. This information includes, but is not limited to, the purpose of the test. It also includes what is currently known about the test, including the quantitative and qualitative significance of a positive (or reactive) test result and the prognostic significance of such a result, even if such data are available only from animal models (Dodd and Busch, 2002). In addition, it is important to advise donors about the risks associated with such a result, including the likelihood of psychological trauma and other potential effects, such as the impact of the release of the results on health insurance eligibility. It is also important to specify the use to be made of the information by the blood collection organization (for example, the possible discarding of donated products and deferral of the donor). Procedures used to notify and counsel donors about test results should also be provided. There should be some mechanism, as well, to permit a donor to opt out of having the test performed (which would imply that his or her blood would not be drawn) or perhaps to decline to receive the results. However, this latter option may be somewhat illusory, as current standards require that a donor be notified of a deferral.

In the United States, there are few exceptions to the rule that donors are notified of their test results. It appears extremely unlikely that a specific or surrogate test for BSE or vCJD would qualify as such an exception. The donor would be advised about the test result and, to the extent possible, its quantitative (i.e., chance that the result is a true positive) and qualitative significance. Provision of counseling and appropriate medical information would be inherent in the notification process. In the event of the use of a surrogate test, it would also be important to provide applicable information about the significance of an abnormal surrogate marker itself, irrespective of its putative relationship to TSEs (as is the case for a markedly abnormal ALT [liver enzyme] level, for example).

Unfortunately, given the characteristics of TSEs and the absence of any organized prospective studies on populations at risk of developing a spontaneous or foodborne TSE, it is unlikely that there will be any meaningful quantitative or clinical information about the prognostic significance of a positive test result at initiation of testing. Thus, the procedures outlined above would be very difficult to put into practice. It would be useful, al-

though daunting, to involve positive donors and, preferably, recipients of their prion blood donations, in long-term follow-up studies, perhaps even to the extent of performing postmortem assessments. At a minimum, periodic assessments of marker levels and neurological status should be performed.

It is difficult to escape the conclusion that if a test for BSE/vCJD were implemented to reduce the risk from current donations, then prior donations from a test-positive donor would also pose some risk (particularly if not previously tested). Thus, some form of look-back would be indicated, suggesting a need for recipient notification. Indeed, the FDA currently recommends "medically appropriate notification and counseling . . . at the discretion of health care providers" for recipients of blood from a donor who is judged to be at (theoretical) risk of transmitting a TSE (FDA, 2002: 23). In the absence of any clear knowledge about the outcome of such transfusions, however, the case for recipient notification is arguable. Such decisions may best be made on a case-by-case basis, although current ethical standards, at least in the United States , would tend to favor notification (Dodd, 2001; Howe, 2001; Steinberg, 2001).

Much of the discussion around this topic is reminiscent of the concerns expressed at the onset of testing for antibodies to HIV. Those concerns and the associated problems were largely overcome and have set the scene for current practice. It must be remembered, however, that AIDS (and of course, HIV infection) had clear and well-established risk factors; many if not most of those who were found to have a positive test result were not completely unprepared for the news. In contrast, those who received indeterminate results were greatly troubled, as they generally had no risk factors. This latter situation may be more akin to the implications of a BSE/vCJD test in the United States, where there is essentially (as of this writing) no risk for indigenously acquired disease.[3] Thus, the prospect of being tested may not deter very many donors at the outset. However, a significant number of well-publicized positive or false-positive results could generate concern and apprehension about donating. The situation may well differ considerably in countries such as the United Kindom, where the vast majority of the population may perceive some degree of risk behavior associated with vCJD. Indeed, surveys have suggested that as much as 50 percent of the population might decline to give blood if a test were to be implemented. This situation will probably depend to a large extent on the future dynamic of the vCJD epidemic.

[3]EDITORS' NOTE: After this report was completed, the first U.S. case of BSE was identified in Washington State and was announced to the public on December 23, 2003.

Regulatory and Commercial Considerations

The technical development and counseling issues discussed above are not the only challenges involved in introducing a new screening test for CJD. There are also commercial marketplace considerations and regulatory requirements that will have to be met. Regarding the marketplace, the health care delivery system in the United States is highly dependent on the private sector, both for the provision of care and for the development of new drugs, vaccines, and medical devices. Generally, demand for a product is a key factor driving investment in the development of new products. The larger the projected sales and profit for a new product, the higher is the priority for that investment. In the case of CJD this disease is very rare; the prevalence of vCJD is near zero; there is no evidence for blood product transmission of either vCJD or sCJD to humans; and there is no mandate to screen blood for CJD by the governing regulatory agency. Thus, any commercial enterprise having finite resources to develop new products would have to weigh those facts as it plans and programs its investment strategy. Market forces clearly will play a role.

If the commercial sector were to develop and market a candidate screening test for CJD, that test would first need to be the approved by the FDA—a methodical and time-intensive process. The FDA uses a variety of pathways and governing legislative codes to evaluate and approve medical products. Screening tests for blood donors are regulated by the FDA as a biological similar to vaccines. At present, the only requirements for testing donors of whole blood and blood components are shown in Box 5-1; no testing is required for CJD.

The manufacturer assumes risk and responsibility for conducting exhaustive studies to demonstrate that the product is safe, reliable, and accurate. Test performance must be demonstrated in human clinical trials. Those clinical trials can begin only after an investigational new drug (IND) application to the FDA has been submitted and approved. The application must show preclinical data that demonstrate proof of principle, performance of the test with reliable reference materials, analytic sensitivity, and the effects of interfering substances (Epstein, 2003). To date, no manufacturer has reached this point for a blood test to detect CJD. Any new biological product, including a blood donor screening test, receives intense scrutiny by FDA regulators. Each test characteristic (see Box 5-2) must be thoroughly evaluated. If one compares this list of characteristics with the status of a screening test for sCJD/vCJD in human blood, significant shortfalls are apparent. There are technical problems involved in achieving the needed sensitivity. The lack of clinical specificity is a concern, and there is no confirmatory test to recheck positive results. Manufacturing processes and tools for prion detection in human systems are not proven. Variability of test

BOX 5-1
Requirements for Testing Donors of Whole Blood and Blood Components

- Agents for which tests are required in the Code of Federal Regulations (CFR), Title 21, part 610.40a
  - –Human immunodeficiency virus type 1
  - –Human immunodeficiency virus type 2
  - –Hepatitis B virus
  - –Hepatitis C virus
  - –Human T-lymphotropic virus type I
  - –Human T-lymphotropic virus type II
- Additional test requirement at 21CFR610.5
  - –Serological test for syphilis

SOURCE: Epstein (2003).

BOX 5-2
Characteristics of New Blood-Donor Screening Tests Considered by the FDA Center for Biologics Evaluation and Research (CBER)

- Analytical sensitivity and specificity
- Clinical sensitivity and specificity
- Chemistry, manufacturing and controls—current good manufacturing practices, including process validation and quality assurance
- Test reproducibility and operator proficiency (variability over time and among different operators or laboratories)
- Stability of reagents
- Instrumentation and computer software
- Product labeling

SOURCE: Epstein (2003).

results could be a problem in detecting CJD since prion distribution in blood may be evanescent and uneven. Variability may also be affected by test reagents that have not been standardized.

In summary, the quest for a screening blood test must overcome many hurdles before such a test can reach the marketplace. Scientists must understand the biology of prions well enough to design and produce a prototype test that is sufficiently sensitive and specific. Multiple testing schemes need to be developed so that the result of one test result can be confirmed by other tests. At the same time stable, standard, and reliable testing reagents must be developed. The biotechnology industry needs to be properly configured to successfully mass-produce a novel test product. Test users need to develop ethically sound counseling and notification policies, especially for those with a positive test result. Developers need to demonstrate and document the performance of the test adequately to achieve FDA approval. And finally, a market must exist, or be created, for the product to attract a commercial manufacturer. While formidable, these obstacles can be overcome with great resolve.

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6 Surveillance for Transmissible Spongiform Encephalopathies in the United States

Comprehensive surveillance for transmissible spongiform encephalopathies (TSEs) in people and animals in the United States is in the best interest of public health and the economy. A number of national and state programs are conducting limited surveillance of both animals and humans. This chapter reviews present TSE surveillance programs and recommends strategies to improve and expand them.

A new human TSE, variant Creutzfeldt-Jakob disease (vCJD), was identified in the United Kingdom in 1996 (Will et al., 1996) (see Chapter 2). Evidence that eating beef products containing the infectious agent of bovine spongiform encephalopathy (BSE) causes vCJD in humans (Coulthart and Cashman, 2001) has led many countries, including the United States, to conduct surveillance for both vCJD and BSE and to try to prevent the entry or spread of BSE within their borders. To date, no case of BSE[1] and no endogenous cases of vCJD have been detected in the United States. Although this country appears to be at low risk for these two TSEs (HCRA and TUCCE, 2001), the evidence that the infectious agent of BSE crossed the species barrier into humans has generated considerable concern about a uniquely North American TSE of animals—chronic wasting disease (CWD). This fatal illness of deer and elk (described in Chapter 2) appears to have spread from its original location in Colorado and Wyoming to 10 more states and two Canadian provinces since the mid-1990s. The U.S. Department of Agriculture (USDA) declared a state of emergency in 2001 after a

[1]EDITORS' NOTE: After this report was completed, the first U.S. case of BSE was identified in Washington State and was announced to the public on December 23, 2003.

CWD-infected cervid was discovered east of the Mississippi for the first time (USDA Office of the Secretary, 2001). Now, virtually every state has or is creating a CWD surveillance program.

For these reasons, the committee believes more surveillance and epidemiological research should be conducted to ensure the early detection of a new human TSE, should one emerge; to monitor scrapie, the likely cause of BSE; and to monitor the spread of CWD. The first two sections of this chapter describe the surveillance programs in place to detect human and animal TSEs in the United States; included are recommendations for research that would strengthen these programs. The final section presents the committee's recommendations for research into the epidemiology and natural history of TSEs; discoveries in these largely uncharted waters would improve U.S. capabilities to conduct surveillance for TSEs.

U.S. SURVEILLANCE FOR HUMAN TSEs

U.S. surveillance of human TSEs relies almost exclusively on mortality data because there is no uniform system for reporting human cases of these diseases as they are diagnosed. Only 12 states have made Creutzfeldt-Jakob disease (CJD) a notifiable disease. Nevertheless, mortality data for human TSEs are a reasonable surrogate for incidence data because these diseases are uniformly fatal and have a relatively short clinical phase—about 4 to 17 months.

The United States conducts nationwide surveillance for human TSEs and studies their epidemiology through two principal organizations: the Centers for Disease Control and Prevention (CDC) in Atlanta and the National Prion Disease Pathology Surveillance Center (NPDPSC) at Case Western Reserve University in Cleveland, Ohio. CDC funds NPDPSC.

Three forms of human TSEs are known to occur in the United States. The most common of these occurs spontaneously as the result of an unknown cause. Sporadic Creutzfeldt-Jakob disease (sCJD) and sporadic fatal insomnia (sFI) fall into this category. The second form is due to mutations in the gene that codes for a prion, the protein thought to be the infectious agent of TSEs (see Chapter 2). The more than 50 distinct subtypes of this genetic form of human TSE are called by one of three names: familial Creutzfeldt-Jakob disease, fatal familial insomnia (FFI), and Gerstmann-Sträussler-Scheinker disease. The third and least common form of human TSE in the United States is due to transmission of the infectious agent through transplanted tissue or tissue extracts from an individual with a TSE or from surgical instruments used on a TSE patient.[2] This illness is called iatrogenic Creutzfeldt-Jakob disease (iCJD).

[2]vCJD is also infectious, but no endogenous cases of the disease have appeared in the United States.

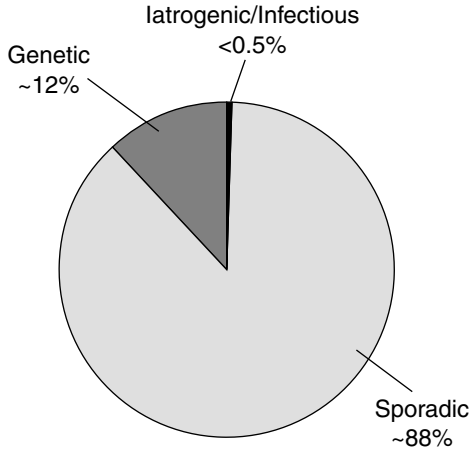

FIGURE 6-1 The relative occurrence of sporadic, genetic, and iatrogenic forms of human TSEs in the United States, 1997–2002.
SOURCES: National Prion Disease Pathology Surveillance Center (NPDPSC) (2003); personal communication, P. Gambetti, NPDPSC, May 2003.

Table 2-1 in Chapter 2 provides some detail on each of these diseases, all of which are rare and fatal. Of the nearly 600 U.S. cases of human prion disease examined neuropathologically from 1997 through 2002, the sporadic forms accounted for approximately 88 percent of cases (the vast majority being CJD), three genetic forms accounted for approximately 12 percent, and iCJD accounted for less than 0.5 percent, as shown in Figure 6-1 (NPDPSC, 2003; personal communication, P. Gambetti, NPDPSC, May 2003).

Through an analysis of mortality data, CDC determined that the rate and characteristics of human TSE deaths identified in the United States between 1979 and 2000 were stable over time and were consistent with the internationally accepted mortality associated with the disease (Belay and Schonberger, 2002). The average annual age-adjusted TSE death rate in the United States from 1979 to 2000 was 1.07 cases per 1 million population (Belay, 2003), close to the worldwide rough annual average of 1 case per 1 million population. This finding suggests that endogenous vCJD has not occurred in the United States.

A comparison of the age distribution of the United Kingdom's vCJD cases with that of the United States' sCJD cases further suggests that endogenous vCJD has not occurred in this country. The median age at death of vCJD patients in the United Kingdom is 28 years (Andrews, 2003). By contrast, the median age at death among sCJD patients in the United States

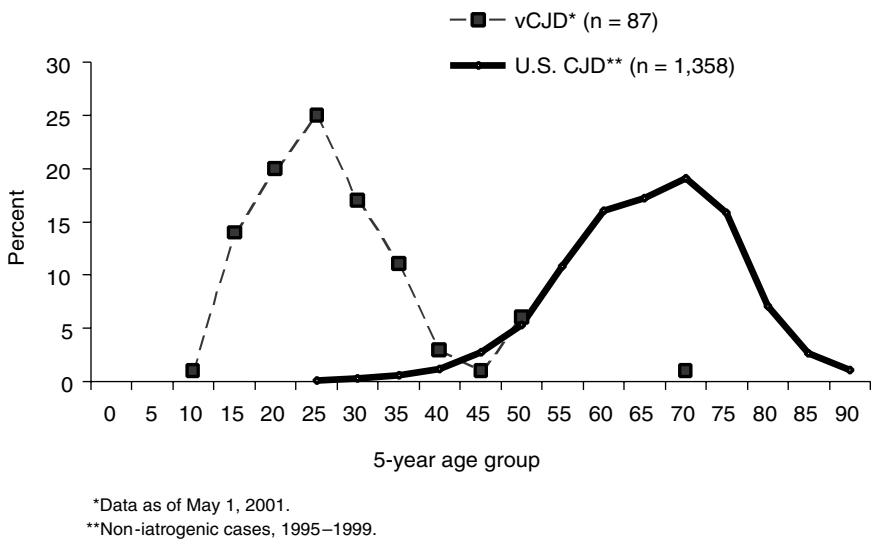

FIGURE 6-2 Percent distribution of vCJD cases in the United Kingdom and sCJD cases in the United States by age group at death, 1995–2001. Reprinted from Belay and Schonberger (2002) with permission from Elsevier. Copyright 2002 by Elsevier.

between 1995 and 2001 was 68 years. Figure 6-2 illustrates the strikingly different age distributions of vCJD and sCJD cases (Belay and Schonberger, 2002). In fact, less than 0.2 percent of noniatrogenic CJD patients in the United States died before the age of 30 (Belay and Schonberger, 2002). Table 4-1 in Chapter 4 provides a more extensive comparison of vCJD and sCJD.

The one known U.S. resident with probable vCJD, a 23-year-old British woman who had resided in Florida since 1992, was born and raised in the United Kingdom during the BSE outbreak and is believed to have contracted vCJD from the consumption of infected beef products in her native country (Belay et al., 2003; CDC, 2002). She was still alive as of January 2004 (personal communication, E. Belay, CDC, January 16, 2004). At least 144 cases of vCJD, including the Florida woman, had been identified globally as of April 2003. The vast majority of these cases occurred in the United Kingdom, but a few were identified in continental Europe, Hong Kong, and Canada.[3]

[3]The single cases of vCJD in Hong Kong and Canada are not considered endogenous because the infected individuals had lived in the United Kingdom for an extended period of time during the BSE epidemic before effective controls were in place. Therefore, it is believed that these people became infected with the BSE agent in the United Kingdom.

BOX 6-1
Tests That NPDPSC Performs on Suspected TSE Deaths

On fixed brain tissue:
1. Histopathology
2. Immunohistochemistry for PrP^{Sc}

On frozen brain tissue:
1. Western blot to determine the presence and type of prion.
2. DNA extraction and *PRNP* sequencing to assess
   a. The presence and type of pathogenic mutations
   b. The methionine/valine polymorphism at codon 129

On cerebral spinal fluid:
1. Determination of whether the protein 14-3-3 is present.

SOURCE: personal communication, P. Gambetti, NPDPSC, 2003.

At present, four scientists conduct surveillance and epidemiology for TSE fatalities at CDC's Division of Viral and Rickettsial Diseases at the National Center for Infectious Diseases. In fiscal year 2003, $3.5 million was budgeted for the TSE group (personal communication, E. Belay, April 2002). In addition to salaries and research, these funds are intended to cover the group's overhead at CDC, operation of NPDPSC, and support for state-based TSE surveillance programs.

The CDC team reviews passively reported cases of human TSE and analyzes national mortality data to determine the trends in human TSE among the U.S. population (Belay et al., 2001). Suspected deaths from TSE are referred to NPDPSC, which makes arrangements for autopsies, collects clinical data, performs neuropathological and neurohistochemical examinations on samples of brain tissue, and analyzes PrP and *PRNP* from brain and other tissues (see Box 6-1).

CDC also monitors populations with a known risk of accidental infection by prions, such as recipients of human growth hormone (Belay, 2003). CDC's epidemiologists have examined mortality records[4] for evidence of

[4]The investigators searched for death certificates that listed as causes of death (1) hemophilia A (International Classification of Diseases [ICD]-9 code 286.0), (2) hemophilia B (ICD-9 code 286.1), thalassemia (ICD-9 code 282.4), and sickle-cell disease (ICD-9 code 282.6) (Holman et al., 1996).

diseases associated with increased exposure to blood or blood products (Holman et al., 1996). Although substantial research has shown that sCJD and other long-known forms of human TSE are not transmissible through blood transfusions (Dodd, 2002; Foster, 2000), it remains unknown whether vCJD is transmissible in this manner.

A hallmark of vCJD at present is the youth of its victims; therefore, CDC investigates all suspected cases of human TSE among those younger than age 55. To date, no evidence of endogenous vCJD or of other new variants of CJD has been discovered.

As alluded to above, concern arose that the infectious agent of CWD had crossed the species barrier into humans after six cases of neurodegenerative disease were detected in the United States in the 1990s among hunters and relatives of hunters (Belay et al., 2001; CDC, 2003). However, follow-up investigations by CDC and state and local health authorities found no causal link between any of the six cases and CWD.

The first investigation concerned three people aged 28 to 30 who died of CJD between 1997 and 2000 and who regularly ate deer or elk meat (Belay et al., 2001). CDC aimed to determine whether the patients might have contracted a TSE by eating venison infected with the agent of CWD. The investigators concluded that the association of the three CJD patients with venison consumption "was more likely coincidental than causal" (Belay et al., 2001:1677–1678).

In August 2002, CDC launched a similar investigation involving three men who died of neurodegenerative illnesses between 1993 and 1999 and who jointly participated in wild game feasts in northern Wisconsin (CDC, 2003). The investigation revealed that the meat eaten at the feasts did not come from CWD-endemic areas. In addition, only one of the men had died of a TSE, and it was a common form of sCJD with no link to CWD. Thus, the investigators concluded that this second set of three cases also provided no evidence of a causal link between CWD and human neurodegenerative disease.

National Prion Disease Pathology Surveillance Center

CDC founded NPDPSC in collaboration with the American Association of Neuropathologists in 1996 to establish a national neuropathology laboratory for the analysis of human prion diseases (Belay, 2003). NPDPSC examines the brain tissue and other informative tissues from all available[5] possible and probable cases of human TSE in the United States. These ex-

[5]Many cases of human TSE in the United States are not identified, reported, or autopsied. This issue is discussed later in the chapter.

aminations, outlined in Box 6-1, provide data for monitoring and characterizing the occurrence of human TSEs. In addition, NPDPSC stores the tissue samples it collects for research purposes.

From 1997 through May 2003, NPDPSC received 1,095 referrals, of which 60.4 percent were definite cases of prion disease. Table 6-1 shows the number of cases diagnosed by the center annually for each type of human prion disease. According to NPDPSC, these figures underrepresent the total number of human TSE cases that have occurred in the United States by about 50 percent.

This conclusion is based on the following analysis. NPDPSC diagnosed 304 U.S. cases of human prion disease among the 511[6] referrals it received during 2001 and 2002 (NPDPSC, 2003; personal communication, P. Gambetti, NPDPSC, July 12, 2003). However, since an estimated 1.07 cases of prion disease per million U.S. population occurred annually between 1979 and 2000 (Belay, 2003), one would expect that about 597 cases occurred in the United States in 2001 and 2002, as shown in Table 6-2. Therefore, only 51 percent of the estimated number of actual U.S. human TSE cases were identified and referred to NPDPSC during 2001 and 2002.

Several factors probably account for the low referral rate to NPDPSC. Some physicians may not recognize human TSEs as such. Some physicians or staff may inadequately document a suspected TSE death. Some individuals who die of TSE may be misclassified as dying from other, more common neurodegenerative conditions. Finally, many diagnosed cases of human TSE are not autopsied.

Increasing the U.S. Autopsy Rate for Human TSE Deaths

The only way to diagnose a human TSE definitively is through a neuropathological examination. The estimated U.S. autopsy and referral rate of 51 percent for human TSEs prevents comprehensive surveillance for these diseases in this country.

Unfortunately, the U.S. autopsy rate has been falling for more than 50 years (Hoyert, 2001). The general autopsy rate in the United States dropped from around 35 percent in the 1960s (Shojania et al., 2003) to 9.4 percent in 1994 (National Center for Health Statistics, 1996), the last year for which CDC tabulated autopsy data. By 2003, the national, nonforensic rate was estimated at 5 percent (Shojania et al., 2003). In contrast, the 51 percent referral rate to NPDPSC is quite an achievement.

Nevertheless, to improve U.S. surveillance for human TSEs, clinicians'

[6]The case of vCJD in 2002 is excluded.

TABLE 6-1 Annual Referrals and Diagnoses of Human TSE Cases in the United States, 1997–May 2003

Year	Number of Referrals	Number of Pending/ Inconclusive Diagnoses	Number of Confirmed Cases by Type of TSE				Total Number of Confirmed Cases
			Sporadic	*Familial*	*Iatrogenic*	*vCJD*	
1997	104	0/0	54	6	0	0	60
1998	94	0/1	44	6	1	0	51
1999	114	0/0	66	8	0	0	74
2000	169	0/1	99	12	2	0	113
2001	247	1/7	138	16	0	0	154
2002	265	4/2	127	22	1	1[a]	151
2003[b]	102	NA[c]	NA	NA	NA	0	61

[a]vCJD likely acquired in the United Kingdom; patient alive as of July 2003.
[b]Through May.
[c]NA = not available.
SOURCE: NPDPSC (2003); personal communication, P. Gambetti, NPDPSC, July 12, 2003.

TABLE 6-2 Actual and Expected Numbers of U.S. Cases of Human TSEs Confirmed in 2001 and 2002

Year	Number of Confirmed Cases of Human TSEs	U.S. Population (millions)	Expected Number of Cases[a] of Human TSEs	Number Confirmed as Percentage of Number Expected
2001	154	277.8	297	52
2002	150[b]	280.3	300	50
2001 + 2002	304	558.1	597	51

[a]Based on the assumption that an average of 1.07 cases of prion disease occur per 1 million U.S. population per year (Belay, 2003).
[b]The case of vCJD is excluded.
SOURCES: NPDPSC (2003); U.S. Census Bureau (2000, 2003).

index of suspicion for this diagnosis must increase. Physicians and public health officials must identify more cases of human TSEs, more of those cases must be autopsied, and the relevant tissues must be sent to NPDPSC for a definitive diagnosis.

Moreover, to identify new phenotypes of human TSEs, more cases of neurodegenerative diseases in general must be autopsied. Doing so would help CDC monitor the U.S. population for the theoretical transmission of CWD to humans. As noted above, there is to date no evidence that CWD is transmissible to humans. Should such transmission occur, however, the human form of CWD might bear little clinical resemblance to known human TSEs—hence the need to autopsy as many atypical cases of neurodegenerative disease as possible. The scientific history of FFI illustrates this point. Unlike any other known TSE, the clinical hallmark of FFI is, as its name suggests, inherited progressive insomnia. Scientists began to suspect that FFI was a TSE only after neuropathological examination of FFI deaths revealed spongiform neurodegeneration (Manetto et al., 1992). Further studies confirmed the presence of proteinase K (PK)–resistant PrP in the decedents' brain tissues and a consistent point mutation (at codon 178) in their PRNP (Medori et al., 1992).

The factors that contribute to the low autopsy rate of human TSE cases in the United States may include a cultural resistance to autopsies and the reluctance of most pathologists to conduct brain autopsies on suspected TSE cases for fear that the pathologist or his/her tools will become contaminated. CDC is studying these issues through its Emerging Infections Programs in New York and California (Belay, 2003). Preliminary data from a survey of pathologists in California indicate that about 80 percent of respondents do not perform autopsies on suspected CJD decedents because of concerns about infection control (Belay, 2003).

In an effort to increase the referral and autopsy rates for suspected cases of human TSEs, NPDPSC began in December 2001 to cover the cost of an autopsy for a suspected CJD case; to identify institutions around the country willing to conduct reimbursed autopsies on suspected CJD cases; and, if necessary, to arrange and pay for the body to be moved to the closest location where an autopsy can be performed. If researchers found a way to inactivate prions on metal surfaces or remove prions from those surfaces, more pathologists probably would be willing to autopsy suspected TSE cases (see Chapter 7).

In addition, the United States should evaluate and support ways to influence neurologists, pathologists, and families to pursue autopsies of all cases of neurodegenerative disease, including suspected TSEs. One solution could be to communicate more frequently with pathologists and neurologists to remind them of both the need to autopsy suspected TSE patients and the free services available for doing so. After the director of NPDPSC

made a presentation about the TSE surveillance program at a national pathology meeting in 2000, the number of referrals to the center increased significantly. As of July 2003, NPDPSC had approached five national associations of pathologists and neuropathologists in an effort to encourage their members to submit cases of suspected human TSEs to the center. Also, NPDPSC and CDC are seeking support from state health departments to urge American neurologists to report cases of suspected human TSEs (personal communication, P. Gambetti, NPDPSC, April and July, 2003).

Recommendation 6.1: Provide funds to promote an increase in the proportion of cases of human neurodegenerative disease, especially suspected cases of transmissible spongiform encephalopathy, that are recognized and autopsied. [Priority 2][7]

The Need for More Epidemiological Research to Improve TSE Surveillance

The committee sees a number of opportunities for the United States to improve its ability to detect a new human TSE, including a theoretical case caused by the infectious agent of CWD. Although CDC and NPDPSC have conducted many valuable laboratory and epidemiological investigations and analyses, the comparison in Table 6-3 of the United States' CJD surveillance program with those of the United Kingdom and Canada illustrates the relatively limited amount of resources that this country devotes to CJD surveillance and epidemiology.

The improvement of U.S. surveillance for human TSEs will depend largely on information gleaned from epidemiological studies that help define the target population and hone survey instruments. The 3 to 4 percent of autopsied cases that fall outside the classified subtypes of human TSEs could be studied further to determine whether a new human TSE lies among these atypical cases (personal communication, P. Gambetti, NPDPSC, March 26, 2003). Risk factors for CJD could be identified by studies that examined differences between CJD cases and controls. Comparing the age and geographic distribution of all neurodegenerative diseases in the United States with the distribution of all CJD cases might suggest regions in which CJD is underreported. In addition, the validity of death certification for CJD could be examined by comparing data on death certificates with results of neuropathological exams. Death certificates also could be examined to determine whether significant differences exist between the demograph-

[7]The committee denotes each recommendation as priority level 1, 2, or 3 based on the criteria and process described in the Introduction.

TABLE 6-3 Comparison of National Surveillance and Epidemiology Programs for Human TSEs in the United States with Those in the United Kingdom and Canada

Characteristic of the Program	United States	Canada	United Kingdom
Funding ($U.S.; FY2003)[a]	$3.5 million	$0.55 million	$3.2 million
Funding per capita[b]	1.2 cents	1.7 cents	5.4 cents
Average annual number of referrals of suspect human TSE cases[c,d]	256	91.3	167.5
Average annual number of referrals of suspect human TSE cases per 1 million population[e]	0.90	2.94	2.86
Staff[f]	13	14 full-time equivalents	26 full-time equivalents
Is CJD a nationally reportable disease?	No	No	No

[a]FY = fiscal year. U.S. data: personal communication, E. Belay, CDC, April 21, 2003; the U.S. 2003 fiscal year is October 1, 2002–September 30, 2003. Canadian data: personal communication, N. Cashman, Health Canada, August 26, 2003; the Canadian 2003 fiscal year is April 1, 2003–March 31, 2004. Currency converted to $U.S. using the interbank conversion rate on April 1, 2003 of $1 U.S. = $1.468 Canadian (FXConverter, 2003). United Kingdom data: personal communication, A. Hunter, National Creutzfeldt-Jakob Disease Surveillance Unit, July 21, 2003; the U.K. 2003 fiscal year is April 1, 2003–March 31, 2004. Currency converted to $U.S. using the interbank conversion rate on April 1, 2003 of $1 = £0.63 (FXConverter, 2003).

[b]Projected resident population of the United States in 2003 = 282.8 million (U.S. Census Bureau, 2000). Projected population of Canada in 2003 = 31.5 million (Statistics Canada, Demography Division, 2003). Projected population of the United Kingdom in 2003 = 59.2 million (Government Actuary's Department, 2002).

[c]The number of years included in the average varies by country and excludes each surveillance system's start-up years. For the United States: 2001–2002; Canada: 2000–2002; United Kingdom: 1997–2002.

[d]Sources: for the United States: NPDPSC (2003); Canada: Health Canada (2003); United Kingdom: National Creutzfeldt-Jakob Disease Surveillance Unit (2003).

[e]Estimated average annual civilian resident population of the United States, 2001–2002 = 285.6 million (U.S. Census Bureau, 2002, 2003). Estimated average annual population of Canada, 2000–2002 = 31.105 million (Statistics Canada, 2003). Approximate average annual population of the United Kingdom, 1997–2002 = 58.6 million (Office of National Statistics, 2003).

[f]Sources: United Kingdom: personal communication, A. Hunter, National Creutzfeldt-Jakob Disease Surveillance Unit, July 21, 2003; United States: personal communication, P. Gambetti, NPDPSC, 2003; Canada: personal communication, N. Cashman, Health Canada, August 26, 2003.

ics of autopsied and nonautopsied CJD cases, as well as what factors are associated with participation or nonparticipation in autopsies, a question on which CDC has launched pilot studies in two states. Other investigations could be conducted to characterize the frequency and types of human exposure to CWD in the United States. For example, studies could be done comparing the mortality rates of human TSEs over time in CWD-endemic areas with the rates in non–CWD-endemic areas. The Colorado Department of Public Health and Environment is presently reviewing the death certificates of state residents from the past 32 years to compare the rates of death due to a range of neurological diseases, including CJD, in CWD-endemic and nonendemic regions of the state (Quirk, 2002). More such studies should now be initiated in other CWD-endemic states. These are just a few of the many epidemiological studies that would provide a firmer scientific foundation for surveillance of human TSEs in the United States.

Recommendation 6.2: Provide funds to increase the number and diversity of epidemiological studies on human transmissible spongiform encephalopathies (TSEs) in the United States. In particular, support research to identify potential cases of variant Creutzfeldt-Jakob disease and new human TSEs possibly caused by the agent of chronic wasting disease. [Priority 2]

U.S. SURVEILLANCE FOR TSEs IN ANIMALS

The three major TSEs of animals—scrapie, BSE, and CWD—pose varying degrees of risk to animal and human health in the United States. First, no scientific evidence has emerged during the past two and a half centuries that scrapie of sheep and goats causes a TSE in humans. However, scrapie spreads easily among sheep and goats, is transmissible to ruminants through animal feed, could possibly be transmissible to cervids, and exists in the United States. Second, the infectious agent of BSE is widely believed to cause vCJD, but, as noted earlier, there has been no known case of BSE in the United States. Considerable epidemiological evidence suggests that the disease does not spread horizontally (from an infected live cow or bull to a healthy one) or vertically (from an infected cow to her fetus).

Finally, existing evidence neither confirms nor rules out the possibility that CWD could infect humans. Scientists in the laboratory of Dr. Stanley Prusiner attempted to transmit CWD to transgenic mice that express human PrP, but the mice remained healthy (personal communication, M. Sear, Institute for Neurodegenerative Diseases, June 20, 2003). Epidemiologists have not identified a causal link between a case of human neurodegenerative disease and CWD after investigations into six suspect cases (Belay et al., 2001; CDC, 2003). Yet two lines of evidence suggest that humans could

conceivably develop a TSE by eating venison infected with PrP^{CWD}. First, investigators from the National Institutes of Health (NIH) induced the conversion of human PrP^{C} to PrP^{CWD} in a cell-free medium (Raymond et al., 2000). Second, as described above, BSE has crossed the species barrier from cattle to humans and has caused vCJD. Finally, scientists have predicted that CWD could potentially wipe out portions of the U.S. wild deer population if the epidemic is not curtailed (Gross and Miller, 2001).

These concerns justify robust surveillance for animal TSEs in the United States. The following sections describe the U.S. surveillance programs for these three diseases and present the committee's recommendations for research to strengthen those programs.

Surveillance of Ovines for Scrapie

Scrapie, a TSE of sheep and goats, was first described in the 1700s (Godon and Honstead, 1998). The first U.S. case was identified in 1952 in an animal in Michigan that was imported from the United Kingdom through Canada (personal communication, L. Detwiler, independent consultant, August 27, 2003). Although all routes of transmission are not known, it has been shown that the scrapie agent can be transmitted laterally (from one unrelated animal to another), maternally (from an infected ewe to her offspring during pregnancy or lambing), and iatrogenically (Detwiler and Baylis, 2003). Clinical signs of the disease appear 2 to 4 years after infection, and affected animals progress invariably to death.

Epidemiological studies, limited primate inoculation studies, and in vitro molecular studies all have indicated that the infectious agent of scrapie is not transmissible to humans, as noted above. Some U.S. cattle that were experimentally inoculated by various routes with the U.S. strain of the scrapie agent appear to have developed an encephalopathy that is not BSE (Clark et al., 1995; Cutlip et al., 1994, 1997).

Scrapie has posed a financial risk to the owners of the United States' approximately 6.35 million sheep and 1.2 million goats (NASS, 2003). Not only does the disease reduce the productivity of infected flocks, but it also prevents the export of breeding stock, semen, and embryos to many other countries (Veterinary Services, 2002). Figure 6-3 depicts the reported occurance of scrapie during fiscal year 2002. Until recently, the estimated prevalence of scrapie in U.S. sheep was 0.07 percent (personal communication, N. E. Wineland, USDA APHIS, September 8, 2003).

USDA's surveillance program for scrapie, which includes goats but focuses on sheep, depends partially on flock owners' participation in a certification program administered by Veterinary Services of USDA's Animal and Plant Health Inspection Agency (APHIS) (Williams, 2002). Approximately 2 percent of the country's farmed sheep, or about 130,000 animals in 1,755

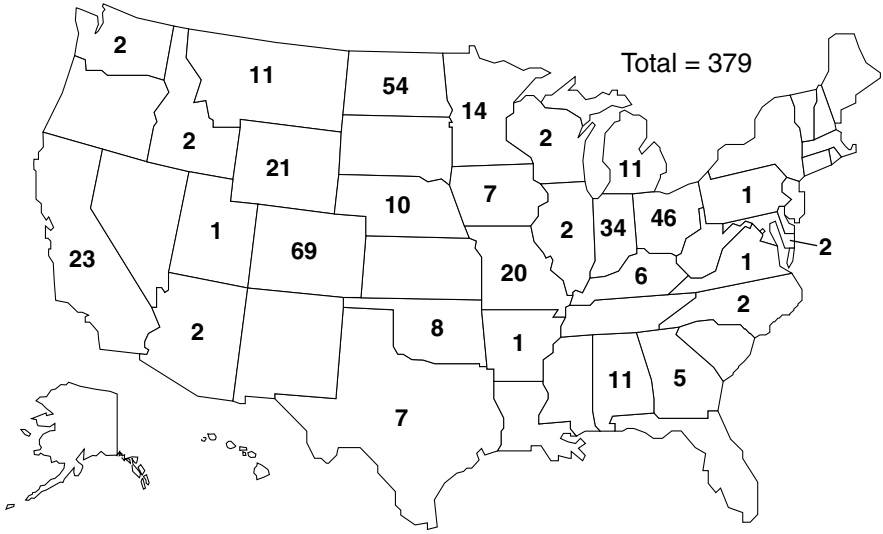

FIGURE 6-3 Locations of 375 of 379 confirmed cases of scrapie reported to USDA APHIS's Veterinary Services during the 2002 fiscal year. The level of scrapie surveillance varies greatly among the states.
NOTE: Four validation cases confirmed in FY 2002 are not shown on map.
SOURCES: personal communcation, E. Williams, University of Wyoming, July 11, 2003; Veterinary Services, USDA APHIS (2003a).

flocks, were registered in the certification program as of July 2003 (NASS, 2003; personal communication, L. Detwiler, independent consultant, August 27, 2003; Veterinary Services, 2003). There were 64,170 U.S. sheep operations in 2002 (NASS, 2003). Figure 6-3 illustrates the reported occurrence of scrapie by state during fiscal year 2002.

Given the small percentage of sheep monitored through the certification program, Veterinary Services launched a year-long study in April 2002 to determine the prevalence of scrapie through the collection and analysis of tissue samples from mature sheep at slaughterhouses (personal communication, L. Detwiler, independent consultant, August 27, 2003). In fact, about 60 percent of sheep tissue samples received by the National Veterinary Services Laboratories (NVSL) during fiscal year 2002 came from the culled slaughterhouse population (Veterinary Services, 2003a). Although the data from the slaughter surveillance study were still under analysis at the time of this writing, it appeared that the results would yield a revised estimate of the prevalence of scrapie in this country (personal communication, N. E. Wineland, Veterinary Services, September 8, 2003).

Veterinary Services also is conducting a small amount of targeted, active surveillance to validate a new antemortem diagnostic test for scrapie and gather data for regulatory approval of the test (O'Rourke et al., 2002; Veterinary Services, 2003a). A mandatory traceback study begun in April 2003 aims to trace infected animals identified during slaughterhouse surveillance back to their source herds (personal communication, L. Detwiler, independent consultant, August 27, 2003). Despite these varied sources of data, however, more surveillance is required to accurately estimate the prevalence of scrapie in the United States (personal communication, E. Williams, University of Wyoming, May 2003).

Goals of Scrapie Surveillance

USDA conducts scrapie surveillance as part of a program begun in the mid-1900s to eradicate the disease from the United States. The eradication program has not succeeded in the United States or in most other countries where scrapie has become endemic (Detwiler and Baylis, 2003); however, Iceland appears close to wiping out the disease (personal communication, L. Detwiler, independent consultant, August 27, 2003). U.S. efforts to eradicate scrapie have intensified since the scientific community reached the prevailing consensus that scrapie-infected sheep offal in cattle feed sparked the BSE epidemic in the United Kingdom (Wilesmith et al., 1988). Through surveillance, USDA aims to prevent scrapie-infected sheep from infecting other animals in their flock, from being sold to owners of a healthy flock, and from contaminating pastures with the infectious agent of the disease. The tissue samples collected during scrapie surveillance also have been used for the validation of an antemortem test that employs immunohistochemistry to detect prions in a tissue sample from a sheep's nictitating membrane (third eyelid)[8] (O'Rourke et al., 2002), as well as in research projects aimed at developing better techniques for scrapie management and control (personal communication, E. Williams, University of Wyoming, May 2003).

Scrapie Flock Certification Program

The Scrapie Flock Certification Program, begun in 1992, helps flock owners identify scrapie-free flocks from which to purchase new animals (Veterinary Services, 1998). About 3 percent of U.S. sheep operations were

[8]Prions have been found in the third eyelids of asymptomatic sheep infected with scrapie (O'Rourke et al., 2000) but not in the third eyelids of cattle or cervids infected with a TSE. This difference is due to the differing paths that prions travel on their way to the central nervous system in different animals.

registered in the program as of July 2003. As mentioned earlier, this corresponds to about 2 percent of farmed sheep.

Participating flocks are monitored for 5 years or more. Owners must record all acquisitions, departures, births, and deaths of their sheep, and must keep these records for a minimum of 5 years after an animal dies or is removed from the flock. These records aid in epidemiological efforts to trace the source of scrapie infections. If no animal in a monitored flock is diagnosed with scrapie during the 5-year period and none shows clinical signs of the disease, the flock is certified as scrapie-free. Since sheep from a certified flock fetch a higher price than other sheep, the market creates an economic incentive to participate in the program.

If NVSL diagnoses a case of scrapie, officials conduct an epidemiological investigation to determine the source of the infection and identify sheep that were exposed to the infected animal. Veterinary Services works with the state and the flock owner to develop a scrapie eradication plan. Those sheep determined to be at high risk of contracting the disease are culled from the flock, and Veterinary Services helps the flock's owner clean and disinfect[9] the sheep facilities.

USDA epidemiologists also conduct traceback studies to determine the source of scrapie infections discovered in animals at slaughter. As increasingly more sheep are registered in USDA's individual animal identification program, epidemiologists will have a better tool for tracking scrapie-infected sheep back through the herds in which they lived (personal communication, E. Williams, University of Wyoming, July 11, 2003). As mentioned earlier, 60 percent of the tissue samples examined for scrapie came from slaughterhouses in fiscal year 2002, but this represents a small amount of slaughterhouse surveillance relative to what Veterinary Services plans to do (personal communication, E. Williams, University of Wyoming, May 2003).

Statistics on Scrapie Infection

NVSL confirmed 193 cases of scrapie between October 1, 2002, and March 31, 2003, and identified 27 flocks that contained one or more cases of scrapie or were the source of a scrapie-infected animal (Veterinary Services, 2003b). A cumulative total of 49 flocks contained scrapie-infected sheep or were the source of an infected animal as of March 31, 2003. As noted earlier, however, these statistics do not reflect the true prevalence of

[9]As discussed in Chapter 7, disinfecting anything that has had contact with an agent of TSE—from a pasture to a scalpel—is extremely difficult. In most cases, scientists do not yet know how to eliminate prion infectivity.

scrapie in the United States because a limited number of flock owners participate in the certification program.

Surveillance of Bovines for BSE

As discussed above, no evidence of BSE has been detected in the United States. The disease became reportable in 1987, meaning that since then, USDA has required notification of all BSE cases (Brown et al., 2001). USDA began to conduct active surveillance for BSE in 1990.

At present, the surveillance program targets the subpopulations of the 45 million adult cattle in the United States that would be most likely to have BSE (Williams, 2002; PL 107-9 Federal Inter-agency Working Group, 2003). These subpopulations include cattle on farms that exhibit signs of neurological disease, cattle condemned at slaughter for neurological disease, cattle with neurological disease that are referred to veterinary diagnostic laboratories, rabies-negative cattle submitted to public health laboratories, cattle that are unable to rise from a recumbent position (often called downer cattle or fallen stock), and cattle that die of unknown causes on farms (Williams, 2002; PL 107-9 Federal Inter-agency Working Group, 2003; USDA APHIS, 2003a). In 2002 USDA significantly increased the proportion of tissue samples obtained for BSE testing from this last subpopulation (USDA APHIS, 2003b).

In addition to the above measures, veterinary pathologists at zoos in the United States conduct neuropathological examinations of animals that exhibited signs of neurological disease before death (USDA APHIS, 2003a). They do so because the United Kingdom's early cases of BSE included some zoo animals (Jeffrey and Wells, 1988; Kirkwood and Cunningham, 1994).

Until 1993, the USDA surveillance program for BSE used exclusively histopathological examinations of brain tissue to search for physical evidence of spongiform encephalopathy. In 1993, the program adopted immunohistochemistry and the Western blot to determine whether prions were present in tissue samples (Detwiler, 2003). Today, all the program's neuropathological examinations rely on immunohistochemistry, and some are performed in conjunction with histopathology and Western blot (Detwiler, 2003).

Historically, USDA and its collaborators have sampled cattle-brain tissues for BSE at approximately double the rate recommended by the Office International des Epizooties (OIE), which sets the world's standards for animal health (PL 107-9 Federal Inter-agency Working Group, 2003). The sampling rate rose to 5 times the OIE standard in 2000 and to more than 10 times the standard in 2001 (PL 107-9 Federal Inter-agency Working Group, 2003).

As shown in Figure 6-4, tissue samples from 19,900 cattle were tested

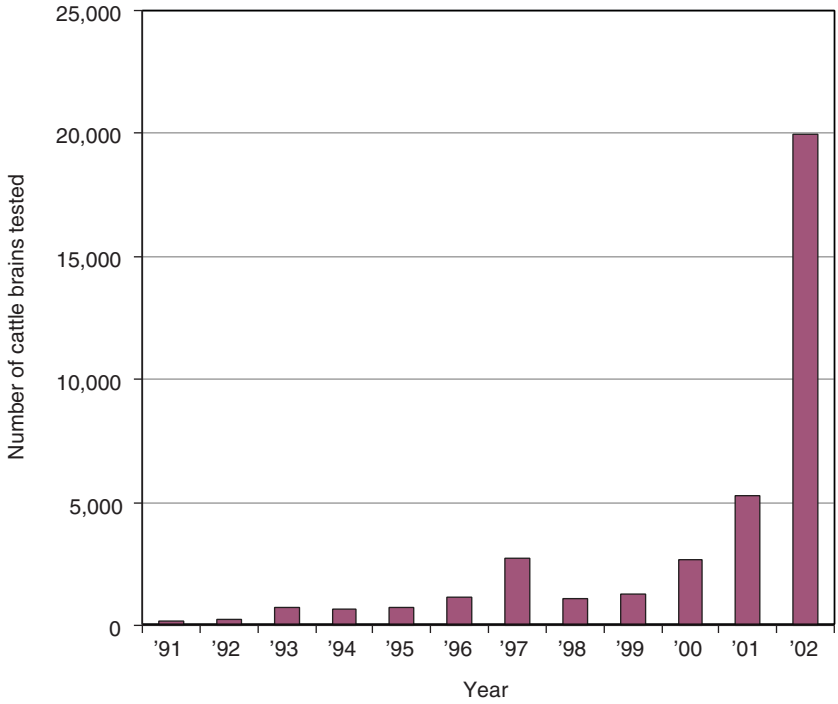

FIGURE 6-4 Number of cattle brains tested for BSE per year in the United States. SOURCE: USDA APHIS (2002).

for BSE in 2002 (USDA APHIS, 2002), surpassing the goal of 12,500 samples set for the 2002 fiscal year. Testing at least 12,500 brain-tissue samples would theoretically permit the detection of one or more clinical cases per million adult cattle, given the estimated number of adult cattle in the U.S. subpopulations most vulnerable to BSE. USDA decided to aim for the level of one per million because classic CJD appears to occur clinically at the rate of one case per million population, and because TSE researchers have hypothesized that other spongiform encephalopathies may also occur at that rate in any given population (Brown et al., 2001; USDA APHIS, 2002). This rationale may be inappropriate for the detection of endemic, foodborne cases of BSE, however, because such cases would occur at a higher prevalence than spontaneous TSEs in cattle.

USDA APHIS locates, monitors, and offers to purchase cattle that were imported to the United States from countries now known to have BSE or to be at high risk of a BSE outbreak, including the United Kingdom, Ireland, countries in continental Europe, and Japan (USDA APHIS, 2003a;

PL 107-9 Federal Inter-agency Working Group, 2003). The purchased cattle are sacrificed and tested for BSE. As of this writing, all of these animals have tested negative for BSE (PL 107-9 Federal Inter-agency Working Group, 2003). As of March 2003, USDA APHIS was aware of and monitoring seven live cattle that had been imported from BSE-reporting countries before the practice was banned.

Between 1991 and 2001, three studies assessing the risk of BSE in the United States led to the conclusion that the country is highly resistant to the introduction and spread of the disease (Health and Consumer Protection Directorate-General, 2000; HCRA and TUCCE, 2001; Veterinary Services, 1991a,b). The most recent study is discussed in detail in Chapter 7, but we address its surveillance aspects here.

The 2001 risk assessment, commissioned by USDA, underscored the importance of surveillance for managing the risk of BSE (HCRA and TUCCE, 2001; PL 107-9 Federal Inter-agency Working Group, 2003). The authors state that an important source of uncertainty in their model was the proportion of clinical cases of BSE that are correctly identified during antemortem surveillance at slaughter (HCRA and TUCCE, 2001:99). The authors' best- and worst-case assumptions for this proportion ranged from 99 percent to 50 percent, with a base level of 90 percent.

Fewer than 4 months after the release of the Harvard/Tuskegee study, the U.S. General Accounting Office (GAO) issued a report, requested by Sens. Tom Harkin, Richard G. Lugar, and Richard J. Durbin, critiquing aspects of the U.S. government's risk-management program for BSE (GAO, 2002). One criticism of the program was that the target population for BSE surveillance included relatively few cattle that died on farms. In unpublished comments addressed to GAO on its draft of this report, USDA responded that, very recently, it had begun to test cattle that died on farms of unknown causes. Plans were in place to increase the proportion of these cattle tested in fiscal 2002, the agency added.[10] USDA reiterated this point in its published response to GAO's recommendations (GAO, 2002:56; USDA, 2002).

Surveillance of Cervids for CWD

The nationwide prevalence of CWD is unknown at present as a result of inadequate surveillance. However, the participants in a federally mandated effort begun in May 2002 aim to change that situation (CWD Task

[10]Unpublished document provided to the committee by L. Detwiler of USDA APHIS in March 2003.

Force, 2002). Indeed, a few states, including South Dakota, Colorado, and Wyoming, have already established highly effective CWD surveillance and eradication programs. Yet a uniform national surveillance program for CWD has been difficult to implement in the United States because most states, federal agencies, and the U.S. Congress did not perceive the disease to be a national problem, and because the regulation of cervids is largely the province of states.

The alarming discovery of CWD in white-tailed deer shot by hunters in Wisconsin in autumn 2001 prompted Congress to instruct USDA and the Department of the Interior (DOI) to establish a national CWD management program (Joly et al., 2003; USDA and DOI, 2002). The two agencies jointly established a multijurisdictional, multisector CWD Task Force[11] that they cochair (CWD Task Force, 2002). The discovery of CWD in Wisconsin deer also prompted Congress to give USDA APHIS line-item funding for CWD for the first time. The fiscal year 2003 budget allocated almost $15 million to APHIS: $7.2 for farmed cervids and $7.7 for free-ranging cervids (personal communication, D. E. Goeldner, USDA APHIS, May 2, 2003). The CWD Task Force used that money to begin to implement its plan, which includes comprehensive nationwide surveillance for the disease (USDA and DOI, 2002). The funds are sorely needed, as many states have to date lacked the resources and infrastructure necessary to conduct CWD surveillance. Congress has allocated more funds to APHIS for CWD surveillance in the 2004 fiscal year (personal communication, Lynn Creekmore, USDA APHIS, October 10, 2003). The committee supports the plan outlined by the task force's surveillance working group, discussed later in this chapter. The congressional allocation also is being used to support epidemiological studies, the testing of thousands of cervid tissue samples from the 2002–2003 hunting season, the evaluation of new rapid-test diagnostics, and the investigation of a vaccine for CWD, among other activities (personal communication, D. E. Goeldner, USDA APHIS, May 2, 2003).

Spread of CWD Across the United States

At least 5.5 million free-ranging mule deer, 14 million free-ranging white-tailed deer, and 1 million free-ranging elk reside in the United States (Nowak, 1999). In addition, 130,000 to 200,000 elk and an unknown num-

[11]The CWD Task Force includes USDA, DOI, 14 state agencies concerned with wildlife management and agriculture, four universities, the Southeastern Cooperative Wildlife Disease Study, and the International Association of Fish and Wildlife Agencies. The task force was formed in response to a congressional directive that USDA and DOI assist state wildlife management and agriculture agencies with the management of CWD (CWD Task Force, 2002).

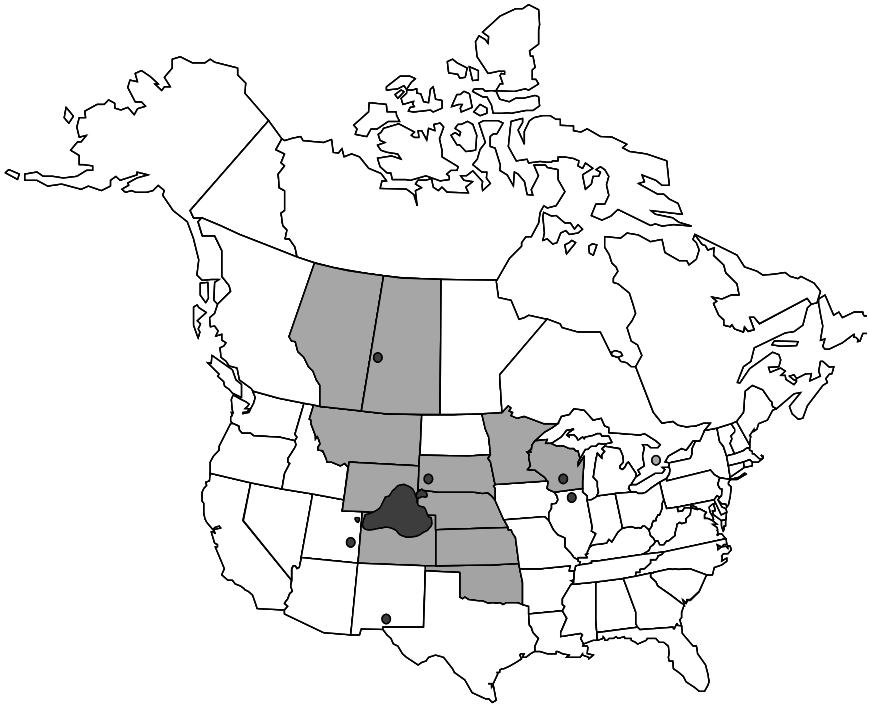

FIGURE 6-5 North American locations where CWD had been diagnosed as of May 2003. The darkest areas represent locations where CWD has been found in free-ranging cervids. The grey areas represent states and provinces where CWD has been diagnosed in captive cervids. Some of these CWD-positive captive herds have been depopulated. The grey circle near Toronto represents a location where CWD occurred in the 1970s and where the disease has not persisted.
SOURCE: personal communication, E. Williams, University of Wyoming, May 2003.

ber of deer live on American game farms, primarily in western, midwestern, and northeastern states (Williams, 2002). CWD was first recognized as a syndrome of cervids at a Colorado research facility in the 1960s (Williams and Young, 1980). It was first observed in free-ranging U.S. cervids in the 1980s (Spraker et al., 1997) and in farmed U.S. cervids in 1997 (Creekmore, 2002). The disease probably arose at least 40 years ago (Miller et al., 2000).

Between 1996 and 2002, CWD apparently[12] spread from the core endemic region illustrated in Figure 6-5—a contiguous area of northeastern

[12]It is also possible that increased awareness of CWD has brought preexisting cases to light for the first time.

Colorado and southeastern Wyoming—to captive and free-ranging herds in Illinois, Kansas, Minnesota, Montana, Nebraska, New Mexico, Oklahoma, South Dakota, Wisconsin, Utah, and the Canadian provinces of Saskatchewan and Alberta (Williams and Miller, 2002; Fischer and Nettles, 2003). Reports of the disease in states east of the Mississippi were particularly alarming because CWD previously had been considered a western problem alone (CWD Task Force, 2002). Figure 6-5 also illustrates the locations of the farmed and free-ranging cervids in North America known to be infected with the agent of CWD.

Helping to Prevent the Spread of CWD Through Surveillance

Scientists who study CWD expect the disease to continue spreading for the foreseeable future and to be found in new locations as surveillance improves. The results of fiscal year 2003 surveillance expanded the known distribution of CWD infectivity among wild cervids in such states as Wyoming and Colorado; however, the disease was not found in wild cervids outside of the eight states where it had been previously identified (personal communication, Lynn Creekmore, USDA APHIS, October 10, 2003).

In addition to the theoretical risk posed by CWD for humans who consume venison, the disease threatens the economic viability of the farmed cervid industry and could significantly impact hunting and its associated economies. Finally, models of CWD dynamics suggest that the epidemic could drive deer populations to extinction if infected herds are not identified and culled while the prevalence of the disease in the herd is low (Gross and Miller, 2001).

Many factors may be contributing to the expanding epidemic. They include the ease of lateral transmission of the infectious agent of CWD; the movement of cervids along natural migration corridors; the unknown mechanism of transmission; environmental contamination by the agent leading to the infection of more cervids (particularly at game farms where sick herds have been eradicated); the lack of efficient live-animal diagnostic tests; the lack of treatments for CWD; and the lack of inactivation techniques for the infectious agent (Gross and Miller, 2001; Williams and Miller, 2002).

Well-planned and -implemented CWD surveillance programs can enable states and other organizations to detect new outbreaks of the disease early and to take actions to prevent its spread. A nationally integrated program of this sort would enable a central organization to efficiently monitor the prevalence, incidence, and distribution of CWD in the United States, to identify national trends in the disease, to support epidemiological studies of CWD, and to evaluate intervention strategies. The fact that each state has jurisdiction over its own deer and elk populations has complicated nationwide data collection on the disease (personal communication, E. Williams,

University of Wyoming, May 2003). For these reasons, the committee supports the development of a comprehensive, unified surveillance program for CWD in cervids.

Recommendation 6.3: Provide funds to support the development of a nationwide surveillance system for chronic wasting disease in the United States. [Priority 2]

Present CWD Surveillance in the United States

Some elements of a national CWD surveillance system are already evolving. USDA APHIS has conducted surveillance for CWD in free-ranging cervids since 1997 in cooperation with a number of state wildlife and agriculture agencies. During the 2002–2003 hunting season, program participants collected more than 126,000 tissue samples from 37 states—more than 10 times the number of samples tested during the 2001–2002 hunting season but primarily from two states: Colorado and Wyoming (personal communication, L. Detwiler, USDA APHIS, April 28, 2003). All 50 states have accepted an offer from USDA APHIS for fiscal year 2004 funds to conduct CWD surveillance and related activities of the states' design (personal communication, Lynn Creekmore, USDA APHIS, October 10, 2003). In addition, the National Park Service conducts targeted surveillance of cervids exhibiting clinical signs of CWD and active surveillance of deer in parks located near confirmed CWD cases (CWD Task Force, 2002).

The extent of CWD surveillance in farmed cervids ranges from mandatory monitoring and certification to no surveillance at all, depending on the state (CWD Task Force, 2002). Since September 2001, APHIS has conducted surveillance of farmed elk herds known to harbor CWD-infected animals (USDA, 2001). As of June 2002, 23 states were testing captive cervids for CWD, and 12 more states were in the process of developing farmed-cervid surveillance programs (CWD Task Force, 2002). More than 6,200 tissue samples from farmed deer and elk were tested for CWD in fiscal year 2002 (Creekmore, 2002).

Goals for Improving CWD Surveillance

The surveillance working group of the CWD Task Force estimates that the United States must test 150,000 cervids for CWD per year to monitor the disease adequately (CWD Task Force, 2002). The country reached at least 84 percent of that goal in 2003, based on the aforementioned number of tissue samples collected. To guide all states toward the level of surveillance needed to adequately monitor CWD, the surveillance working group established the four broad goals and action items shown in Box 6-2. The

BOX 6-2
Goals and Action Items for Nationwide Surveillance of CWD

1. *Sampling plans:* Develop sampling design that specifies numbers of animal to be sampled by area and year, and assist agencies with surveillance strategies.

2. *Early detection:* For cervid populations and herds in which no infection has been detected, the primary surveillance objective is early detection of new CWD foci.

3. *Determination of prevalence rates:* For cervid populations in which infection has been detected, estimate CWD prevalence over time and space.

4. *Epidemiological investigations:* Conduct surveillance to support research investigations and trace-back (tracing movement into the herd) or trace-forward (tracing movement out of the herd) efforts for the purpose of identifying transmission mechanisms.

SOURCE: CWD Task Force (2002, section III-F).

working group aims to foster the development of techniques for enabling each state to share the results of its unique CWD surveillance program such that the combined data could be analyzed on the national level (personal communication, Lynn Creekmore, USDA APHIS, October 10, 2003). In addition, the working group proposed a national program to certify herds of farmed cervids as being CWD-free (CWD Task Force, 2002). This program would require owners to report all cervid deaths and to have CWD testing performed on all animals (except calves) that are slaughtered or die on the premises.

The committee supports the aims of the CWD Task Force's surveillance working group. We encourage the National Prion Research Program (NPRP) to fund projects that complement the group's activities.

TSE Surveillance as an Antidote to Terrorism

Adequate surveillance for TSEs is essential to discount and discredit terrorist hoaxes of planting BSE (World Health Organization, 2002). Research that helps produce better TSE diagnostics and better screening tests for infectivity would improve the United States' capability to conduct TSE

surveillance and provide convincing reassurance to the public, thereby undermining TSE agents as a tool for terrorism.

The committee determined that TSE agents would be ineffective tools for terrorism if the objective were to create an epidemic of TSE. The difficulty of artificially infecting animals or people with the agents, the inefficient transmission of the agents within and between species (except cervids), and the long incubation period of TSEs make TSE agents unlikely weapons. However, the lack of reliable diagnostic tests for TSEs and the limited understanding of the epidemiology and pathogenesis of these diseases could provide the conditions for an effective terrorist hoax (National Research Council, 2002). For example, if either a BSE hoax or a real case of BSE were effectively planted, it could damage consumer confidence in beef products, resulting in significant losses to the beef and cattle industries.

ESSENTIAL RESEARCH TO IMPROVE U.S. CAPABILITIES TO CONDUCT SURVEILLANCE FOR TSEs

Many unanswered questions regarding the epidemiology, natural history, and prevalence of animal and human TSEs in the United States hamper the nation's ability to conduct the best possible surveillance for these diseases. The limitations of current diagnostic tools for TSEs also pose obstacles to comprehensive surveillance. The committee recommends that NPRP support the research outlined in Box 6-3 because the results of such research would not only improve the quality of TSE surveillance in the United States, but also would advance prion science in general. These research recommendations are discussed in detail in the following sections.

Transmission Characteristics

Although not a single case of CWD has been diagnosed in humans, BSE has crossed the human species barrier. Therefore, it is important to determine whether the theoretical risk of CWD transmission to humans is real. Making this determination will require a substantial amount of experimental and epidemiological research, although work along these lines has begun. For example, studies in a cell-free conversion model have shown that the infectious agent of CWD can convert human prion protein (Caughey, 2001). New animal models, such as the ferret (Sigurdson et al., 2003) and mice with CWD transgenes, are being developed for use in CWD transmission experiments, as are cynomologous macaques (Lasmézas et al., 2001).

Nonhuman primates are particularly valuable surrogates for assessing the risk of transmission of the CWD agent to humans. The utility of cynomologous macaques as human surrogates in TSE transmission studies was demonstrated through experiments that further established BSE as the

BOX 6-3
Priority Research on the Epidemiology and Natural History of TSEs

Transmission Characteristics:

➤ Determine whether nonhuman TSEs, including chronic wasting disease (CWD), are transmissible to humans.
➤ Identify the determinants of interspecies transmissibility.

Exposure Characteristics:

➤ Identify mechanisms and routes of human and animal exposure to TSE agents.
- Determine the sources and magnitude of risks associated with human exposure to the agents of bovine spongiform encephalopathy (BSE) and CWD.

Host Susceptibility:

- Identify the determinants of host population susceptibility and resistance, including genetic factors.

Distribution, Prevalence, and Host Range:

- Determine the national and international geographic distribution of CWD by well-designed surveillance methods.
- Determine the breadth of strain diversity for agents of known TSEs.
- Search for previously unidentified TSEs.

NOTE: ➤ Indicates the most critical, urgently needed research.

cause of vCJD (Lasmézas, 2003). Lasmézas and colleagues showed that both the BSE and vCJD agents were readily transmitted to macaques by either the intracerebral (i.c.) or intravenous (i.v.) route, and that the transmission of either agent by either route resulted in the same histopathological lesions in the macaques' brains. Further, the scientists observed identical histopathological lesions in second-order macaques that were infected i.c. or i.v. with either the vCJD or BSE agent from the previously infected macaques. These observations supported the hypothesis that the same prion strain causes both BSE and vCJD (Lasmézas et al., 2001).

Moreover, Lasmézas and colleagues observed prions in the tonsils, spleens, and lymph nodes of the macaques—observations also made in humans with vCJD (Lasmézas, 2003). This finding suggests that cynomologous macaques would be excellent models for evaluating the risk

of iatrogenic transmission of vCJD to humans and the transmissibility of the CWD agent to humans by various routes.

Studies are needed to determine the transmissibility of CWD not only to humans, but also to other animal species. Several investigations are under way to determine whether the CWD agent is transmissible to cattle. In one such study, 5 of 13 cattle inoculated i.c. with CWD-infected tissue from the same mule deer developed a disease resembling TSE (Hamir et al., 2001; Hamir and Miller, 2002; USDA and FDA, 2003). In another ongoing study, cattle exposed in 1997 to the CWD agent by either oral inoculation or contact with CWD-affected cervids had shown no evidence of transmission as of July 2003 (personal communication, E. Williams, University of Wyoming, July 11, 2003). Similar studies should be performed with other domestic and wild animals using various routes of transmission, including the oral route. For example, CWD transmission studies in cervids' feline predators, such as cougars, would be valuable.

Exposure Characteristics

The natural routes and vehicles of exposure to TSE agents and the mechanisms by which the agents invade a host are well defined for some TSEs and poorly defined for others. Considerable epidemiological and experimental evidence underscores the importance of the oral route, ingestion, and alimentary uptake of the agents of scrapie in sheep, BSE in cattle, vCJD in humans, and transmissible mink encephalopathy in mink. Yet little is known about the routes and vehicles by which cervids are exposed to the CWD agent. Nor is it known whether the agent of CWD can infect primates (as a proxy for humans) after oral or cutaneous exposure to CWD-infected cervid tissues.

Investigations into the route or routes by which healthy cervids become exposed to CWD and into the mechanisms of CWD infection are under way at a few U.S. laboratories and field sites. The committee urges NPRP to support these long-term investigations and fund complementary ones. For instance, experiments should be performed to determine whether cynomologous macaques can become infected with the CWD agent through oral or cutaneous exposure.

Host Susceptibility

Susceptibility to a TSE is believed to involve multiple host, agent, and environmental factors. Which factors play the most significant roles and how those factors interact are poorly understood. A principal host factor is the composition of the gene that codes for the PrP protein. Genetic variability along the PrP gene sequence in animals suggests that evolutionary pres-

sure has led to the development of PrP sequences that are resistant to prion diseases. One of the best such examples is in sheep.

Genetic resistance of sheep to the scrapie agent has been studied for decades. The polymorphisms at codons 136, 154, and 171 along sheep *Prnp* have been recognized as major factors in determining resistance to scrapie. Three amino acids—alanine (A), glutamine (Q), and arginine (R)—are encoded at those three sites. Sheep encoding ARR, especially those that are homozygous (ARR/ARR), are extremely resistant to scrapie. There has been only a single case of a sheep with an ARR/ARR genotype that developed scrapie in nature. The sheep industry in the United Kingdom is taking active steps to inbreed ARR/ARR resistance into its flocks. Recently, however, experimental i.c. inoculation of ARR/ARR sheep with the BSE agent led to the surprising observation that 3 of 19 sheep developed clinical symptoms of TSE (Houston et al., 2003). This finding raises questions about the advisability of such inbreeding and suggests the need to investigate whether there is a difference in susceptibility to the scrapie and BSE agents in sheep.

Much also needs to be learned about the genetic factors that determine susceptibility to human TSEs. We know that the *PRNP* gene in humans is polymorphic at certain locations; for instance, PrP may have either methionine or valine at codon 129. A recent report by Mead and colleagues (2003) suggests that human resistance to prion disease may be traced to early humans, whose practice of cannibalism placed them at risk of developing fatal, kuru-like TSEs. The investigators hypothesized that this, in turn, resulted in selective survival of those resistant to TSEs. Individuals who are heterozygous at codon 129 of human *PRNP* appear to be more resistant to TSEs than those who are homozygous at that codon, according to this report, especially those who are homozygous for methionine. This hypothesis could explain the high frequency worldwide of heterozygosity at *PRNP* 129, the authors suggest, especially among the surviving female members of the Fore people who practiced ritual cannibalism (see Chapter 2).

Distribution, Prevalence, and Host Range of TSEs

The global distribution of TSEs is unknown because prion diseases could be occurring in areas where cases are unobserved or undiagnosed. Determining the extent of TSEs worldwide will require not only better diagnostic tools, but also more extensive surveillance. Ecological research to improve sampling strategies, study animal movements and behavior, and develop novel tracking methods would enhance the quality of TSE surveillance.

It is likely that novel, undiscovered TSEs exist. They may be occurring in animals not previously known to have TSE, or known TSE agents may be presenting differently in new animal hosts. Surveillance systems must have

sufficient sensitivity to identify newly emergent TSEs or new presentations of TSE disease expression. The development of this capacity will require a multitude of surveillance methods and programs, and research to develop and sustain the necessary tools.

Recommendation 6.4: Provide funds to expand research on the natural history, prevalence, distribution, exposure and transmission characteristics, host susceptibility, and host range of transmissible spongiform encephalopathies, especially chronic wasting disease. [Priority 1]

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7 Assessment of Strategies to Prevent and Treat Transmissible Spongiform Encephalopathies

The prospects for successfully treating an established prion disease are so disheartening at present that the most effective strategy for man-aging the threat of transmissible spongiform encephalopathies (TSEs) is to avoid preventable exposure to the infectious agents. This chapter begins, then, by describing the strategies and policies adopted by the United States to prevent human and animal exposure to the agent of bovine spongiform encephalopathy (BSE) through food and feed. Next, the chapter describes means of preventing human and animal exposure to the agent of chronic wasting disease (CWD) in food and the environment in the United States. We then discuss the challenges of preventing exposure to TSE agents by inactivating them in blood, blood derivatives, and tissue, as well as on surfaces and in the environment; this section also addresses the potential for vaccination as a preventative strategy. The final section of the chapter reviews the therapeutic agents used to date in attempts to treat TSEs. The development of a successful therapy will require a level of innovation and effort as exceptional as that needed for the development of antemortem diagnostics, described in Chapter 4.

MEASURES TO PREVENT THE BSE AGENT FROM ENTERING THE U.S. FOOD CHAIN

The United States has built a multilayered preventive barrier during the past 15 years against the introduction of the BSE agent into the U.S. animal

and human food chains.[1] This barrier has three components (PL 107-9 Federal Inter-agency Working Group, 2003):

1. Prevent the agent of BSE from entering the United States and infecting U.S. cattle.
2. If the agent of BSE penetrates U.S. borders and infects cattle, prevent the amplification of the agent throughout the U.S. cattle herd.
3. Prevent the exposure of U.S. residents to the agent of BSE through food and other products that come either partially or completely from cattle.

According to a 3-year risk assessment by Harvard and Tuskegee Universities, this trilayer barrier keeps animals and humans in the United States at very low risk of exposure to the BSE agent—despite imperfect compliance with and enforcement of certain prevention strategies. "If BSE were somehow to arise in the U.S.," the study concludes, "few additional animals would be infected, little infectivity would be available for human exposure, and the disease would be eradicated" (HCRA and TUCCE, 2001:97–98).

Although U.S. policies toward BSE effectively safeguard animal and human health, their effectiveness in protecting the U.S. economy is less certain. According to a recent congressionally mandated analysis by the U.S. Department of Agriculture's (USDA) Economic Research Service, the identification of just a single case of BSE in the United States could be more costly to this country than the BSE outbreak has been to the United Kingdom to date (Mathews and Perry, 2003). The analysis does not give a dollar amount for the potential U.S. cost, but for perspective, farmers alone in the United Kingdom lost an estimated $700 million (Mathews and Perry, 2003), not to mention the losses to the beef processing and related industries. The authors of the USDA analysis based their prediction in part on the fact that the U.S. population is 5 times that of the United Kingdom, the U.S. beef sector is 10 times greater, and U.S. beef exports far exceed the amount of beef exported from the United Kingdom before the BSE outbreak.

The very low risk that a case of BSE would enter the U.S. food chain and spread to other cattle would not mitigate the predicted financial impacts of a BSE case in the United States, the analysts forecasted, especially if the cow were a native-born animal (Mathews and Perry, 2003). Domestic consumption of beef products would likely decrease,[2] U.S. renderers would

[1]EDITORS' NOTE: After this report was completed, the first U.S. case of BSE was identified in Washington State and was announced to the public on December 23, 2003.

[2]By contrast, the USDA Foreign Agricultural Service reports anecdotal evidence that Canadians responded to the BSE outbreak there by consuming more beef, not less (Myles, 2003).

have to find new ways to use or dispose of cattle offal[3] and other edible waste if these materials were banned from use in animal feed, and countries with BSE policies similar to those of the United States would stop importing U.S. beef and ruminant products. In addition, such industries as cosmetics, pharmaceuticals, and medical supplies that use livestock by-products or rendered products might need to find alternative sources of these materials for a period of time. Later in this chapter, we discuss the impact of the single Canadian case of BSE on that country's beef, cattle, and related industries.

This section describes the policies behind each layer of the United States' preventive barrier against the infectious agent of BSE (with the exception of surveillance, which is discussed in Chapter 6). Table 7-1 provides a chronological overview of the implementation of many of these policies. In the discussion that follows, we note salient criticisms regarding certain policies and describe how federal agencies have responded to those criticisms. While policy recommendations and cost-benefit analyses are beyond the scope of this committee's mandate, we recommend research that would further strengthen the present safeguards against BSE.

Restrictions on Imports

It is widely believed that the exportation of BSE-infected cattle and cattle-derived products from the United Kingdom spread the infectious agent of BSE to countries in Europe and beyond. Beginning in 1989, therefore, USDA's Animal and Plant Health Inspection Service (APHIS) banned the importation of live ruminants[4] and most ruminant products from all nations that had identified a case of BSE (USDA APHIS, 2003d). Twenty-three countries fall into that category as of this writing (Office International des Epizooties, 2003). The Harvard/Tuskeegee risk assessment cites the import ban as one of the most effective tools for keeping the agent of BSE out of the United States (HCRA and TUCCE, 2001).

USDA expanded the ban in 1997 to all of Europe, regardless of whether a country had reported a case of BSE (USDA APHIS, 2003d). The ban was further expanded the following year to include any country "at risk" for BSE, meaning countries that conduct inadequate surveillance for BSE or regulate imports related to BSE in a less restrictive manner than does the United States (USDA APHIS, 1999). Subsequently, after the European Union

[3]Offal is the parts of butchered animals not processed into human food. These parts generally include blood, internal organs, legs, heads, and spinal cords (Harlan, 2003).

[4]Ruminants are hoofed, even-toed, usually horned animals that characteristically have a four-chambered stomach and chew their cud.

(EU) announced in 2000 that feed made in EU countries from the offal of nonruminants may have been contaminated with the agent of BSE, USDA prohibited the import of all rendered protein and rendering wastes originating from or processed in the EU, regardless of the animal species (USDA APHIS, 2003d).

A 2002 report by the U.S. General Accounting Office (GAO) on U.S. vulnerabilities to BSE asserts that the United States lacks sufficient capacity to inspect all cattle imports, a weakness in the enforcement of the import bans (GAO, 2002). USDA responded with a description of several efforts under way to remedy this problem:

- USDA proposed using a portion of its fiscal year 2003 budget to increase the number of its inspectors at ports of entry from 2,500 to 4,000 people (USDA and Department of Health and Human Services [DHHS], 2002).
- To "strengthen coordination and documentation" among agencies that inspect products at U.S. ports of entry, USDA noted that it had obtained funds through the 2002 Defense Appropriations Act to integrate its computer technologies with those of the other relevant agencies (USDA, 2002:1).
- USDA would invest in new detection systems, such as x-ray equipment (USDA and DHHS, 2002).

Feed Ban

While the import bans described above attempt to keep BSE out of the United States, restrictions on the ingredients of feed products intended for ruminants aim to prevent BSE from spreading in the United States should it be introduced through imported goods, through a spontaneous case of BSE in a U.S. cow, or through other means. In 1997, the U.S. Food and Drug Administration (FDA) prohibited the use of most mammalian protein in animal feed intended for ruminants (FDA CFSAN, 1997). This prohibition is often termed simply the feed ban.

There is a consensus among scientists that cattle can contract BSE by eating animal feed made from the offal of scrapie-infected sheep or of BSE-infected cattle. This opinion stems largely from epidemiological work by Anderson and colleagues (1996), who conclude that the widespread consumption of cattle feed contaminated with the infectious agents of scrapie and BSE was the most likely cause of the BSE epidemic in the United Kingdom.

The Harvard/Tuskegee risk assessment indicates that the feed ban is one of the most important elements of the U.S. barrier against BSE. In fact, the authors conclude, the effectiveness of the feed ban influences the risk of

TABLE 7-1 Measures Taken by the United States to Prevent the Introduction, Spread, and Consumption of the Infectious Agent of BSE

Date	Measure Taken
1987	BSE made a reportable disease.
1989	Ban on importation of live ruminants and most ruminant products from BSE-reporting countries instituted.
1990	USDA's Animal and Plant Health Inspection Service (APHIS) launches active surveillance for BSE and a BSE-education program.[a]
1992	U.S. Food and Drug Administration (FDA) recommends that manufacturers of dietary supplements avoid materials that could contain BSE or scrapie infectivity.
1993	Nonambulatory cattle added to targets of BSE surveillance. FDA requests that most bovine source materials[b] used in the manufacture of regulated products come from scrapie-free countries.
1994	FDA requests that bovine-derived materials for animals, cosmetics, or dietary supplements come from BSE-free countries.
1997	Ban on importation of live ruminants and most ruminant products from all of Europe instituted. FDA bans the feeding of most mammalian proteins to ruminants. FDA requests that bovine gelatin from countries reporting BSE not be used in certain products.[c]
1998	Ban on importation of ruminants and ruminant-derived products from countries at risk for BSE instituted.[d] Vermont quarantines two flocks of imported sheep possibly exposed to the BSE agent from contaminated feed in Europe.
1999	USDA proposes a new rule to intensify scrapie-eradication efforts.
2000	Ban on import of rendered animal protein products originating from or processed in Europe instituted. BSE surveillance more than doubles from 1,300 to nearly 2,700 cattle brains tested. USDA seizes Vermont sheep after four die with an atypical TSE of foreign origin.

BSE to the United States more than any other factor. They note: "A single breach of the feed ban can introduce . . . cattle to a substantial amount of BSE infectivity" (HCRA and TUCCE, 2001:97).

Challenges of Enforcing the Feed Ban

Before and during 2001, FDA had serious problems with monitoring and enforcing compliance with the feed ban. A significant percentage of animal rendering plants and feed mills failed to meet at least one major requirement from 1998 through 2000, according to an FDA report on the

TABLE 7-1 Continued

Date	Measure Taken
2001	Precautions enacted to protect safe, edible ruminant products from contamination while passing through countries reporting or at risk for BSE en route to the United States.
2002	Food Safety and Inspection Service (FSIS) issues a directive for routine inspection of advanced meat recovery (AMR) systems and for regulatory actions if spinal cord is detected in beef products produced by AMR.
2003	USDA APHIS solicits public comment on ways to control the risk that dead and nonambulatory ruminants could facilitate the spread of BSE.[e] USDA APHIS closes a loophole[f] to prevent the importation of live ruminants from Canada immediately after the May 20 announcement of a BSE-positive cow in Canada. On August 8, USDA lifts part of the import ban on Canada by allowing the importation of hunter-harvested wild ruminant products intended for personal use and accepting applications for import permits for a number of ruminant products.[g]

[a]The education provided involves teaching veterinarians, farmers, and others who work with cattle to recognize the clinical signs of BSE.

[b]Excluding gelatin.

[c]Includes injectable, implantable or ophthalmic products. Also, FDA asked that manufacturers take special precautions when using gelatin for oral and topical use.

[d]An at-risk country is one that conducts inadequate surveillance for BSE or that regulates imports related to BSE in a less restrictive manner than does the United States (USDA APHIS, 1999).

[e]Source: USDA APHIS (2003b).

[f]The original rule had exempted certain regions, including Canada, under certain circumstances, from the requirement to obtain a permit to import live ruminants into the United States (USDA APHIS, 2003a). The import ban automatically applies to ruminant meat, ruminant meat products, and ruminant by-products from Canada as of May 20.

[g]Source: USDA (2003c).

SOURCES: Adapted from Brown et al. (2001) and USDA APHIS (2003d).

9,947 inspections of the rendering plants, feed mills, and related businesses[5] conducted during those years (FDA CVM, 2001a). For instance, the report states that 28 percent of the inspected rendering plants lacked a system to prevent commingling of mammalian protein with other materials, and 20 percent of the inspected, FDA-licensed feed mills did not place a required

[5]These include ruminant feeders (operations that feed and care for ruminants), and protein blenders (GAO, 2000; FDA, 2001b).

caution label on animal feed containing mammalian protein. However, these data do not capture the true rate of compliance because, according to the report, state and FDA officials had not inspected 30 to 40 percent of all U.S. renderers and feed mills (FDA CVM, 2001a).

The Harvard/Tuskegee risk assessment addressed these shortcomings and incorporated them into its analysis. Subsequently, FDA boosted its efforts by inspecting more firms that handled mammalian protein and by reinspecting more of the firms previously found to be out of compliance (FDA CVM, 2001b). FDA's Center for Veterinary Medicine (CVM) gave businesses easy access to the checklist used by inspectors to determine compliance with the feed ban by placing a link to the checklist on the center's Web site (FDA CVM, 2001c). CVM also hired a contractor to restructure the database used to manage the information reported by the state officials and FDA field officers who conducted inspections for compliance with the feed ban (FDA CVM, 2001b). Nevertheless, GAO's 2002 report sharply criticized FDA for its poor enforcement of the feed ban and for its "severely flawed" database, and recommended a number of ways in which the agency could improve compliance rates (GAO, 2002).

FDA continued to improve its methods of enforcement. Feed mills that used mixed-species meat and bone meal came under increasing scrutiny because of the risk that mammalian protein could contaminate feed destined for ruminants (personal communication, D. Harlan, Excel Food Solutions Company, March 25, 2003). Some firms decided to stop using mammalian proteins altogether. By March 2002, CVM reported, the compliance rate for 2,153 U.S. firms handling materials prohibited for use in ruminant feed had reached 95 percent (FDA CVM, 2002). The next month, CVM began using a new database to better manage the information on nationwide inspections and enforcement activities related to the feed ban.

While criticisms of enforcement of the feed ban had subsided by 2003, they had not been altogether eliminated. At least one major U.S. rendering firm, Darling International Inc. of Irving, Texas, stated in February 2003 that FDA should take "more vigorous enforcement actions against violators" of the feed ban (Ransweiler, 2003:2). At the same time, industries affected by the feed ban have taken steps to monitor themselves and to make changes that reduce the risk of transmitting the BSE agent to ruminants. For instance, in 2001 the American Protein Producers Association and the American Feed Industry Association began to hire outside auditors to conduct inspections of plants and mills (Ransweiler, 2003). A number of firms subject to the feed ban stopped using mixed-species meat and bone meal (Harlan, 2003). Some producers voluntarily stopped feeding mammalian-derived meat and bone meal to all their livestock, reducing the risk that ruminants on a farm would accidentally be given the banned feed. The

combination of stronger FDA enforcement and self-monitoring by industry bolsters the effectiveness of the feed ban.

Tools to Detect Mammalian Protein in Animal Feed

An additional way to prevent ruminants from eating feed containing mammalian protein is for ruminant producers to test their purchased feed for the banned material. At least one test that detects some banned material has been on the U.S. market since 2002: Agri-Screen® for Ruminant Feed, manufactured by Neogen Corp. of Lansing, Michigan (Neogen Corp., 2002a). According to Neogen, its product enables feedlots, dairies, marketers of feed products, regulators, and auditors to verify that ruminant feed and feed supplements do not contain ruminant muscle proteins, a marker for the presence of ruminant tissue. Since the product does not detect mammalian muscle protein in general, however, the test cannot verify whether a sample of feed made for ruminants is in compliance with the feed ban.

The company describes Agri-Screen as a single-step, lateral-flow immunochromatographic assay (Neogen Corp., 2002b). It consists of an absorbent strip with a reagent area containing color-tagged antibodies that are specific to heat-stable ruminant muscle protein, and a control area farther upstream. Like a pregnancy test, the strip wicks the extract through the reagent and control areas. Within about 10 minutes, a colored line always forms in the control zone, but a second line forms in the reagent zone only if the feed sample contains ruminant muscle protein. Although the company claims the test can detect ruminant muscle protein present in concentrations as low as 1 percent of a sample, an industry source who has used the test says its lower limit in practice ranges from 1 to 5 percent (personal communication, D. Harlan, Excel Food Solutions Company, May 2003).

Feed tests such as Agri-Screen provide a worthwhile, additional line of defense against the introduction of BSE into the food chain. It would be even better to have a test that could detect mammalian protein, not just ruminant protein. The development of such products for an affordable price should be encouraged, as should their use by farmers.

Additional Ways to Prevent the BSE Agent from Entering Ruminant Feed

Policy recommendations are beyond the charge of this committee. However, it is worth noting that additional precautions beyond those in the FDA feed ban would further reduce the risk of amplification of the BSE agent should the disease arise in the United States. For instance, FDA could prohibit the use of mammalian protein in feed for *all* animals, not just rumi-

nants. The United Kingdom instituted such a policy[6] in 1996, and the EU[7] followed in 2001 (Brown et al., 2001). This more stringent measure makes sense in countries that, unlike the United States, have diagnosed cases of BSE or are at high risk for the disease.

At least one challenge posed by the potential prohibition of mammalian protein in animal feed is to find alternative means of eliminating the 3.6 billion pounds of ruminant meat and bone meal left over from meat processing each year (Harlan, 2003). Present U.S. research into alternative fuels may offer at least a partial solution. Some scientists in this field are developing an experimental industrial boiler powered by agricultural by-products, including meat and bone meal, blood meal, and tallow (The Energy Institute, 2002). Although the boiler may be a more efficient disposal mechanism than incineration, it would yield less energy than the amount required to create the meat and bone meal—from producing feed, to raising animals, to processing parts into by-products (personal communication, D. Cliver, University of California, Davis, June 21, 2003). The potential prohibition of mammalian protein in all animal feed also would challenge animal producers to find alternative affordable sources of nutrients for their livestock.

How Beef Processors Prevent the BSE Agent from Entering the Food Chain

The beef processing industry and USDA have developed procedures and regulations to prevent tissue infected with the BSE agent from entering the food chain should a cow infected with the BSE agent go undetected on the farm and be sent to slaughter. These measures are not foolproof, however. The sheer number of cattle involved—U.S. beef processors slaughtered more than 36 million cattle in 2002 (see Figure 7-1)—makes BSE detection at the slaughterhouse a formidable task. Beef processors reduce the risk of BSE-infected tissue entering the food chain by focusing on three aspects of their operation: procurement, antemortem inspection, and the removal of central nervous system (CNS) tissue.

[6]Specifically, the United Kingdom bans mammalian meat and bone meal from all animal feed and fertilizer (Brown et al., 2001).

[7]The EU prohibits the use of most animal protein in feed for any farmed animal species. Exempted proteins include those in milk, blood, and gelatin (Brown et al., 2001).

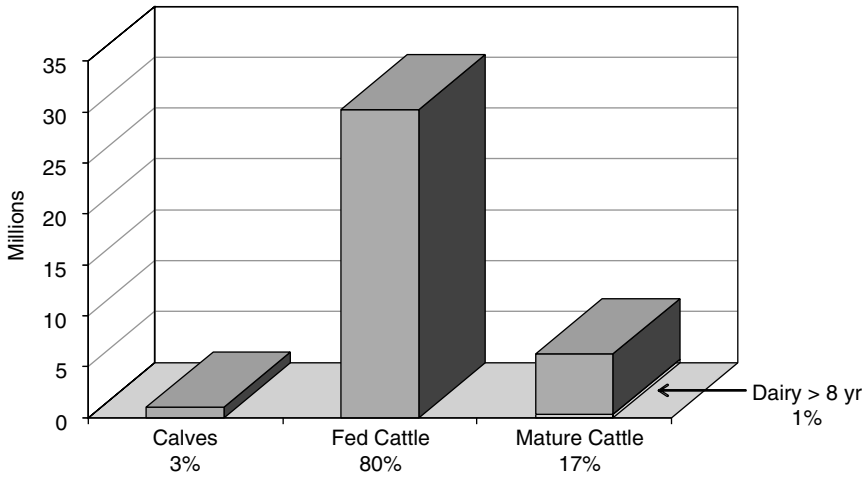

FIGURE 7-1 The 36.75 million U.S. cattle slaughtered in 2002 comprised calves (3 percent); fed cattle (80 percent); and mature cattle (17 percent), which included a small percentage of dairy cattle older than age 8. Fed cattle are 1 to 2 years old. Mature cattle are more than 2 years old.
SOURCE: Harlan (2003).

Procurement Controls

Some processors have limited their risk of exposure to BSE through company policies that specify the kinds of cattle that will or will not be purchased. For instance, a processor may decline to purchase nonambulatory cattle (Harlan, 2003), that is, cattle that cannot rise from a recumbent position. These so-called downers can no longer stand because they are ill, and BSE is always suspect as the cause of illness in downer cattle. As noted in Chapter 6, nonambulatory cattle are the targets of active U.S. surveillance for BSE.

Other procurement controls include buying only cattle of North American origin (Harlan, 2003), although, as noted in Table 7-1, the United States banned the importation of cattle from Canada as of May 2003 because a case of BSE was discovered in Alberta (USDA and FDA, 2003). Buyers may also request that the producer certify the BSE-free status of an animal, and such certification is provided frequently. However, the committee sees little value in this sort of certification at present because only a neuropathological exam can establish the BSE-free status of a cow. An antemortem test would make the BSE-free certification of live cattle meaningful.

Antemortem Inspection

Officials from USDA's Food Safety and Inspection Service (FSIS) inspect all incoming cattle at all U.S. slaughterhouses for signs of neurological disease (USDA and FDA, 2003). In general, if an animal shows such signs, it is condemned, and its meat may not be used for human consumption (HCRA and TUCCE, 2001). However, if the signs are not pronounced or typical, an inspector may designate the animal as suspect but not condemned (personal communication, D. Cliver, University of California, Davis, July 2003). After FSIS notifies USDA Veterinary Services of the suspect animal, laboratory staff at one of USDA's 15 National Veterinary Service Laboratories analyze the animal's brain tissue for evidence of BSE or some other TSE. FSIS tracks instances of antemortem or postmortem condemnation due to signs of disease (HCRA and TUCCE, 2001).

As mentioned earlier, no case of BSE has been identified in the United States to date. However, antemortem inspections can identify only clinical cases; they will not identify infected cattle during the incubation period, which lasts 2 to 8 years. The Harvard/Tuskegee risk assessment could not determine with certainty what percentage of clinical BSE cases inspectors might miss. In fact, the authors note that this was one of the most important sources of uncertainty behind the study's estimates of human exposure to the BSE agent.

Condemned animals are rendered or incinerated (HCRA and TUCCE, 2001). Rendered by-products could be turned into feed for nonruminant animals or an ingredient for cosmetics, among other products.

Removal of CNS Tissue from Slaughtered Cattle

BSE infectivity becomes concentrated in CNS tissue during the later stages of the disease. Therefore, in case a BSE-infected animal should fail to be detected by antemortem inspection, meat processors can reduce the risk of human consumption of the BSE agent by removing all CNS tissue from cattle.

The air-injection captive bolt pistol, a tool used to render cattle unconscious before slaughter, has been implicated in the inadvertent spread of CNS tissue to the blood and thereby to the heart, lungs, and liver. The pistol would thrust a bolt under high pressure into the skull of an animal to render it unconscious (TSE BSE Ad Hoc Group, 2001). Projecting a volume of air into the cranial cavity at high speed would displace small but visible pieces of brain into the bloodstream. Most U.S. meat processors have not used air-injection stunning devices since at least 2000 (HCRA and TUCCE, 2001). FSIS plans to complete a direct final rule in 2003 prohibiting the use of those devices (USDA, 2003b). From the standpoint of BSE risk, a safer

tool is the nonpenetrating captive bolt stunner, which literally knocks the animal unconscious without penetrating the skull.

Once the animal has been slaughtered and exsanguinated, the body is split open. At some plants, workers vacuum the spinal cord out of the spinal canal, then visually check for any remaining spinal cord and remove what is found using a hand-held scraper. However, this method does not remove dorsal root ganglia, which are directly in contact with the spinal column and would likely contain BSE infectivity if the spinal cord did. Other plants use a saw that removes the intact spinal column. Some processors conduct hourly quality checks to verify the complete removal of the spinal cord.

In the 1990s, the beef processing industry embraced a new technology called advanced meat recovery (AMR). The technology mechanically recovers from bones meat that otherwise would be rendered or discarded, increasing the amount of meat obtained from—and thus the value of—each animal. The use of AMR has created controversy, however. Opponents have asserted that the technology slices residual spinal cord tissue off neck bones and backbones, and that beef processors have mixed that CNS tissue with muscle tissue to sell as meat.

As early as 1997, USDA FSIS issued a directive explicitly reminding beef processors that "product that contains spinal cord does not come within the definition of 'meat' in . . . the regulations" (FSIS, 1997). The directive instructed inspectors to determine whether processors were completely removing spinal cord from neck and back bones before sending those bones to AMR. If a processor did not appear to be doing so, the inspector was to send a quarter-pound (113.4 gram) sample of finished product to an FSIS laboratory for verification. The GAO (2002) report mentioned earlier asserted that USDA had not rigorously enforced this directive, that inspectors tested AMR-derived products too infrequently, and that FSIS did find spinal cord tissue in a significant number of samples taken.

In 2002, FSIS surveyed 34 beef processing plants that use AMR systems and found that approximately 35 percent of the final product samples tested positive for spinal cord and associated tissues (FSIS, 2003b). Consequently, FSIS issued a directive in December 2002 with new instructions for the "routine" sampling of beef products produced by AMR and new enforcement actions should spinal cord be found in those samples (FSIS, 2002, 2003:1). On the day the directive took effect—March 3, 2003—FSIS announced that the survey had provided enough data to support the creation of a new rule on AMR systems (FSIS, 2003b). This rule will include specifications for the removal of CNS and associated tissues. Meanwhile, FSIS determined that the amount of noncompliance with existing rules regarding AMR and spinal cord tissue demanded that the 2002 directive be substantially rewritten "to reiterate that establishments whose AMR system repeat-

edly fails to produce product that is free of spinal cord will not be allowed to produce AMR meat from beef vertebrae, and that product containing spinal cord tissue will not be allowed to enter commerce labeled as meat" (FSIS, 2003a).

An effective, inexpensive, and convenient test for verifying the absence of CNS tissue in a beef product is the Ridascreen® glial fibrillary acidic protein (GFAP) test developed by Schmidt and colleagues (personal communication, D. Cliver, University of California-Davis, March 20, 2003; Schmidt et al., 1999). GFAP is the most abundant protein in the glial filaments of differentiated astrocytes, which appear only in CNS tissue. The test, an enzyme-linked immunosorbent assay, can detect as little as 1.0 ng of CNS tissue in a sample (Schmidt et al., 1999). According to an industry source whose company uses the test as back-up verification for spinal-cord removal, the assay reliably detects CNS tissue concentrations as low as 0.1 percent of raw meat (Harlan, 2003). This sensitivity is sufficient to keep BSE risk materials out of the food supply in BSE-free countries such as the United States (personal communication, D. Cliver, University of California-Davis, March 20, 2003).

An independent study found that the Ridascreen® Risk Material 10/5 test is 10 times more sensitive than another CNS-tissue assay, the ScheBo® Brainostic™ kit, which uses immunoblotting to detect a different CNS-specific antigen (Hajmeer et al., 2003). However, the Ridascreen® test cannot distinguish between GFAP from cattle and GFAP from pigs. This means the test would yield a positive result if a meat product containing tissue from both cattle and pigs included porcine CNS tissue.

Recommendation 7.1: Fund research to improve rapid, accurate, and affordable screening assays for central nervous system (CNS) tissue such that the assays can specifically identify CNS material from cattle in processed meat products. [Priority 2][8]

Rendering of Ruminant Tissue and the Potential Spread of the BSE Agent

Many animal rendering plants use discarded tissue from multiple sources to manufacture a variety of edible products for animals and humans and inedible products for people. These products include meat-and-bone meal (MBM), poultry feed, gelatin, and cosmetics ingredients. The raw materials that are rendered in the United States include certain animals

[8]The committee denotes each recommendation as priority level 1, 2, or 3 based on the criteria and process described in the Introduction.

that were condemned during slaughter surveillance, leftover cattle tissue from beef processors (Harlan, 2003), and plate waste (HCRA and TUCCE, 2001).

According to the Harvard/Tuskegee risk assessment, the rendering of dead-on-farm and downer cattle is one of the most likely routes by which the BSE agent would enter the animal feed chain (HCRA and TUCCE, 2001). Present U.S. policies permit the rendering of these at-risk cattle for further processing into animal feed. By contrast, scrapie-suspect sheep and cattle condemned because of neurological signs are incinerated (personal communication, L. Detwiler, independent consultant, August 2003). USDA APHIS acknowledges that if BSE or scrapie were present in the rendered tissue of dead-on-farm cattle, downer cattle, or sheep, and if that rendered material were used in cattle feed (deliberately or accidentally in violation of the feed ban), healthy cattle could contract BSE (USDA APHIS, 2003b). In January 2003, USDA APHIS wrote, "[W]e believe that dead stock and downer animals represent the most significant potential pathway that has not been addressed in previous efforts to reduce BSE risks" (USDA APHIS, 2003b:2704).

Another possible source of TSE infectivity in rendered material is the offal of scrapie-infected sheep or BSE-infected cattle that are presymptomatic. Other countries—Canada most recently—have dealt with this issue by requiring that ruminant tissues known to harbor TSE infectivity be excluded from the human food and feed chains. This so-called specified risk material (SRM) includes the brain, skull, eyes, tonsils, vertebral column, dorsal root ganglia, and distal ileum (CFIA and Health Canada, 2003; Government of Canada, 2003b). A review of U.S. policy on SRM would be appropriate.

Plate waste represents a third potential source of TSE infectivity in rendered material. Plate waste consists of food products that were inspected by FSIS or an equivalent state agency, cooked, and presented for humans to eat (e.g., leftovers from meals served to restaurant patrons) (HCRA and TUCCE, 2001). Such material may contain CNS tissue from sheep or cattle. The Harvard/Tuskegee risk assessment considered plate waste to be a low-risk source of BSE infectivity, primarily because about 90 percent of all plate waste consists of bakery products and because it is "extremely unlikely" that plate waste would contain high-risk animal tissues (HCRA and TUCCE, 2001:32). Yet USDA APHIS expressed support for an unsuccessful effort by FDA to amend regulations such that plate waste would be prohibited from ruminant feed (Medley, 1997). The committee believes such a prohibition should be reconsidered.

The rendering processes at U.S. plants would not eliminate BSE infectivity. In most cases, the raw materials are heated for a specified time at a temperature and pressure that would reduce the infectivity of the BSE agent—if it were present—by 1 to 2 logs (Harlan, 2003; USDA APHIS,

2003b). Fewer than 5 percent of rendered animals undergo processing that would reduce BSE infectivity by 3 logs (USDA APHIS, 2003b). It is noteworthy, however, that part of a respected hypothesis on the origin of the BSE outbreak in the United Kingdom is that changes made in the 1970s and 1980s to rendering processes may have permitted the scrapie agent to retain its infectivity, whereas before those changes were made, rendering processes had inactivated the agent (Wilesmith et al., 1988). Later in this chapter, we discuss the few successful methods of inactivating the TSE agent and recommend research to develop new approaches to this problem.

Renderers, like beef processors, may reduce the risk of spreading the BSE agent by accepting tissue only from ambulatory cattle of U.S. origin that have passed antemortem inspection at the abattoir. In addition, some rendering plants and beef processing operations are run by the same company, and these companies may render only tissues from their own beef processors to control the quality of BSE risk-reduction through the entire process. In addition, some rendering plants may divert CNS tissues from mature cattle (Harlan, 2003). However, the extent to which U.S. renderers take these voluntary risk-reduction measures is unclear.

In early 2003, USDA APHIS issued an advance notice of proposed rulemaking indicating that it wants to develop a new regulation to reduce the BSE risk posed by dead-on-farm cattle in the context of rendering (USDA APHIS, 2003b).

A Case Study: One BSE-Positive Canadian Cow

To demonstrate the impact of a single case of BSE on a country and its major trading partners, we review here the BSE case discovered in northern Alberta, Canada. On May 20, 2003, Canadian agricultural officials announced that a native 8-year-old black Angus beef cow from a farm in Wanham, Alberta, sent for slaughter in January 2003 had been condemned during antemortem inspection and tested positive for BSE (Government of Canada and Government of Alberta, 2003). An inspector had noticed that the animal was unusually thin, and he suspected the cow had pneumonia (Krauss and Blakeslee, 2003a). Immediately after the announcement, the United States and several other countries prohibited the importation of Canadian live cattle, beef, beef products, and cattle by-products (USDA and FDA, 2003). This ban was obviously a blow to Canada's cattle, beef, and rendering industries, especially in the loss of trade to the United States, which had until then received 80 percent of Canada's exported beef and nearly all its exported live cattle (Agriculture and Agri-Food Canada, 2003). In 2002, Canadan farm cash receipts for the export of beef and cattle totaled about $4 billion (U.S. $2.96 billion) (Agriculture and Agri-Food

Canada, 2003), almost 63 percent of the total output[9] of those industries (Myles, 2003). Those exports accounted for less than 1 percent of the country's 2002 gross domestic product, however (Central Intelligence Agency, 2003).

U.S. stock markets reacted immediately to the case. Fast-food hamburger chains were particularly affected. McDonald's stock fell by 6.7 percent, Wendy's International by 6.6 percent, and Tyson's Food by 4.9 percent (Day, 2003). Futures and commodity markets in beef, cattle, and feed products were affected in the United States, Canada, and elsewhere.

Because the BSE-positive cow, dubbed the index case, was condemned during antemortem inspection, Canadian policies prevented its meat from entering the human or ruminant food chains (Evans, 2003). The carcass was rendered into meat and bonemeal, and possibly tallow as well. The Canadian Food Inspection Agency (CFIA) is tracing the path of this rendered material. The agency has reported that at least some of the material was used in the production of certain brands of dry dog food manufactured in Alberta (Government of Canada, 2003a). Figure 7-2 illustrates the path under investigation. Some of these pet-food products crossed the U.S. border. A week after the incident, FDA published a warning that a pet-food product sold by Pet Pantry in the United States might contain rendered material from the infected cow. The company reportedly planned to retrieve the product from its customers (Carlisle, 2003).

The BSE-positive cow had come from a commercial herd of 150 cattle established 3 years earlier (Evans, 2003). CFIA depopulated the case herd and tested the cattle for BSE; all the results were negative (USDA and FDA, 2003). The cow's farm of origin was unclear, however. Epidemiologists identified two potential farms of origin, one in Saskatchewan and one in Alberta. Consequently, CFIA quarantined 18 Canadian farms that were the potential source farm or were at risk for BSE (Evans, 2003). Tests on the brain tissue of a sampling of cattle from those herds yielded negative results (CFIA, 2003a). As of June 4, 2003, approximately 1,500 cattle had been killed, and most had been tested for BSE, with negative results (CFIA, 2003a). Within 2 $^1/_2$ weeks after the case of BSE had been announced, CFIA lifted the quarantines on the farm where the BSE-infected cow had commingled in a pasture, and on three herds containing offspring from the case herd (CFIA, 2003b).

A Montana ranch purchased 5 bulls from one of the possible birth herds of the index case. These 5 were among 24 bulls that left the farm between 1997 and 2002 (USDA and FDA, 2003). During a technical brief-

[9]Total output is measured as all beef produced plus the beef equivalent of all live cattle destined for slaughter.

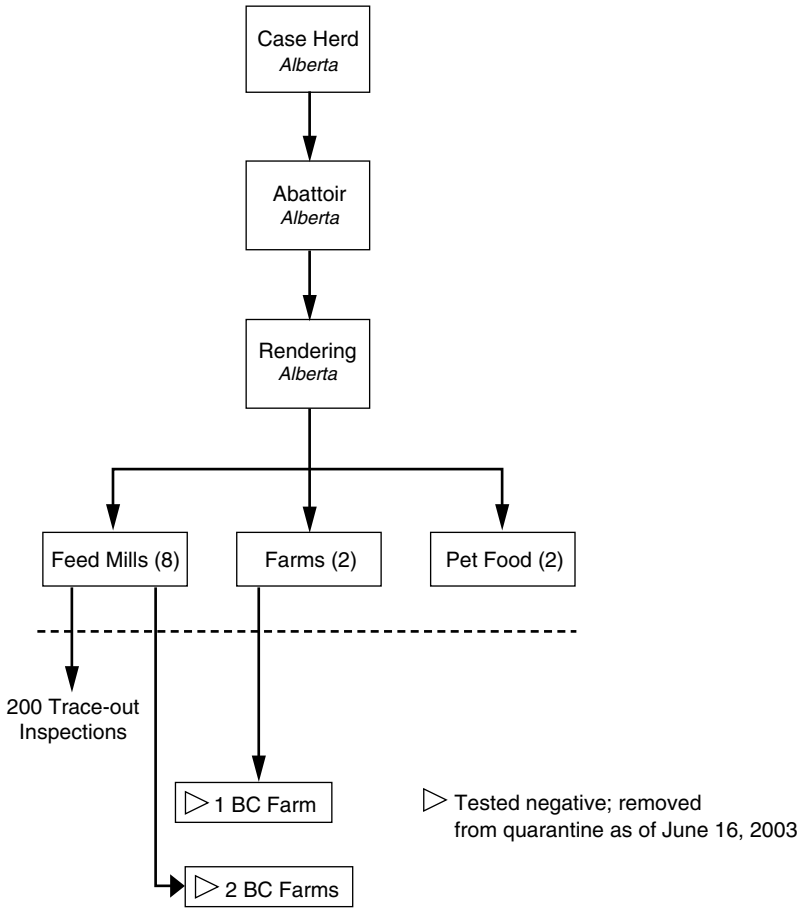

FIGURE 7-2 The path of the Canadian forward trace of rendered tissue from the BSE-positive cow found in Alberta. This diagram reflects findings as of June 16, 2003. Reprinted with permission from the Canadian Food Inspection Agency (2003). SOURCE: CFIA (2003b).

ing on the BSE investigation, the deputy administrator of Veterinary Services at USDA APHIS said, "We think it is very unlikely that any five of those bulls were infected" (USDA, 2003a:5). Of the 24 animals, 22 have been traced to abattoirs in Minnesota, Nebraska, Texas, and South Dakota. One bull was sent to a location in Wyoming, and another was slaughtered for personal consumption (USDA and FDA, 2003).

In August 2003, USDA partially lifted the import ban by allowing several types of ruminant products from Canada back into the United States.

These include boneless sheep or goat meat from animals under 12 months of age, boneless bovine meat from cattle under 30 months of age, boneless veal from calves that were 36 weeks or younger at slaughter, fresh or frozen bovine liver, vaccines for veterinary medicine for nonruminant use, and pet products and feed ingredients that contain processed animal protein and tallow of nonruminant sources when produced in facilities with dedicated manufacturing lines (USDA, 2003c). Even with this change in U.S. policy, however, USDA's Foreign Agricultural Service predicts that Canada will export 25 percent less beef in 2003 than it did the year before (Myles, 2003).

The long-term economic effects of the Canadian BSE case remain unclear. It is difficult to predict how long the United States' and Canada's other trading partners will keep their import bans partially or fully in effect. However, the short-term impacts are evident. According to the Canadian Beef Export Federation, each day after the case was announced, Canada's beef industry lost U.S. $8 million (Krauss and Blakeslee, 2003b). Layoffs inevitably followed. For example, one beef processor and exporter to the United States laid off 75 to 100 employees just a day after the announcement (Olsen et al., 2003). Another consequence of the case of BSE in Canada was the decision by CFIA to ban SRMs from the rendering process to help prevent the inadvertent spread of the BSE agent (CFIA and Health Canada, 2003; Government of Canada, 2003b). The EU took the same step in 2000 (Brown, et al., 2001).

The lessons learned from the impact of this single BSE-infected cow, discovered among the 3 million Canadian cattle slaughtered annually, are sobering. If the United States experienced a similar case of BSE, U.S. trading partners would impose an import ban on this country. The ban would have less of an effect on the U.S. beef industry than has been the case for the equivalent Canadian industries because the United States exports only about 9 percent of its beef production (Van Eenoo et al., 2000), while, as mentioned earlier, Canada had exported almost 63 percent of its beef and live beef cattle[10] before the discovery of the BSE case (Myles, 2003). Nevertheless, the United States is the world's second-largest beef exporter, with those exports, including variety beef (internal organs), totaling $3.2 billion annually (Canfax, 2003; U.S. Meat Export Federation, 2003).

If large cattle ranches were involved in a U.S case of BSE, the costs associated with quarantine, culling, destruction, and testing of cattle could be enormous. The case would require extensive investigations into its lineage and movement, as well as traces of all rendered beef by-products of suspected cases. Given the relatively high proportion of cattle, beef, beef

[10]On a beef-equivalent basis (Myles, 2003).

products, and beef by-products exported from the United States, import bans on those products would shake financial and commodity markets worldwide. Within the United States, a case of BSE would lead to a short-term oversupply of the banned products, causing their prices to fall domestically. Layoffs would likely follow. Retail sales of all beef end-products might plummet if consumer confidence faltered.

These likely effects underscore the importance of preventing even one case of BSE in the United States.

Plans for Responding to a BSE Outbreak in the United States

The Harvard/Tuskegee risk assessment concluded that if the BSE agent were to enter the United States and infect a cow, the trilayer barrier described in this chapter would very likely prevent the agent from entering the human food supply. Nonetheless, as the Canadian BSE case demonstrated, the potential consequences of an outbreak on the economy, on animal and human health, and on public confidence would demand a swift, coordinated, transparent response. For that reason, the federal government has developed plans for how to respond to a confirmation of BSE in the United States.

USDA would have primary responsibility for managing a BSE outbreak. *BSE Emergency Disease Guidelines* (APHIS and FSIS, 2002), informally called the BSE Red Book, describes in detail the laboratory and field activities to be performed in an emergency. In addition, a joint working group composed of representatives from APHIS and FSIS have a BSE Response Plan (APHIS and FSIS, 2002) to coordinate communication with the public, with other federal agencies, and among the departments within USDA that would carry out the laboratory and field activities. Similarly the FDA has prepared a BSE Response Plan much like that of USDA (personal communication, D. Asher, FDA, August 2003).

MEASURES TO PREVENT THE CWD AGENT FROM ENTERING THE U.S. FOOD CHAIN

The principal question regarding CWD and human food is whether the infectious agent of CWD can cause a TSE in humans. As described in Chapter 3, the answer is that no one knows. Without a definitive answer, the scientific consensus is to proceed on the assumption that tissue infected with the agent of CWD could theoretically be fatal to hu mans. Therefore, venison processors and consumers should avoid CWD-contaminated meat at present. The committee notes a number of precautions in the discussion that follows. When it comes to processing cervid meat, however, the wide range of practices, the lack of regulation, and the many opportunities for

spreading the CWD agent suggest the need to research and document the human risks of contact with the infectious agent.

Methods to Avoid Contact with the Agent of CWD

As a first step, hunters should be aware of known CWD-endemic areas. States can help hunters locate these areas through surveillance. Colorado and Wyoming, for example, conduct excellent surveillance of both wild and captive cervids, providing good guidance for hunters from a food-safety standpoint. As described in Chapter 6, however, the lack of adequate nationwide surveillance for CWD means that neither scientists nor hunters of wild venison know all the locations where the disease presently occurs. Moreover, CWD is emerging in new locations with increasing frequency as the disease appears to spread. For example, Utah reported a new case of CWD in April 2003 near the town of Moab, more than 100 miles south of the first case of the disease identified in the state (Knowles, 2003).

As a second precaution, hunters could be trained to recognize clinically ill cervids. As is the case with other TSEs, however, a significant amount of infectivity develops in CWD-positive animals before the onset of clinical disease. The brain, spinal cord, lymph nodes, and spleen of a CWD-positive cervid would contain infectivity at various stages of the disease. In addition, given that the disease is transmitted laterally, some material shed by the animal (saliva, feces, urine, or hair, for instance) is likely to contain infectivity as well.

To avoid contact with and eating of CWD-infected tissue, processors theoretically could test cervids with one of three USDA-approved rapid postmortem diagnostics for CWD: an enzyme-linked immunosorbent assay (ELISA) from Bio-Rad Laboratories Inc. of Hercules, California (USDA APHIS, 2002); the Dot Blot ELISA from VMRD Inc. of Pullman, Washington (USDA APHIS, 2003c; VMRD Inc., 2003); and the IDEXX HerdChek® CWD Antigen Test Kit from IDEXX Laboratories Inc. of Westbrook, Maine (IDEXX Laboratories, 2003) (see also Chapter 4). All three tests can detect PrP in peripheral lymphoid tissues (personal communication, Rick Hill, USDA APHIS Center for Veterinary Biologics, November 25, 2003). The Bio-Rad test is approved for use on the tissues of white-tailed deer, mule deer and elk, the VMRD test for use on the tissues of white-tailed and mule deer, and the IDEXX test for use on white-tailed deer tissue (personal communication, Rick Hill, USDA APHIS Center for Veterinary Biologics, November 25, 2003). As of November 2003, however, USDA had licensed the tests for use exclusively by approved veterinary laboratories for surveillance purposes only. It would be desirable to have a USDA-approved test for CWD in brain tissue designed for use at any cervid-processing facility. The easier such a test was, the more likely a processor would be to use it.

Processing of Cervids

There is little documentation on or uniformity to cervid processing in the United States. The committee gleaned most of its information on the subject from Dr. Warrie Means, an associate professor of animal science at the University of Wyoming, Laramie (Means, 2003). He is the source of information on cervid processing in this chapter unless otherwise noted.

An estimated 4,510 meat processing plants in the United States process game meat (Means, 2003).[11] This figure is based on the assumptions that about one-third of the country's 7,500 to 8,000 small FSIS- or state-inspected plants process venison, and that about 85 percent of the country's 2,200 custom processors do the same. The latter type of establishment generally is not licensed or inspected by USDA and is not necessarily inspected by the state, either.

Commercial Processing of Cervid Meat

The processing of cervids takes one of two paths, depending on whether the animal is harvested commercially or for sport. A flow chart for each path appears in Figures 7-3 and 7-4.

Commercial game processors work with deer harvested specifically for commercial sale. Edible tissue is fabricated into cuts or processed into such items as sausage, jerky, and snack sticks, and is sold to restaurants and individuals. Inedible tissue is sent to renderers or landfills.

From a food-safety standpoint, the commercial processing of venison is probably safer than any other method. Most game processors use a technique called boneless fabrication, which minimizes contact with the brain, spinal cord, and lymph nodes if the animal was not quartered first. Based on the practices in Wisconsin and Wyoming, these commercial establishments also process an average of 115 to 260 head of beef per year, or 1 to 3 percent of all cattle slaughtered annually in the United States. Thus, CWD-infected tissue could potentially cross-contaminate a small percentage of U.S. beef tissue or equipment at processing sites.

The responses of deer and elk processors to the risks posed by CWD have ranged from apathy to serious concern. Not surprisingly, the actions taken by various establishments to reduce the risk of CWD infectivity range from minimal to dramatic, as outlined in Box 7-1. Only about half a dozen plants are taking the most extreme measures listed at the bottom of the box (personal communication, W. Means, University of Wyoming, March 26,

[11]Dr. Means cited a personal communication with the American Association of Meat Processors for the figures in this paragraph.

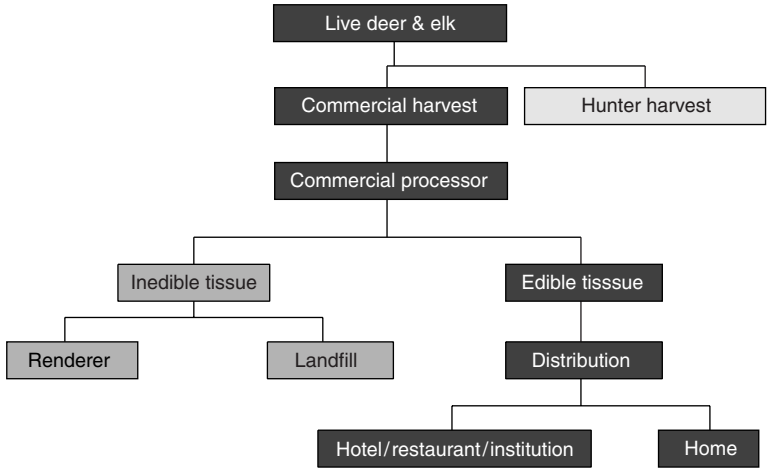

FIGURE 7-3 Commercial processing of cervid tissues in the United States.
SOURCE: Adapted from Means (2003).

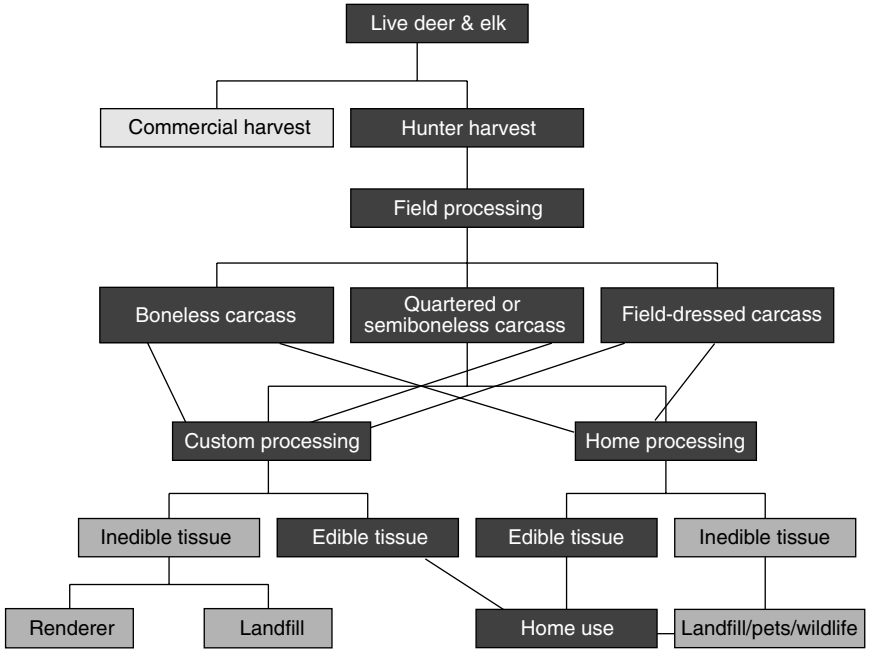

FIGURE 7-4 Paths taken by hunter-harvested cervid tissues during processing in the United States.
SOURCE: Adapted from Means (2003).

BOX 7-1
Actions Taken by Game Processors to Minimize Contact with the Infectious Agent of CWD

In order of increasing rigor:

- No changes.
- Minimize contact with brain, spinal cord, lymph nodes, eyes, and spleen.
- Wear rubber gloves and use a separate saw or knife when removing antlers. Avoid splitting midline. Process using boneless fabrication procedures. Remove visible lymph nodes.
- Liberally trim gunshot areas involving the spinal cord.
- Sanitize utensils and equipment with strong bleach.*
- Process animals individually to avoid cross-contamination.
- Process only animals accompanied by a negative CWD-test result.
- Process game in separate plant facilities.
- Stop processing game from established CWD areas.
- Stop processing wild game.

*It is questionable whether this technique deactivates CWD infectivity. Inactivation is discussed later in this chapter.

SOURCE: Means (2003).

2003). However, there has been a noticeable increase in the number of cervids being tested for CWD.

Hunter Harvesting of Cervids

Hunters may process cervids to various degrees in the field, then complete the fabrication at home or give the animal to a custom processor to fabricate into cuts of meat and/or processed meat, such as sausage. Most hunters "field dress" the animal by removing its internal organs. Some hunters further process the animal, depending on its size, the hunter's skill, and the distance the hunter (and his or her horse) will have to carry the animal. The traditional method of field processing is to remove the head and quarter the animal. In the process, the hunter bisects the spinal cord, exposing CNS tissue. An alternative, infrequently used method called field butchering does not bisect the spinal cord and is recommended in areas known to have CWD. Obtaining the trophy antlers that hunters often prize exposes the brain. The tools used for this purpose in the field may include a chain

saw or battery-powered saw (both of which scatter tissue into the air), a handsaw, or a hatchet.

When the field work is done, the cervid tissues left behind sometimes include the spinal column, the head, and the spleen. These organs could theoretically contaminate the environment or cause TSE in a scavenger animal that ate them if the slain cervid were infected with the CWD agent.

As noted, hunters complete the fabrication of the meat into cuts or other products either at home or at a custom processor. These processors are typically very small establishments. Nevertheless, when a hunter arrives to pick up his or her butchered and processed venison, it is not necessarily the meat from the animal he or she killed, but rather an amount equal to what the hunter dropped off. Therefore, even if the hunter was very careful in the handling of risky tissues, the safety of his or her meat depends also on how other hunters handled their cervids, as well as how the processing facility is run.

Regulation of Live Cervids, Venison, and Venison Products

There is relatively little regulation of live cervids, venison, and venison products in the United States. Each state is responsible for the regulation of such products produced and sold within its borders. To the committee's knowledge, few states regulate the industry; however, some states require venison to be inspected before being sold.

USDA regulates interstate commerce in live cervids, cattle, sheep, swine, goats, and horses, as well as meat and poultry, while FDA regulates interstate commerce in cervid meat and meat products. Although beef, pork, and mutton must be inspected by USDA to be sold across state lines, FDA does not require such inpection of venison. Federal regulations simply require that the venison come from an approved source when intended for sale in retail stores and restaurants. Approved sources are licensed food establishments, federally inspected food plants, and state-inspected meat plants (Deer Farmer's Information Network, 2003).

Conclusion Regarding the Prevention of CWD Transmission

There is a dearth of scientific information on cervid processing, despite the opportunities for contamination by the CWD agent during cervid processing and the theoretical possibility that the infectious agent might be transmissible to humans.

Recommendation 7.2: Fund risk assessments that characterize the exposure of hunters, cervid processing establishments, and consumers to the infectious agent of chronic wasting disease. [Priority 3]

PREVENTING TSE TRANSMISSION THROUGH BLOOD, BLOOD DERIVATIVES, AND TRANSPLANTED TISSUES

In addition to measures to prevent TSE agents from entering the food chain, steps must be taken to prevent transmission of such agents by other means. This section reviews measures to prevent transmission of TSE agents through blood products, blood derivatives, and transplanted tissues.

Preventing TSE Transmission Through Blood Products

Regulatory bodies throughout the world, including FDA, have taken steps to prevent the potential transmission of TSEs among humans through blood, blood components, and blood derivatives (Foster, 2000). FDA has instituted a number of policies, listed in Table 7-2, to prevent the infectious agents of human TSEs from entering U.S. supplies of these biological products.

FDA keeps the blood collection and processing industry informed of its policies through "guidance to industry" documents that convey the information necessary to comply with policy decisions (FDA, 2002). The center within FDA that regulates biological products is the Center for Biologics Evaluation and Research (CBER). CBER has a TSE Scientific Advisory Committee and a Blood Products Advisory Committee, composed of national experts in the field (with community representation), that provide the scientific underpinnings for new regulatory policy regarding TSEs.

In addition to developing preventive policies regarding deferral of high-risk donors, FDA provides guidance to industry and evaluates manufacturing processes to ensure the production of pure, safe, and effective products. Studies show that the processes involved in manufacturing blood derivatives, such as precipitation, filtration, and chromatography, remove between 1 and 6 logs of TSE infectivity, or an average of 3 to 4 logs (Brown et al., 1999). This reduction of potential infectivity is highly desirable, but its adequacy will remain unclear until scientists develop an estimate of the size of an infectious dose of the TSE agent in human blood. Meanwhile, the blood industry is evaluating methods for inactivating the TSE agent.

Scientists have invested much effort in pursuing the goal of inactivating TSE agents in blood. An important challenge is to inactivate these prions within living tissue, such as blood, without exacting collateral damage on other tissues. Most of this effort has focused on ways to inactivate or remove prions from blood and, in particular, blood derivatives. This focus is reasonable because existing tests are unable to detect small amounts of prions that may be circulating in blood, and it is therefore impossible at present to identify asymptomatic, TSE-infected blood donors.

Gamma radiation has been used experimentally to inactivate hamster scrapie prions added to human albumin. A dose of 50 kGy (100 rads = 1

TABLE 7-2 Measures Taken in Response to Concern That TSE Agents May Be Transmissible via Human Blood Products

Date	Event
1987 (Nov.)	FDA defers recipients of human pituitary–derived growth hormone from donating blood.
1995 (Aug.)	FDA extends donor deferral to those with a family history of CJD and to recipients of dura mater. Plasma derivatives prepared by using a donation that would now be excluded must be withdrawn.
1996	FDA recommends withdrawal of blood plasma and plasma products made from pooled blood donations to which persons who later died of CJD have contributed.
1996 (Dec.)	FDA clarifies which donors are at risk of developing CJD.
1998 (Sept.)	FDA recommends that plasma derivatives manufactured from a donor who develops vCJD be withdrawn, but not plasma derivatives involving donors with other forms of CJD.
1999 (Aug.)	FDA extends donor deferral to those who spent 6 months or more in the United Kingdom between 1980 and 1996.
1999 (Nov.)	FDA revises donor deferral to include recipients of bovine insulin unless the product was not manufactured since 1980 from cattle in the United Kindom.
2000	FDA holds public discussion regarding the possible risk associated with vaccines produced with bovine-derived materials from countries experiencing BSE.
2002 (Nov.)	FDA extends donor deferral to those who have spent 3 months or more in the United Kingdom between 1980 and 1996, and those who have spent 5 years or more in France or any of 29 other European countries between 1980 and the present.[a]

[a]Page (2002).
SOURCE: adapted from Foster (2000: Table 1).

Gray [Gy] = absorbed radiation dose) was used in the experiment. This is a massive dose that would, if applied to food, kill all microorganisms present, including viruses. In the experiment this dose caused an estimated reduction of 1.5 log10 ID_{50} of the scrapie prion level based on a delay in onset of clinical symptoms in a hamster infective assay. The radiation did not appear to cause excessive damage to the albumin, and it delayed clinical end points in hamsters inoculated intracerebrally (i.c.) with the albumin, but it failed to prevent infection by the scrapie agent (Miekka et al., 2003).

A new chromatography-based process for manufacturing immune globulin to be given intravenously (IGIV) was evaluated to determine whether the process would remove hamster scrapie prions added to the source plasma material. Western blot analysis measured a large reduction in PrP^{Sc}, and assays in hamsters showed no infectivity after i.c. inoculation of six hamsters followed for 250 days (Trejo et al., 2003). This result looks very promising for this isolated end product. However, blood fractionation studies (Brown et al., 1998) indicate that the potential burden of prions in the immunoglobulin fraction is much less than would be found in other plasma derivatives. Also these findings can not be generalized to other blood products manufactured using different processes.

Another strategy used to eliminate prions is to filter them out of the product during the manufacturing process. Nanofiltration has shown great promise in eliminating viruses and prions from plasma products (Burnouf and Radosevich, 2003). In a recent study, Planova® filters of 15 nanometer (nm) pore size reduced mouse-adapted scrapie prions that had been added to human albumin to such a low level that infectivity was eliminated as determined by a mouse infectivity assay (Tateishi et al., 2001). It is possible, however, that test conditions may create aggregation of the prions that favors their removal. Burnouf and Radosevich (2003) reported that in one experiment by Tateishi's group, residual infectivity was found in the 10 nm filtrate when the detergent sarkosyl (1 percent) was added to the plasma, reducing aggregation of prions. However, in another experiment, when Tateishi's group added sarkosyl (0.5 percent) to plasma, their 15 and 10 nm filters appeared to remove the infectivity as demonstrated in mice inoculated with filtrates at dilutions of 10^{-1} or less (Tateishi et al., 2001). Although this application of the technology is heartening, it does not fully address the needs. Advancing this method and others, however, would be an important step in the direction of having safer blood derived products.

Recommendation 7.3: Fund research to develop novel methods for removing prions from or inactivating prions in blood products and tissues in vitro, using physical, chemical, or immune mechanisms alone or in combination. [Priority 2]

Preventing Transmission of TSE Agents in Transplanted Human Tissues

As previously noted, many iatrogenic CJD cases have been attributed to infected processed dura mater allografts and a small number to infected corneal transplants (Hogan et al., 1999). Thus, processed human dura mater, corneas, and other eye tissues (e.g., retinas) are thought to present the greatest risk of TSE transmission by implantation (FDA TSEAC, 2001). In addition, substantial amounts of PrP^{Sc} accumulate in the lymphoid tissues of vCJD patients (Bruce et al., 2001).

Several measures taken by FDA and other organizations have reduced the risk of iCJD due to tissue implants in the United States. Nearly 20 years ago, FDA ceased approving the U.S. sale of human cadaveric pituitary hormones, responsible for a large number of iCJD cases worldwide (personal communication, D. Asher, FDA, August 2003). In 1990, FDA issued its first safeguards to minimize the possibility of TSE transmission through processed dura mater allografts (FDA CDRH, 1999). There has been no confirmed U.S. case of iCJD due to this product since March 1997. In fact, NPDPSC has seen only 4 cases of iCJD out of nearly 1,100 suspect human TSE cases since 1997, when the center was established (Chapter 6, Table 6-1). FDA does not require providers of human cells, tissues, and cellular products (HCT/Ps) intended for human transplantation to register with FDA or to give the agency a list of their products, although the agency proposed these requirements 7 years ago (FDA CBER, 1997).

The World Health Organization (WHO) recommended in 1997 that processed human dura mater grafts no longer be used, especially in neurosurgery, unless no alternative was available (FDA CDRH, 1999). This recommendation was endorsed by FDA's TSE Advisory Committee (TSEAC) with the caveat that "the final decision regarding the use of processed human dura mater should be left to the discretion of the treating neurosurgeon" (FDA CDRH, 1999:3). Since then, FDA's Center for Devices and Radiological Health (CDRH) has issued guidelines, not regulations, to minimize the risk of TSE transmission by processed human dura mater implantation (FDA CDRH, 1999). Among other precautions, this guidance recommends that manufacturers:

- Obtain a medical history to select dura mater donors who are free of neurological disease at the time of death.
- Use suitability criteria similar to those for donors of blood (see Box 5-1 in Chapter 5).
- Perform gross and histological examination of the brain to exclude donors with possible evidence of TSE-related changes.
- Include a disinfection step in the manufacturing process.
- Prevent cross-contamination of dura mater from different donors by blocking commingling during collection and processing.

CDRC is developing updated guidance regarding processed dura mater allografts (FDA CDRH, 2002).

FDA's Center for Biologics Evaluation and Research (CBER), which regulates corneas and other human cells and tissues intended for transplant, requires these materials to be tested for various infectious diseases (FDA, 1997). Since there is no antemortem test for TSEs in humans, however, providers of corneas for implantation generally reject prospective donors

who would be deferred from donating blood because they resided in Europe during the high-risk period for BSE transmission to humans, possibly exposing them to the infectious agent (see Chapter 9, Table 9-2) (personal communication, D. Asher, FDA, August, 2003). Stricter deferral criteria could entail the exclusion of older donors, who are more likely to have sCJD, or the examination of potential donors' brain tissue for signs of TSE. However, TSEAC concluded in 2001 that imposing more stringent selection criteria on potential donors of corneas would prevent the United States from meeting the demand for corneal transplants (FDA TSEAC, 2001).

To further minimize the risk of disease transmission, CBER prohibits the commingling of human tissues from different donors during processing (FDA, 1997). In addition, the Eye Bank Association of America and the American Association of Tissue Banks have adopted voluntary standards to prevent the transplant of TSE-infected tissue (personal communication, D. Asher, FDA, August, 2003).

TSE infectivity has been detected in a variety of human organs besides brains and eyes, albeit less consistently and in much smaller amounts. Primate bioassays of tissue homogenates from the organs of human TSE cases (mostly sCJD) revealed infectivity in 10 percent of tested human spleens, 11 percent of livers, 18 percent of kidneys, 20 percent of lymph nodes, and 50 percent of lungs (Brown et al., 1994). The regulation of human solid organs intended for transplantation is conducted by the United Network for Organ Sharing under contract with the Health Resources and Services Administration, U.S. Department of Health and Human Services.

INACTIVATION OF PRIONS ON SURFACES AND IN THE ENVIRONMENT

Avoidance of exposure to TSE agents is a principal preventive strategy. However, there are sites in which the presence of prions may be unavoidable. These sites include hospitals and veterinary clinics; research and reference laboratories; and operating rooms having contact with TSE-infected people, animals, or tissues. In such situations, it is appropriate to use inactivation procedures to eliminate prions so they will not contaminate equipment or infect people. Inactivation becomes an especially critical issue when a prion-exposed surgical instrument is being used on a patient.

Today, hospitals lack both an antemortem test for human TSEs (except a brain biopsy) and satisfactory methods to disinfect medical equipment potentially contaminated with a TSE agent. The few disinfection methods believed to have the power to eliminate TSE infectivity on medical equipment (Department of Health, 2003; Weber and Rutala, 2002; WHO, 2000) are caustic and potentially harmful to both hospital personnel and the equipment itself. New disinfection methods are sorely needed. It would be ideal

to have a safe, inexpensive disinfection procedure against prions or the TSE agent for routine use in all hospital disinfection protocols.

Since human TSEs incubate for years, it is not unusual for a patient diagnosed with suspected TSE to have undergone an invasive medical procedure during the years when the suspected infection was incubating. Under such circumstances, hospital staff must trace back all patients who may have been exposed to the instruments used on the suspect TSE case, determine the patients' level of risk, and decide what to tell them (CJD Incidents Panel, 2001; Weber and Rutala, 2002). Anecdotal evidence suggests that many surgeons are reluctant to perform invasive procedures on a suspected TSE patient, leaving hospitals to undertake draconian infection-control measures for what may or may not be a case. Finally, as mentioned in Chapter 6, most pathologists in the United States will not perform an autopsy on suspect TSE cases—mainly for fear of contaminating their equipment (Belay, 2003). Clearly, the lack of a safe, routine, affordable procedure for inactivating the TSE agent on surfaces has significant consequences.

Prions are unusually difficult to inactivate—a unique characteristic that differentiates them from infectious living organisms such as viruses, bacteria, and fungi. Excellent reviews regarding prion inactivation have been published (Taylor, 1999, 2000). Table 7-3 summarizes the types of agents used to inactivate prions and their effectiveness. Dry and steam heat, organic and nonorganic chemicals, and various types of ionizing and nonionizing radiation have been employed. In addition to the agent used, certain factors associated with the source material can influence inactivation. Factors that would increase resistance to inactivation include a larger amount or a higher ID_{50} level of infectivity of the material being treated, macerated material versus intact tissue, dried material, contact with glass or steel surfaces, or protein fixation by a chemical or heating (Taylor, 1999). These factors promote thermostability and thus greater resistance to inactivation. Also, different prion strains respond differently to the same inactivation procedure (Taylor et al., 2002).

Studies in the 1980s (Brown et al., 1986; Kimberlin et al., 1983) employed mouse assays and mouse-adapted prion strains to develop recommendations on the effectiveness of inactivation procedures. Since some prions from certain mouse strains were more resistant to inactivation than others, the recommendations were based on the conditions that would inactivate the hardiest strain tested. These recommendations included providing for an additional margin of safety by increasing the concentration of the chemical agent and lengthening the time of heat treatment.

Recommendations from these earlier studies were later shown to be inadequate for other prion strains or in other test conditions. For instance, testing showed that autoclaving inactivated prions, but that a subpopulation of the protein in test material could become "fixed" and be more resis-

TABLE 7-3 Agents Used to Deactivate Prions

Class of Agent	Agent/Conditions	Evidence for Complete Inactivation: Not Successful	Evidence for Complete Inactivation: Successful
Radiation			
Microwave		X	
Ultraviolet		X	
Ionizing		X	
Dry Heat			
600°C/5–15 min[a]		X	
360°C/24 hr			
220°C/20 min		X	
160°C/24 hr		X	
Moist Heat (autoclave)			
Gravity displacement	121°C/90 min	X	
	132°C/90 min		X[b]
	132°C/4.5 hr		X[c]
Porous loading	134–138°C/18 min	X (debated)	
Chemicals			
Acid/bases	2 M NaOH/2 hr	X	
Alkylating agents	Formalin	X	
	Glutaraldehyde	X	
	Acetyl ethylenimine	X	
	β propyl lactone	X	
	Ethylene oxide	X	
Detergents	Sodium dodecyl sulfate	X	
Halogens	2% iodine/4 hr	X	
	Sodium hypochlorite (20,000 ppm/1hr)		X[d]
	Dichloroisocyanurate	X	
Organic solvents	Chloroform	X	
	Ethanol	X	
	Phenol	X	
	Hexane	X	
	Heptane	X	
	Perchlorethylene	X	
	Petroleum	X	

TABLE 7-3 Continued

Class of Agent	Agent/Conditions	Evidence for Complete Inactivation: Not Successful	Evidence for Complete Inactivation: Successful
Chemicals (continued)			
Oxidizing agents	Chlorinedioxide	X	
	Hydroperoxide	X	
	Peracetic acid	X	
Salts	Sodium metaperiodate	X	
	Potassium permanginate	X	
Chaotropes	Guanidine thiocyanate >4 M		X (debated)
	Guanidine hydrochloride	X	
	Urea	X	
Proteolytic Enzymes			
	Trypsin	X	
	Pronase	X	
	Quiagen	X	
	Proteinase K	X	
Combinations			
	1 M NaOH/121°C/30–60 min GD		X[e]
	1 M NaOH/121°C/90 min GD		X[f]
	2 M NaOH/121°C/30 min GD		X[g]
	1 M NaOH/boiling/1min		X[h]

[a]Brown et al. (2000b).
[b]Ernst and Race (1993).
[c]Prusiner et al. (1984).
[d]Kimberlin et al. (1983).
[e]Ernst and Race (1993).
[f]Prusiner et al. (1984).
[g]Taylor et al. (1997).
[h]Taylor (2000).

NOTE: GD = gravity displacement autoclave; M = molar; NaOH = sodium hydroxide.
SOURCE: Adapted from Taylor (2000).

tant to temperature inactivation. That phenomenon was demonstrated in a study in which 60 percent of the animals injected with infectious material previously treated at 134°C became infected, compared with 72 percent that were injected by material treated at 138°C (Taylor, 1999). This study revealed that autoclaving alone is not a sufficient method for inactivating prions. One study that appeared to show the ability of sodium hydroxide to inactivate prions was subsequently found to have significant design pitfalls. In a later study, when the caustic chemical was neutralized before injection into the assay animal, the material was found to contain residual infectivity (Taylor et al., 1994). Earlier studies had diluted the sodium hydroxide so it could be injected into the assay animals without acute toxicity. Unfortunately this procedure also reduced the prion levels so that infectivity was not detected.

Failure to identify fully satisfactory methods for inactivating prions with a single type of deactivation treatment led investigators to believe that combination methods might be a more effective approach. Several studies have since shown that a combination of heat and sodium hydroxide is a reliable way to inactivate prions. However the latter chemical is caustic to some equipment and must be handled carefully because of safety concerns.

Most of the methods shown in Table 7-3 have little if any effect in inactivating prions. Some can reduce infectivity many-fold but only a very few are reliable. Because of this concern, it is recommended that surgical instruments having been in contact with known CJD-positive patients or patients known to be at a much elevated risk of acquiring CJD be discarded rather than disinfected (Taylor, 2000). The rarity of CJD and the high stakes associated with infection make this an attractive option. The Department of Health in the United Kingdom recommends that, at a minimum, for brain biopsies involving nonfocal lesions, the surgical equipment should be quarantined until CJD can be ruled out. This policy was established in response to an unfortunate incident in which 24 patients were exposed to the same surgical equipment used on a CJD patient whose diagnosis was initially unknown (Mayor, 2003). Policies recommending disposal or quarantining of equipment are problematic, however, when dealing with fiberoptic equipment, which is both expensive to replace and difficult to disinfect, especially when contaminated with prions (Dombrovski et al., 2003).

The concern about transfer of infectivity through instrumentation is not merely theoretical. The same surgical stereotactic encephalographic electrode implanted into the brain of a patient with CJD infected two subsequent patients, aged 17 and 23, despite the use of typical sterilization procedures (Bernoulli et al., 1977). Proof that this electrode was the source of the prion infection was convincingly obtained in a subsequent experimental animal study. Two years had passed since the silver electrode had been used in the last human patient. The electrode had been cleaned three times and

had been disinfected repeatedly with both ethanol and formaldehyde vapor. Yet when that same electrode was implanted in the brain of a chimpanzee, the agent was transmitted to the animal (Gibbs et al., 1994).

The evidence for iatrogenic transmission of prions has been summarized (Brown et al., 2000a) and is mentioned in Chapter 5, but the special concern about transferring the prion agent on surgical instruments merits additional discussion. The type of surface appears to be an important feature. Steel and glass are extremely common surfaces for surgical equipment and in surgical environments generally. Steel and glass have both been shown to thermostabilize prions from inactivation (Asher et al., 1986, 1987).

A series of intriguing studies in mice has revealed just how intractable prions are once they have become fixed onto a steel surface (Flechsig et al., 2001; Weissmann et al., 2002). The model for those studies involved the insertion of prion-contaminated stainless steel wires into the brains of uninfected mice. The initial experiment revealed that it took as little as 5 minutes of contact to contaminate the wire and transfer the agent to a recipient mouse with the same degree of infectivity as occurred by direct injection of 30 µl of a 1 percent brain homogenate into the brain. In a second experiment, recipient mice were exposed to contaminated wire for different time periods. It took only 30 minutes of exposure to transfer the agent to four of four mice in that exposed group although the incubation period was extended, indicating a lesser degree of infectivity from the brief contact. The investigators indicated that they had demonstrated, in unpublished studies, similar infectivity using gold wire, revealing that prions coated on plastic surfaces transmitted infection in cell culture assays (Weissmann et al., 2002). This model may be useful in screening new methods for inactivating prions, but the investigators caution that larger surface-contact with brain material, use of the vCJD prion strain, and testing in nonhuman primates would ultimately need to be performed to ensure the efficacy of any new sterilization technique for surgical instruments (Weissmann et al., 2002). Such research is worthy and could prevent the unnecessary quarantining or discarding of valuable surgical equipment. It also might offer a more environmentally friendly method of decontaminating surgical operatories.

Recommendation 7.4: Fund research to develop standard assays for the detection of PrP^{Sc} or TSE infectivity on the surfaces of reusable medical instruments and materials, as well as research to develop better methods to disinfect such instruments and materials. [Priority 2]

The inactivation of prions contaminating a relatively small contained space, such as a laboratory work surface, an operating room table, or a

surgical instrument, poses challenges, but the inactivation of prions in a large contained space, such as an abattoir or a large open space, such as a pasture, poses far greater ones. Knowledge of the natural degradation of prions in the environment is extremely limited. Studies from Iceland showed that scrapie-free sheep brought into a pasture where scrapie had been prevalent 3 years previously, but where no animals had grazed in the interim, came down with scrapie, suggesting that pasture land could remain infected for many years (Palsson, 1979). More recently, Brown and Gajdusek (1991) showed that scrapie agent from a hamster brain homogenate mixed with soil could survive burial in a garden for 3 years. Standard methods for evaluating the presence and decay of TSEs in the natural environment are not available, and this has hampered our understanding of the threat posed by long-term environmental contamination by prions.

Recommendation 7.5: Fund research to develop standard test methods for detecting prion contamination in environmental samples. [Priority 3]

The longevity of TSE infectivity, specifically in soil, has tremendous implications for the proper disposal of animals infected with a TSE agent, as well as the offal and rendered material from these animals. For years, burial of dead farm animals has been a common and acceptable practice. However, large-scale burial of animal material potentially contaminated by TSE agents poses a significant risk (Scientific Steering Committee, 2003b). New methods are being evaluated to avoid the need for burial of TSE-contaminated material. One method that has received much attention is treatment at 150°C for 3 hours in concert with high-pressure alkaline hydrolysis. The European Commission's Scientific Steering Committee (2003a) could not certify this method as effective; however, that committee cited laboratory studies demonstrating that under similar conditions, large reductions in infectivity occurred but the method did not eliminate all infectivity. The committee urged further study suggesting that if such studies showed evidence for continued infectivity, residual waste products would need to be incinerated and placed into a control landfill.

One cannot overrstate the importance of identifying safe methods for disposing of animals and animal tissues infected with a TSE agent. Disposal would immediately become an issue if the United States were to experience an outbreak of BSE; the United Kingdom and Europe already face the problem. Recognizing this, USDA APHIS (2003b) proactively published a notice of proposed rulemaking in January 2003 in which it requested public comment on safe, reliable ways to dispose of animals and tissues infected with a TSE agent. Present research on this subject has not progressed far enough to provide a sufficient scientific foundation for policymaking. Therefore, the United States must intensify research into disposal methods for infected

animals and animal tissues. Given that the United Kingdom and Europe need such methods urgently, this area of research appears ripe for international collaboration.

Recommendation 7.6: Fund research to identify safe, cost-effective disposal mechanisms for animals and rendered waste infected with agents of transmissible spongiform encephalopathies. This research would best be conducted with a multidisciplinary approach involving experts in such fields as prion biology, biochemistry, environmental engineering, and commercial disposal technology. [Priority 2]

VACCINATION AS A PREVENTIVE STRATEGY

For more than 200 years, people have successfully combated infectious diseases by using vaccines to mobilize the immune system to protect against invading pathogens. The approach has worked so well that immunization is among the 10 most successful achievements in public health in the twentieth century (CDC, 1999). Yet vaccination has a dubious role in the prevention of prion diseases. Most experts believe the body is immunologically tolerant to prions because the aberrantly folded protein has the same primary protein structure as normal body protein. The basic substrate prion protein appears to be poorly immunogenic (Heppner et al., 2001). The immune response to bacteria or viruses which are much larger and contain foreign proteins is considerably more robust and much better understood. The host recognizes antigens on bacteria and viruses and elaborates protective antibodies. Attenuating or manufacturing these identical antigens so that they are harmless to the host yet create the protective antibody response is the essence of vaccination. This classic immune response does not occur with prions.

Investigators appear to be undaunted by the special challenges introduced by the atypical immune response seen in prion infections. One group has immunized mice with a recombinant PrP protein, which resulted in antibody production that slightly prolonged the incubation period (Sigurdsson et al., 2002). The mice were challenged with a mouse scrapie strain by intraperitoneal (i.p.) inoculation. The animals produced measurable antibody, and those with the higher antibody titers experienced longer delays in their incubation period before their uniform demise. This result suggested a dose-response relationship between the antibody and the longer survival. Additionally, the survival time was slightly prolonged when mice were vaccinated before their exposure as compared with 24 hours following exposure (Sigurdsson et al., 2002).

Another team of investigators, using a mouse model, demonstrated that

injecting mice i.c. with a relatively low dose of SY, a mild strain of mouse-adapted CJD, followed by challenge with FU, a more virulent CJD strain, delayed death and suppressed the expression of clinical disease in these animals by many months, up to approximately 2 years following inoculation of the SY agent (Manuelidis and Lu, 2003). These results support earlier work by Dickinson and colleagues (Dickinson et al., 1968, 1972). Manuelidis and Lu speculated that the innate immunity possibly involving myeloid microglial cells may have been involved in the host response they observed.

The exploration of ways to elicit active immunity by vaccination as a strategy for TSE prevention, while intriguing, will probably not progress much further, even in animals, without a better understanding of the basic immunobiology and molecular biology of PrP^{C} and PrP^{Sc}. Even if animal experiments clearly showed that vaccination prevented prion disease, the transfer of that information to human trials and to a human vaccine would be many years away.

It is clear that prions behave differently from classic pathogens. But even if prions or prion fragments do not make good immunogens, there is a growing body of evidence that the host does have an immune response to them. B-lymphocytes and follicular dendritic cells of the immune system appear to play a significant role in the extraneural pathway of prion diseases (Mabbott and Bruce, 2001). Thus it is conceivable that modulation of this immune activity, in a manner to be determined, might be effective in preventing or treating prion diseases.

The most promising near-term strategy employing the principles of immunity involves the development of specific antibodies. If antibodies could be tailored to bind to certain specific locations on prion protein or preferably prions themselves, such that further conversion of PrP^{C} to PrP^{Sc} were disrupted or a critical pathogenic molecular pathway were inhibited, this might serve as an excellent prophylactic or therapeutic approach. Significant advances are being made in this area, as will be discussed in the next section.

PROGRESS IN THERAPY FOR TSEs

The evidence for success in treating TSEs is meager and incomplete. The reasons for this limited success are multiple and include uncertainty regarding the underlying pathophysiology of prion diseases, the difficulty of identifying disease at an early preclinical stage without sensitive detection tests, the problem of getting any treatment agent past the blood–brain barrier, the toxicity associated with therapeutic agents, and the enormous challenge of translating gains from cell culture or animal studies to human

use. Adding to these important obstacles is the reality that prion diseases, which are devastating to victims and their loved ones, are quite rare; thus the allocation of research funds for work on TSEs has historically been limited as well. However, research directed at this protein-folding disorder would likely have crossover value in advancing understanding of other, more common neurodegenerative disorders, such as Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease, and Parkinson's disease. And fortuitously for sufferers of prion diseases, the reverse is also true. This section briefly reviews progress made in prion disease therapy. It addresses not only the use of drugs, but also gene therapy and treatment with engineered antibodies.

A recent review of drug therapy for TSEs (Brown, 2002) notes the large number of animal studies seeking to discover candidate therapeutic agents. The large majority of those studies led to dead ends, but a few showed some promise. A broad array of studies has been conducted with many different classes of therapeutic drugs. Most have shown little efficacy. Brown (2002) lists of more than 60 drugs that have been studied, highlighting those few agents that showed some efficacy in modifying the disease course. These older experiments in animals were aimed at a putative unknown infectious agent. More recent experiments involving cell-free conversion systems, cell cultures, and animals, undertaken after prions were discovered, have been directed at preventing prions from entering the brain or modifying them once in brain cells within the central nervous system (Brown, 2002).

The timing of the therapeutic dosing can limit interpretation of drug efficacy studies. Drugs being studied have often been given shortly before or shortly after challenge with infectious prions. The efficacy of a drug used in that fashion may be quite different from that of the same drug given later, at the time of disease expression. The longer is the delay in giving the therapeutic agent after the infectious challenge, the less favorable are the results (Brown, 2002).

Many of the newer experiments have been conducted in cell cultures systems as well as animals. Cell culture systems serve as excellent models for screening new drug agents, but they are limited utility because the kinetics of drug absorption, distribution, inactivation, degradation, and excretion cannot be studied (Brown, 2002). Thus any positive effects must be further demonstrated using in vivo systems.

The mechanisms of action of the drugs used to suppress or reverse prion progression are variable and in many cases not fully characterized. The general concept is that there are multiple targets of opportunity to attack prions in the body. A listing of those targets appears in Box 7-2.

Many drugs appear to have a direct or indirect effect on the protein folding capacity and convertibility of prion protein to prions. Some operate

BOX 7-2
Therapeutic Strategies for TSEs

- Prevent infection at the primary site of replication.
- Prevent infection of lymphoid organs.
- Prevent infection of follicular dendritic cells.
- Block the neuro-immune interface.
- Inhibit progression of TSE agents to the central nervous system (CNS) from the peripheral nervous system
- Inhibit progression of TSE agent inside the CNS.
- Provide protection against neuronal death (neuroprotectors).
- Restore neuronal populations (growth factors, stem-cell grafting?).
- Inhibit conformational change of PrP^{C} to PrP^{res}.
- Enhance PrP^{C} degradation.

SOURCE: Dormont (2003).

within the periphery, while others have their effects in the central nervous system. A list of drug classes and drug agents showing some level of effectiveness appears in Table 7-4.

The translation of a scientific discovery in the laboratory to animals and then on to human studies prior to commercial use of a new drug is a laborious, lengthy, and highly structured process that takes many years. One shortcut strategy has been to administer drugs already FDA-approved for some other medical conditions to patients having a TSE. Although this strategy is sound, the results have been highly disappointing. These attempts have involved very few patients with a small number of drugs (Brown, 2002). Also, because of the rarity of human TSEs, the ability to test a candidate drug in hundreds of individuals, as is done in clinical trials, is limited.

The dilemma of not having an approved drug to treat human TSEs, yet having drugs in the formulary that have shown some positive effect against prions in cell or animal testing, has led to some controversial decisions on the appropriateness of administering such drugs to patients dying from CJD. An example was reported recently in the United Kingdom (Dyer, 2003). A teenage boy, symptomatic from a vCJD infection, was given pentosan polysulfate, a drug used in North America to treat interstitial bladder infections. Studies conducted a decade ago in mice showed that this drug, when given prior to prion exposure, protected animals challenged with 100 LD_{50} of prions (Diringer and Ehlers, 1991; Doh-ura et al., 2002). Since the drug is not thought to cross the blood–brain barrier, it had to be administered directly into the brain by a catheter, a procedure that carries a risk of cere-

TABLE 7-4 Drug Classes and Agents Used Experimentally to Treat TSEs

Drugs Whose Action Occurs in Peripheral Stages of Disease	Drugs Whose Effect Is in Both the Peripheral Stages of Disease and the Central Nervous System
Polyanions	Polyene antibiotics
Dextran sulfate	MS-8029
HPA-23	
Pentosan polysulfate	Branched polyamines
Sulfonated dyes	Cysteine protease inhibitors
Congo red	
	Acridine derivatives
Tetrapyrroles	Quinacrine[a]
Porphyrins and phthalocyanines	
	Phenothiazines
Anthracyclines	
Iodotoxorubicin	Antiparasitics
	Suramin
Lymphotoxin β-receptor	
	β-breaker peptides
IgG fusion protein	
	Synthetic peptides
Antibacterials	
Dapsone	Anti-PrP antibodies
Tetracycline[b]	

[a]Korth et al. (2001)
[b]Forloni et al. (2002)
SOURCE: Adapted from Brown (2002), Tables 1 and 2 and supplementary table.

bral hemorrhage and infection. This case was widely debated and legally challenged, eventually leading to a court decision that the drug could be given to this patient.

Despite the controversy over using existing formulary drugs for human prion diseases, research continues to offer new possibilities. A recent study using chronically scrapie-infected mouse neuroblastoma cells (ScN2a) showed that tricyclic derivatives of acridine and phenothiazine may have some potential for treatment of patients with CJD (Korth et al., 2001). The investigators screened several candidate drugs, two of which had already been approved for human use: quinacrine used as an antimalarial, and chlorpromazine, used as an antipsychotic. Both drugs inhibited PrP^{Sc} formation and cleared the prion-infected N2a cells. The quinacrine was 10-fold more potent than the chlorpromazine. The fact that both drugs cross the blood–brain barrier is an additional reason for their use in treating patients with

CJD. At least two patients with CJD apparently have been treated with quinacrine, and a more extensive study is planned (Love, 2001).

Some caution, however, is appropriate given the recent case report of serious liver toxicity in a patient receiving quinacrine for sCJD (Scoazec et al., 2003). Very recently, this acridine class of drugs was manipulated to create a dimeric motif, bis-acridine. This new class and its derivatives, when studied in cell culture systems, appeared to show even greater potency than its relative, quinacrine (May et al., 2003).

Another team, building on results seen in cell culture studies, evaluated quinacrine in a murine model (Collins et al., 2002). The study results were disappointing, failing to show prolonged survival of mice when treated with quinacrine orally. All mice survived the same length of time whether they were treated at 5 days, 65 days, or not at all following i.c. challenge with a mouse-adapted CJD prion agent. The authors concede that using an alternative route of exposure, such as parenterally, might have resulted in a different outcome. They caution that different prion strains can respond differently to therapeutic agents tested in the same animal species (Collins et al., 2002). Barret and colleagues (2003) also evaluated quinacrine. They studied the drug's effect in both ScN2a cells and a murine model. Like previous researchers, they show that quinacrine inhibited the formation of PrP^{Sc} in cells but did not appear to be effective in the animal model. They note that this result supports observations reported by others in both animal and human studies, and they question its use for CJD monotherapy.

A commonly used antibiotic, tetracycline, has shown the ability to hinder the formation of amyloid fibrils and reverse protease resistance of the PrP^{Sc} in human strains of sCJD in cultured cells (Tagliavini et al., 2000). A later study using vCJD and BSE prion strains in hamsters showed that when tetracycline was incubated with the infectious prions prior to intracerebral inoculation, it partly suppressed the agent and prolonged survival of the hamsters (Forloni et al., 2002). The survival outcomes were correlated with to the amount of infectivity and the concentration of the drug. When the drug was incubated with dilute inoculum and then injected into the hamsters, one-third did not develop disease. The researchers suggest that in situations in which an exposure was recognized, such as an iatrogenic exposure, the drug could be useful since the prions would initially be at low levels. They comment on the structural or chemical similarities of tetracycline to other drugs known to suppress prions, such as iododoxorubicin, Congo red, tetrapyrroles, and acridine derivatives. Finally, they indicate that minocycline and doxycycline forms of the drug are available and cross the blood–brain barrier (Forloni et al., 2002). There are no published reports at present that show the effectiveness of tetracycline in human patients with TSEs, however.

Another therapeutic strategy is to attack structural targets along the

prion/prion protein interfaces with the hopes of inhibiting conversion. Synthetic peptides with the same amino acid sequences as those from the central region of the hamster's prion protein inhibited conversion of PrP^{C} to PrP^{Sc} in both mice and hamsters (Chabry et al., 1999) in cell-free conversion systems, and in ScN2a cell culture studies. A similar study of synthetic peptides in cell-free systems revealed that the inhibition was due to binding of the peptide on the PrP^{C} that blocked conversion to PrP^{Sc} (Horiuchi et al., 2001).

Other synthetic peptides have been used to disrupt the prominent β-sheet structure of the prions. A synthetic 13-residue peptide based on amino acid residues from the conserved central region (spanning residues 115–122 and additional proline residues) was incubated with purified mice and human strains of PrP^{Sc} and then introduced to Chinese hamster ovary cells and to mice (Soto et al., 2000). When the PrP^{Sc} was incubated with the peptide, resistance to proteinase K resistance was decreased in the cell studies, and longer incubation periods were observed in the mouse infectivity assay. The investigators estimated that there had been a 90 to 95 percent reduction in infectivity. They also claimed a reduction of the β-sheet structure of the PrP^{Sc} and an increased proportion of α helix structure (Soto et al., 2000).

Gene Therapy for TSEs

The application of gene therapy to treat prion disease in humans appears feasible yet quite far in the future. Gene therapy, even when it involves well characterized vectors, genes, and disease conditions is in its infancy. A recent incident involving a retroviral vector in blood stem cells may have led unexpectedly to the development of a leukemia-type condition in two children. That incident has resulted in even greater caution and oversight regarding this new mode of therapy (FDA, 2003).

Nevertheless, some innovative experimental work in the laboratory holds promise for this method of therapy. A mutant *Prnp* gene was engineered that resulted in the deletion of eight amino acids between residues 114 and 121 of the PrP^{C} prion protein. This deletion spanned most of the central amyloidogenic region of PrP^{Sc}. Using a mammalian viral vector to carry the mutant and normal *Prnp* genes, investigators transfected scrapie-infected mouse neuroblastoma cells. Expression of PrP^{C} protein having this deletion resulted in a dominant negative inhibitory effect on the PrP^{Sc}. The altered protein would not serve as a substrate as would normal PrP^{C}, and it reduced the existing levels of PrP^{Sc} in the infected cells (Holscher et al., 1998).

Another group investigated this dominant negative inhibition in vivo. They inserted *Prnp* genes having amino acid substitutions into transgenic

mice. The inserted substitutions were known to be the polymorphic alleles Q171R and Q218K, which confer resistance to prion disease in sheep and humans, respectively. The prion protein produced by these mutant genes successfully inhibited the PrP^{Sc} inoculated into the brains of transgenic knockout mice, as well as mice that had both null and wild-type *Prnp* genes (Perrier et al., 2002).

Another highly innovative experiment was aimed at disrupting the PrP^{C}–PrP^{Sc} interface. The investigators used a soluble, dimeric form of PrP created in transgenic mice. Vectored genes inserted in these mice resulted in their PrP^{C} being expressed as two full-length PrP^{C} molecules fused to the heavy chain of a human immunoglobulin. This Fcγ (PrP–Fc_2) dimer was reported to interact with PrP^{Sc} that was delivered by i.c. and i.p. routes to these experimental mice. The interaction significantly delayed onset of disease in the mice, although all succumbed eventually (Meier et al., 2003).

These encouraging cell and animal experiments suggest that modification of gene expression can deter PrP^{Sc} conversion. However, all the molecular events that result from gene modifications need to be understood. A single point mutation can lead to familial CJD. Unfortunately, the altered mechanisms introduced as a result of that point mutation are still not clear. Until those mechanisms are clarified, the use of replacement genes as a therapeutic modality is unacceptably risky.

Agents That Modulate or Augment Immunity

In the near term, a more promising treatment strategy is based on the use of agents that affect immune mechanisms. Such strategies might involve a broad effect on the host's innate immune system or highly specific synthesized antibodies designed to attack a precise epitope on prion protein or its isoform.

Regarding the more generic approach, such immune modulators as oligodeoxynucleotides containing CpG dinucleotides (CpG DNA) have been used to activate the innate immune system (Agrawal and Kandimalla, 2002). These dinucleotides are contained in bacteria, but only rarely in the eukaryotic cells of animals. Because these agents activate the host immune response they have been used to boost the effects of vaccines, antibodies, allergens, and antigens (Agrawal and Kandimalla, 2002). Several different kinds of cytokines are activated by CpGs, as shown in Plate 7-1. Multiple pathways are at work simultaneously. The application of CpG oligonucleotides in prion therapy was demonstrated by Sethi and colleagues (2002). After infecting mice with the scrapie agent i.p., they administered CpG by i.p. injection either at the time of scrapie inoculation or 7 hours later. CpG was given daily for 4 days except in one group which was treated for 20 days. In all treatment groups, but not controls, survival was extended; in

addition, the group receiving the 20 days of treatment survived without illness beyond 330 days. The investigators were unable to determine the mechanism for these effects, but speculated that they were due to the stimulation of toll-like receptor (TLR) 9–expressing cells, such as macrophages, monocytes, and dendritic cells (Sethi et al., 2002).

A more direct approach is to modulate a specific protein of interest, such as prion protein, with a specific agent, namely antibodies. As previously noted, there appears to be no natural antibody response by a host to prions. However, antibodies can be produced exogenously by novel methods and administered prophylactically to the host organism. There are encouraging studies showing that antibodies directed at PrP apparently block conversion to PrP^{Sc}. For example, researchers used a monoclonal antibody, 6H4, previously shown to bind PrP in the region spanning amino acid residues 144 to 152 (Korth et al., 1997) to block infection of mouse neuroblastoma (N2a) cells with mouse scrapie agent (Enari et al., 2001). When these cells were preincubated with 6H4 antibodies at the time of exposure to scrapie PrP^{Sc}, infection of the cells was not observed. When the antibody was added after the cells had been infected and were in a static state, the antibodies caused a reduction in the amount of PrP^{Sc}. This result suggested that equilibrium of PrP^{Sc} production and degradation existed and that it could be altered (Enari et al., 2001).

Another study, also using scrapie-infected cells (ScN2a), screened several possible recombinant antibody fragments, known as Fabs, for their ability to clear PrP^{Sc}. The investigators noted significant activity associated with Fab 18, and also observed that the decrement of PrP^{Sc} was dose related. They caution that future in vivo studies must recognize that Fabs have a short half-life in the body and do not cross the blood–brain barrier (Peretz et al., 2001).

In vivo studies have proceeded using antibodies. One group examined the administration of several different monoclonal antibodies in mice. Mice were inoculated with antibodies i.p. at the same time they were inoculated i.p. with a mouse scrapie agent (Sigurdsson et al., 2003). The antibodies were readministered weekly until sacrifice. The antibodies prolonged survival of the mice compared with controls given no antibody or standard IgG. The result of one antibody in particular, 8B4, was notable in that 10 percent of the animals receiving a diluted level of prions did not develop disease, and no toxicity was observed during the study (Sigurdsson et al., 2003).

Another encouraging report was recently published by a group studying monoclonal antibodies in mice. In this study, using two different test antibodies, the investigators showed that even when the i.p. administration of the antibodies was delayed to 7 or 30 days after i.p. inoculation of the scrapie agent, all the mice survived and remained healthy for more than 500

days—which was 300 days longer than control mice (White et al., 2003). While very hopeful, the researchers offer caveats that the antibodies did not work when given after the onset of symptoms, suggesting that the blood–brain barrier may limit their use to prophylaxis during the incubation period. The auhors note further that although they saw no evidence for autoimmunity, it is a possibility to be considered (White et al., 2003).

At least one research team combined concepts of gene therapy and antibody therapy by creating transgenic mice that could produce antiprion antibody endogenously. Using some clever genetic engineering, they transferred genes into knockout mice. The transgene was derived from a hybridoma that expressed monoclonal antibody to murine PrP^{C}. Once in the mouse, the transgene expressed a single-chain variable antibody fragment (scFv) that had anti–PrP binding attributes (Heppner et al., 2001). Following i.p. inoculation of these transgenic mice with scrapie prions, no infectivity was seen in either the knockout mice ($Prnp^{0/0}$) or mice that had one null allele and one *Prnp* allele ($Pnrp^{0/+}$). Nor could PrP^{Sc} be detected in the spleen (Heppner et al., 2001). The study team observed no overt symptoms of autoimmune disease but were cognizant of that potential. The investigators are hesitant to recommend such complex gene-altering strategies as those they described but are optimistic about the potential for active and passive immunization strategies used in prophylaxis or therapy for prion diseases (Heppner et al., 2001).

Recommendation 7.7: Fund research to develop new therapeutic agents, including antibodies, that either block the conversion of PrP^{C} to PrP^{Sc} or disrupt the molecular mechanisms of pathogenesis of transmissible spongiform encephalopathies after this conversion has taken place. The most promising approach appears to be rational drug design, which begins with knowledge of the tertiary structure of the protein or molecule that the therapeutic agent will target. [Priority 1]

Summary of Outlook for TSE Therapy

The work in progress to develop therapeutic agents for TSEs is revealing that, in cell culture and animal models, experimental agents can affect the accumulation of prions and prolong the survival of animals. At present, drug treatment in humans is limited to drugs that have been used for other medical conditions and been shown to be relatively safe. To date, no drugs or other agents have demonstrated consistent or prolonged success in treating human TSEs. This failure relates in part to the use of candidate therapies very late in the course of disease. Also, it is unknown whether the efficacious outcome of one therapeutic agent for a particular TSE can be extrapolated to other TSEs.

Significant acceleration in identifying effective therapeutic agents for TSEs will require scientific breakthroughs. The main obstacle to rapid progress is the same as that which is constraining rapid development of diagnostics (see Chapter 4): fundamental knowledge gaps with regards to the molecular mechanisms, immunobiology, and pathogenesis of prion disease. The heartening news, however, is that breakthroughs in TSE diagnostics will likely translate quickly into progress in the development of therapeutic agents because both diagnostics and therapeutics will target the structural peculiarities of prions. Diagnostics and therapies are inextricably linked for another vital reason: Therapies will likely be more successful if administered early in the preclinical stage of infection, when prions exist in the host at very low titers. Thus, having a diagnostic test sensitive enough to detect prions very early in the incubation period, long before the onset of symptoms, will likely lead to the best outcomes for persons or animals being treated for prion diseases.

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8 Infrastructure for Research on Transmissible Spongiform Encephalopathies

The chief goals of research on transmissible spongiform encephalopathies (TSEs) in the United States are to prevent an outbreak of bovine spongiform encephalopathy in this country; to prevent the further spread of chronic wasting disease; to eradicate scrapie; to reduce the incidence of Creutzfeldt-Jakob disease; and to develop better diagnostic tools, chemoprophylactic agents, and treatments for prion diseases. Although these goals may be easy to defend, they will not be easy to achieve. This chapter begins by reviewing the present U.S. infrastructure for research on TSEs. It next addresses the need for consistent, science-based standards for biological safety levels in laboratories conducting such research. The need for standard reagents and materials is then discussed. The final section examines opportunities for international collaboration in TSE research.

PRESENT U.S. INFRASTRUCTURE

The infrastructure for TSE research in the United States is small, aging, and funded at a level below that needed to achieve the research goals cited above expeditiously. At present fewer than 20 principal investigators conduct TSE research funded by the National Institutes of Health (NIH), the largest sponsor of TSE research in the United States. In fiscal year 2002, NIH spent $27.2 million dollars on TSE research from the total NIH budget of $23.2 billion (personal communication, Robert Zalutsky, NIH, June 2003). Approximately 75 percent of the funds provided for TSE research goes to two laboratories (personal communication, R.T. Johnson, The Johns

Hopkins University, 2002). In addition to the funds provided by NIH, the Agriculture Research Service of the U.S. Department of Agriculture (USDA) spent approximately $6.6 million dollars on TSE research in fiscal year 2002 (Rexroad, 2003).

This level of effort is quite different from that supported by the European Commission (EC). One recent EC report listed 58 delegations of researchers conducting TSE research (European Commission, 2001). It is estimated that the countries of the European Union invest manyfold greater amounts of money in TSE research annually.

Moreover, few U.S. scientists are involved in TSE research for a number of reasons, including but not limited to the paucity of available funds. First, the small number of TSE research laboratories in the United States limits the number of opportunities to obtain training, experience, and expertise—especially for new investigators. Second, the costs of conducting prion-related research are generally higher than those of conducting other kinds of infectious-disease research. The animals needed for prion bioassays are expensive to maintain, and the long incubation periods associated with prion diseases necessitate relatively long time frames for a single experiment. Furthermore, laboratory equipment used in this research must be dedicated solely to TSE research and cannot readily be shared for other research purposes because of decontamination difficulties (see Chapter 7).

The biohazardous nature of prions also leads to delays, frustration, and extra costs associated with adherence to safety regulations and compliance requirements. TSE research laboratories are extremely expensive to build or expand because special safeguards are required to protect both investigators and the public. USDA and institutional policies require TSE laboratories to meet the biological safety standards at biological safety level (BSL) 2 or 3. These additional costs and the lengthy periods of time required for prion research can discourage young investigators who are in relatively short doctoral or postdoctoral training programs. Moreover, investigators who have just completed their training and wish to start up their own laboratories must weigh the high initial costs, and the long-term investment involved, as well as the uncertain availability of funds for TSE research as compared with those for other types of research.

Efforts to reestablish an intramural NIH laboratory on human prion diseases should be encouraged. Research conducted by such a laboratory would complement the animal prion research work at NIH's Rocky Mountain Laboratories. The new laboratory also could become a center for training new investigators. Moreover it could better handle issues of infrastructure investment, sustained funding, and investigator security than extramural programs in universities and private institutes.

Recommendation 8.1: Provide funds to attract and train more investigators in prion disease research. In addition, for investigators conducting prion bioassay research, provide grants for 5- to 7-year periods. [Priority 1][1]

Recommendation 8.2: Provide funds to boost the capacity of the U.S. infrastructure for research on transmissible spongiform encephalopathies by expanding or upgrading existing laboratories, animal facilities, and containment laboratories (biological safety levels 2 and 3), and by building new ones. [Priority 1]

NEED FOR CONSISTENT, SCIENCE-BASED STANDARDS FOR BIOLOGICAL SAFETY LEVELS IN TSE LABORATORIES

At present, neither USDA nor the Department of Health and Human Services (DHHS) has published regulations specifying the BSLs required for laboratories conducting various types of prion research. USDA provides non-regulatory guidance to TSE investigators on laboratory BSL requirements and regulates the movement of prion-contaminated specimens of animal origin across state lines. DHHS has published guidance for appropriate BSLs in laboratories conducting prion research (CDC and NIH, 1999), but the manual has no regulatory authority.

The BSL research requirements for USDA-funded projects may differ from those for projects funded by agencies of DHHS (primarily NIH and the Centers for Disease Control and Prevention [CDC]). This lack of consistent and regulatory guidance from USDA and DHHS creates confusion within the prion research community. As a result, it is unclear when research involving TSE agents must take place within a BSL 2 laboratory versus a BSL 3 laboratory. The difference is significant because the costs of building, upgrading, and maintaining the two types of laboratories differ notably.

In addition, there are far fewer BSL 3 facilities than BSL 2 facilities in the United States. This limits the amount of TSE research requiring BSL 3 conditions that can take place in this country.

Recommendation 8.3: Provide funds to develop scientifically based biological safety level standards for laboratories conducting research that involves infectious agents known to cause transmissible spongiform encephalopathies. [Priority 2]

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[1]The committee denotes each recommendation as priority level 1, 2, or 3 based on the criteria and process described in the Introduction.

If embraced by the community of organizations that fund TSE research, such BSL standards could serve as a common, consistent framework for regulations governing laboratory biosafety for the full spectrum of TSE research.

NEED FOR STANDARDIZED REAGENTS AND MATERIALS

Few of the basic materials used in prion research have been standardized or commercialized, a fact that creates additional challenges for TSE investigators. The reagents used to conduct individual studies are often made by the laboratory conducting the study or borrowed from a fellow investigator's laboratory. The consequent lack of standardization has hampered the ability of one laboratory to replicate the results of another , thus delaying opportunities to validate key discoveries.

The same lack of standardization characterizes the animals used in TSE research. Many of these animals are specially inbred or are genetically altered to have specific mutations, have the genes of other species embedded in their chromosomes (transgenic animals), or have specific genetic coding areas deleted (knockout mice). The processes and level of quality control required to produce these engineered animals are not well established.

The issue of standardized materials is also a concern for researchers in Europe and elsewhere. In September 1999, the World Health Organization (WHO) held a consultation meeting and recommended that a working group be established to address this problem (WHO, 2000). The working group is organizing a systematic collection of animal and human reference materials for TSE research from a variety of prion research centers (WHO, 2001). That effort is laudable, but progress has been slow. In addition, barriers relating to the importation of potentially infectious materials into the United States would preclude, delay, or complicate the retrieval of material from this WHO reference center. Therefore, there needs to be a mechanism in the United States for giving investigators access to research reference standards.

Reference Repositories

NIH sponsored a meeting in February 2002 to establish a TSE reagent repository. At that meeting, various mechanisms to improve the availability of reagents for TSE research were discussed, including the establishment of a centrally run NIH repository. A good model for this is the AIDS reagent repository at NIH. A government–commercial partnership involving one or more private companies could also establish a repository. Or a government contract for establishing a repository could be awarded to a central organization, which could subcontract requirements of the contract to other orga-

nizations. This mechanism has worked well with the Vaccine Development Program at the National Institute of Allergy and Infectious Diseases (personal communication, R.T. Johnson, The Johns Hopkins University, 2002).

The TSE research community in the United States needs not only standard reference materials but also reference centers. These centers do not necessarily need to be stand-alone facilities, nor does any one center need to contain all the various types of required reference materials. For example, one repository might contain diagnostic assay reference material, one might contain different reference strains of prions, and another might contain transgenic or specially engineered animals. The preferred model is to use existing repositories (for example, the Jackson Laboratory, an animal production repository) and add prion-related materials to their collections. The preexisting building, equipment, personnel, and database infrastructure associated with this approach would make the marginal costs far less than those associated with building new repositories.

The funding required to start up such repositories may initially need to come from government research funds or scientific foundations. Once the repositories had been established, the fees paid by investigators using the materials would cover the general operating costs.

New NIH-Based TSE Reagent Repository

On July 9, 2003, NIH announced its intent to establish a TSE reagent repository (Beisel and Nunn, 2003), satisfying a goal of the DHHS TSE Action Plan (DHHS, 2001). The reagents will be available free of charge to scientists around the world. The committee applauds NIH for establishing this international resource for advanced TSE research.

The repository will be a collaborative project of the National Center for Allergy and Infectious Diseases (NIAID) and the National Institute of Neurological Disorders and Stroke (NINDS). The collection will include nonhuman materials containing TSE agents; noninfectious materials, such as antibodies, plasmids, and cell lines; and live animals, such as transgenic mice. It is anticipated that TSE investigators will have relatively easy access to the contents of the new repository, since that has been the case with the AIDS Research and Reference Reagent Program at NIH. More than 2,000 investigators from 63 countries are registered with the AIDS Reagent Program (Beisel and Nunn, 2003).

The announcement encourages TSE investigators to register with the repository and to donate reagents. Investigators will be expected to assume liability for the reagents they borrow, to abide by the safety standards that are established, and to agree to a donor-assigned release for discoveries that are made using the reagents and later commercialized. Donors will receive

semiannual reports of how their reagents are used and acknowledged in relevant publications, on the repository's Web site, and in the repository's annual catalog (NIH, 2003).

Recommendation 8.4: Provide funds to support new or established transmissible spongiform encephalopathy (TSE) repositories that contain a collection of reference materials and genetically engineered animals (including transgenic mice), as well as reagents useful for developing TSE diagnostics and for other TSE research. All registered investigators involved in prion research should have access to these collections. [Priority 1]

Standardized Reagents for Validating TSE Diagnostic Tests

The Food and Drug Administration (FDA) can play an important role in assisting the TSE research community, as well as the commercial sector, by maintaining panels of reference reagents for validating the performance characteristics, such as sensitivity and specificity, of new tests for the detection of both PrP^{Sc} and infectivity. These panels would consist of reagents known to contain TSE-related material (positive controls), as well as reagents known to be free of TSE-related material (negative controls). FDA has developed such panels in the past for validating antibody-screening tests for HIV and hepatitis C virus.

Recommendation 8.5: Provide funds to support the U.S. Food and Drug Administration's development of panels of reference reagents needed to evaluate the performance characteristics of tests designed to detect the prion protein and TSE infectivity. These panels would be used to confirm the performance characteristics of test kits before they are approved for public use, as well as to perform quality control on test kit lots before their release to the market. [Priority 3]

The pace of progress in research on prion diseases will be determined not only by what is studied, but also by the capacity to pursue scientific inquiry. A small, albeit dedicated, effort is proceeding in the United States, and that effort will continue to make contributions, but at a pace that ultimately may not accomplish the nation's goals in a timely manner. To accomplish the broad goals cited at the beginning in this chapter, the capacity to conduct TSE research must be enhanced significantly. Doing this will require more laboratories that can serve as training platforms, more researchers who can enter the field of study, a larger and more reliable funding environment, and better scientific tools.

It is noteworthy that the secretary of Health and Human Services ap-

proved an action plan on August 23, 2001, to increase the infrastructure for TSE research (DHHS, 2001). Achieving the laudable goals set forth in that plan will require sustained attention, effort, and funding.

OPPORTUNITIES FOR INTERNATIONAL COLLABORATION

A quick remedy for the shortage of U.S. laboratory space for prion investigators is unlikely. However, several large European laboratories conducting prion research may represent opportunities for collaboration with U.S. investigators and might even allow U.S. investigators to use their laboratory space. Many TSE investigators have been engaged in international collaborative research efforts for years. These efforts should continue and be expanded. There is a significant TSE infrastructure research base in Europe and other sites around the world. This base should be leveraged not only to conduct collaborative studies, but also to provide training opportunities. The French government has already established 35 fellowships for foreign TSE researchers and is actively seeking U.S. applicants (personal communication, R. T. Johnson, The Johns Hopkins University, 2002). France has a BSL 3 facility that can house 60 macaques, and the government is building a dedicated prion research facility that will house 120 monkeys and provide laboratories for visiting scientists (personal communication, R.T. Johnson, The Johns Hopkins University, 2002). At a meeting of this committee, a scientist from a large Swiss TSE research facility indicated that a great deal of collaborative TSE research is occurring on both a national and an international scale (Raeber, 2002).

The EC maintains an online database of TSE research that is being funded by the the European Union and includes project descriptions and contact information (CORDIS Project Database, 2002). Also, in 2001 the EC published a reference document assembled by a group of TSE experts that lists all the European laboratories conducting TSE research at that time, as well as points of contact and descriptions of the research projects (European Commission, 2001). These contacts may be able to provide information to interested investigators about present opportunities for collaboration with their research groups or with other groups in their countries.

Another resource for TSE investigators seeking funding and the opportunity to collaborate with colleagues abroad is the Human Frontier Science Program (HFSP). Based in Strasbourg, France, this organization promotes collaborative international research by requiring that proposed projects include investigators from different scientific disciplines and different countries (HFSP, 2003). The principal applicant must be from an eligible member country: Austria, Belgium, Canada, Denmark, Finland, France, Germany, Greece, Italy, Japan, Luxembourg, the Netherlands, Portugal,

the Republic of Ireland, Spain, Sweden, Switzerland, the United Kingdom, or the United States. HFSP supports novel, interdisciplinary, basic research involving complex biological systems, including TSE-related studies. The organization awards grants to project teams and provides fellowships to individual scientists so they can work in foreign laboratories. The fellowships are aimed at young investigators seeking experience in a field outside their specialty.

Further opportunities for U.S. scientists to conduct TSE research with foreign investigators outside Europe are offered by the University of Melbourne (personal communication, C. Masters, University of Melbourne, May 4, 2003).

The United States could foster international collaboration by sponsoring one or a series of international conferences on advancements in prion science. Investigators from around the world would be invited to attend and present their research findings. This would facilitate both the timely dissemination of emerging scientific information and the face-to-face interactions among scientists that could foster new collaborative research initiatives. Funds from the National Prion Research Project might be able to provide some or all of the support for such conferences.

Recommendation 8.6: Provide funds to enable U.S.-based investigators of transmissible spongiform encephalopathies (TSEs) to collaborate or train with TSE investigators internationally and to use TSE research facilities abroad. Exploiting such opportunities will expand the range of TSE research that U.S. scientists can conduct. [Priority 3]

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9 Risk of Transmissible Spongiform Encephalopathies to the U.S. Military

U.S. forces are continually deploying around the globe. More than 250,000 military personnel[1] were deployed to more than 130 foreign countries on March 31, 2003 (DOD, 2003), including several countries where bovine spongiform encephalopathy (BSE) had been reported and where variant Creutzfeldt-Jakob disease (vCJD) subsequently occurred. In addition, U.S. forces are frequently accompanied by their families when they are deployed on noncombat missions over an extended period. As a result, U.S. military personnel and their families deployed to countries having reported the occurrence of BSE were at increased risk of exposure to BSE-contaminated food products for several years starting in the early 1980s. Likewise, deployed U.S. military personnel may receive blood transfusions if they are injured in combat or under other circumstances. These two factors—exposure to BSE-contaminated food and exposure to BSE-contaminated blood products—constitute the focus of this chapter. Specifically, we examine how much risk is faced by U.S. forces of acquiring a foodborne BSE infection and under what circumstances that risk would occur. Additionally, we evaluate the risk of acquiring a TSE from a blood transfusion administered to a deployed member of the U.S. forces being treated for trauma or other emergency condition.

[1]This figure excludes the number of military personnel participating in Operation Iraqi Freedom.

RISK OF EXPOSURE TO BEEF PRODUCTS CONTAINING BSE INFECTIVITY

Department of Defense Military Food Supply System

In assessing the military's risk of exposure to beef products containing BSE infectivity, a brief description of how food is supplied to military personnel is appropriate. All beef products supplied to U.S. forces come from approved suppliers. The forces receive a majority of their food, including beef and beef products, from U.S. producers. Food is prepared and prepackaged in a variety of ration sets served during training or combat operations. Some meals are served fresh, and regulations dictate that vendors selling food destined for troops be closely inspected and regulated.[2]

Commanders of U.S. military units have the authority to purchase local food products, including beef. A commander might exercise this authority if his or her troops had been eating prepackaged rations for an extended period of time to offer variety and to maintain high morale. If local beef were purchased in a country where BSE had been reported,[3] the troops who consumed it would be at risk of exposure to BSE, although the practice of procuring local beef is the exception rather than the rule. Current policy prohibits the purchase of beef from a country reporting cases of BSE, but it does not prohibit the purchase of beef from other countries, as long as the source is approved by the Department of Defense (DOD) Veterinary Services. Some beef was purchased from the United Kingdom, Italy, Germany, and Japan before it was recognized as potentially being infected with the BSE agent.

DOD Commissary Food System

Military personnel, as well as their families, have access to beef products through several other outlets as well. The first is the commissary system. Commissaries are military supermarkets stocked primarily with food products from the United States. U.S. producers generally supply all the beef sold in the commissaries. In some European countries reporting BSE, however, some beef sold in commissaries was procured locally for certain periods of time. From 1980 to 1989, the monthly foreign beef procurement from non-U.S. suppliers averaged 2.5 million pounds. Of this amount, 35

[2]U.S. Army Regulation AR40-657 (1997); U.S. Navy Regulation NAVSUPINST4355.4F (1997); U.S. Marines Regulation MCOP10110.316 (1997).

[3]EDITORS' NOTE: After this report was completed, the first U.S. case of BSE was identified in Washington State and was announced to the public on December 23, 2003.

percent was from the United Kingdom and 65 percent was from other European countries. Of the product from the United Kingdom, approximately 300,000 pounds was delivered each month to commissary stores north of the Alps (Germany, Belgium, Netherlands, and the United Kingdom) and approximately 575,000 pounds was delivered each month to commissary stores south of the Alps (Italy, Spain, Greece, and Turkey). Supply contracts for 112 stores located on 21 delivery routes were written on a monthly basis. Thus, the source of supplies for a specific store could and did change monthly. Records of specific delivery dates and locations no longer exist. Thus it is impossible to determine which stores received beef from the United Kingdom, but it must be assumed that all stores received some product from that country. These contracts were for carcass beef, which was split into forequarters and hindquarters at the packinghouse and further processed into cuts for retail sale in the meat market of the commissary store.

In 1990 the Beef to Europe Program was initiated for commissary stores north of the Alps. This program, which was congressionally mandated and not related to BSE, entailed the shipment of boxed beef (vacuum-packaged wholesale cuts) of U.S. origin to Europe. During a supply failure, beef was purchased on an emergency basis within Europe. Of these emergency contracts, 99 percent were given to German meat packers. All commissary stores within the United Kingdom, with the exception of the commissary in Edzell, Scotland, participated in the Beef to Europe Program.

Shipments to the Edzell Commissary and areas south of the Alps continued to be carcass beef from the United Kingdom. These contracts were converted to boxed beef in 1994. After March 1996, all procurement of beef from the United Kingdom ended, and in March 2000, all procurement of European beef stopped.

Other Sources of Beef Products

Beef products are also sold to members of the U.S. military and their families at post exchanges (PXs), which are located on U.S. military posts and bases. The Army and Air Force Exchange Service (AAFES), which manages the exchange system, is not able to provide estimates of the total number of pounds of beef procured in Europe during the same time frames mentioned above. They did, however, use cuts of carcass meat and distribution procedures similar to those described above for the commissary system. AAFES food service outlets used European beef, and approximately 20 percent of this beef was from the United Kingdom.

A third outlet for the purchase of beef by members of the U.S. military and their families is hamburger franchises. Before the reduction of troop strength in Europe, more than 50 hamburger franchises were operating as concessions. These operations used preformed patties from the United King-

dom through 1989. From 1990 to March 2000, either U.S. beef was used, or beef was ground in an AAFES-operated facility in Germany; the latter was a combination of U.S. beef and beef from European countries other than the United Kingdom. Between March 1996 and March 2000, most of the beef originated from European countries without cases of BSE, and some came from the United States. Since March 2000, the beef has been of U.S. or non-European origin.

Risk of Exposure to BSE Agent

Members of U.S. forces, including their family members, commonly enjoy the local culture and consume locally prepared foods while they are stationed overseas. They purchase food in local markets and dine in local eating establishments. However, the majority of food they consume comes from either the system used to feed troops or the commissary system.

The greatest period of risk for exposure to BSE-contaminated beef products occurred between 1980 and 1996 in the United Kingdom. For continental Europe, that period of risk was extended beyond 1996. In the early 1980s, the BSE outbreak was not apparent, yet cattle were infected with BSE. Effective controls to prevent further contamination of the food supply were put in place iteratively, first in the United Kingdom and then throughout other European countries. During that period, 4,428,572 military personnel and their family members were potentially exposed to BSE-contaminated beef products (Table 9-1).

U.S. military members and their families living in the United Kingdom and Europe between 1980 and 1996 were at increased risk of exposure to the BSE agent as a result of their consumption of locally procured beef or their consumption of beef in local eating establishments as compared with the risk to their counterparts in the United States. The committee judged, however, the risk of acquiring foodborne-associated vCJD to be relatively small compared with that of the local population due to the lesser consumption local beef and the shorter period of exposure. Notification and

TABLE 9-1 DOD Active Duty Personnel and Dependents in Europe

Period	Number of Active-Duty Personnel	Number of Dependents	Total Number of Individuals
1980–1996	1,932,179	2,496,393	4,428,572
January 1, 2001	215,778	317,231	533,009

SOURCE: Severin (2002).

active prospective surveillance are not warranted for these military members or their families. However, the committee encourages passive monitoring of the incidence of Creutzfeldt-Jakob disease (CJD) among military personnel.

Recommendation 9.1: Use existing passive surveillance systems to monitor the incidence of Creutzfeldt-Jakob disease and variant Creutzfeldt-Jakob disease among individuals receiving medical care from the health systems of the U.S. Department of Defense and the Department of Veterans Affairs. [Priority 3][4]

RISK OF TSE INFECTION FROM BLOOD PRODUCTS

Blood transfusions could also place deployed forces at theoretical risk of infection by the agent of BSE or other transmissible spongiform encephalopathies (TSEs) (see also Chapters 5 and 7). In a situation in which a deployed service member is wounded or otherwise injured and needs a blood transfusion, where does that blood come from and what is the likelihood that it contains the agent of vCJD or another TSE?

The DOD Blood Supply System

DOD's blood supply is under the management of the Armed Services Blood Program (ASBP). The collection, processing, tracking, storage, and distribution of blood are closely managed (DOD, 1996). The majority of blood used by U.S. forces is collected at 24 blood collection sites: 18 sites in the United States and 6 sites overseas (Sparks, 2002). More than 90 percent of the blood collected at these sites comes from active-duty service members (Sparks, 2002). Some of the military's blood is frozen for longer-term storage and use. This stockpile was collected in the early 1990s, before the current blood donation deferral policy was in place (personal communication, LTC R. D. Sylvester, Armed Services Blood Program, November 5, 2002) and would be used only in a major military contingency situation in which fresh blood was unavailable.

The U.S. military deploys its own health care system in support of U.S. forces overseas. That system includes medical providers, fixed and mobile hospitals, and medical supplies, including blood. In general, any blood given to a deployed service member would be collected and controlled by ASBP. Thus, the committee concluded that the risk that a service member would

[4]The committee denotes each recommendation as priority level 1, 2, or 3 based on the criteria and process described in the Introduction.

be transfused blood from a donor having preclinical vCJD would be quite remote.

Under some circumstances, blood products are supplied to U.S. facilities by the host nation. Examples of such blood products are platelets, which have a very short shelf life, and products whose supplies are exhausted or unavailable. Additionally, U.S. forces deployed overseas use local emergency rooms or hospitals when medical care is not available from the DOD health care system. Should a U.S. service member have an injury serious enough to warrant a blood transfusion from a local hospital in a country reporting BSE, there would be a theoretical risk of exposure to the vCJD agent in the blood, but those situations are uncommon.

DOD Blood Donation Deferral Policies

Although the risk of a member of the deployed armed forces acquiring a TSE infection from a blood transfusion is presumed to be very low, individuals who were deployed to Europe during the period of risk are not able to donate blood, according to DOD policy (Sparks, 2002). The Food and Drug Administration (FDA) and the American Red Cross have similar blood donation deferral policies (see Table 9-2). This policy results in the deferral of 18 percent of DOD donors and has placed a significant burden on DOD's ability to maintain its blood supply. However, special recruiters at blood donor sites have increased collections by 9 percent, helping to offset these losses (Sparks, 2002). If an antemortem blood test were available and sensitive enough to detect prions in blood, it might be possible to return to the blood donor pool more than 4 million donors whose DOD service in Europe precludes them from donating blood, as well as half a million deferred civilians (5 percent of the national blood donor pool) (Sparks, 2002).

SUMMARY OF OVERALL RISK

This chapter summarized the risk to deployed U.S. forces of acquiring a TSE as the result of eating a food product or receiving a blood product containing TSE infectivity. Both risks are deemed small. Nevertheless, the level of risk is unknown, so the precaution of deferring individuals who were potentially exposed to BSE-contaminated meat from donating blood is justified. Research clarifying the infectious potential of blood products as a vehicle for transmitting prions would help immensely in addressing this issue.

TABLE 9-2 Comparison of Deferral Policies

Assistant Secretary of Defense for Health Affairs (DOD)	U.S. Food and Drug Administration	American Red Cross
United Kingdom (UK)		
Cumulative time ≥ 3 months 1980–1996	Cumulative time ≥ 3 months 1980–1996	Cumulative time ≥ 3 months 1980–present[a]
Transfusion in UK 1980–present	Transfusion in UK 1980–present	Transfusion in UK 1980–present
Europe and other countries associated with BSE by USDA		
Europe 1980–present cumulative time ≥ 5 years (applies to DOD after 1997)	Europe 1980–present cumulative time ≥ 5 years (applies to DOD after 1997)	All of Europe, regardless of USDA rating, cumulative time ≥ 6 months 1980–present
DOD stationed in Europe 1980–1996 cumulative time ≥ 6 months.	DOD stationed in Europe from 1980–1990 (north of the Alps) cumulative time ≥ 6 months.	
	DOD stationed in Europe from 1980–1996 (south of the Alps) cumulative time ≥ 6 months.	

[a]The American Red Cross recently changed its policy to defer donations of blood from individuals who spent 3 months or more living in the United Kingdom between 1980 and 1996 (personal communication, R. Dodd, The American Red Cross Holland Laboratory, June 27, 2003).
NOTE: USDA = U.S. Department of Agriculture.
SOURCE: Sparks (2002).

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Appendixes

A Agendas of Open Sessions of Committee Meetings

The committee gathered information about the state of prion science from journal articles and sections of reports provided by committee staff, as well as from presentations and group dialogues during three meetings held in the summer and fall of 2002. The agendas of the open sessions of those meetings appear below.

Meeting 1
July 17–18, 2002
500 5th Street, NW, Room 101, Washington, DC

Purpose of the Meeting

- Discuss and reconcile any bias issues with committee members
- Orient members and consultants to the National Prion Research Project
- Orient members and consultants to any U.S. Department of Defense (DOD) concerns regarding the threat of transmissible spongiform encephalopathies (TSEs) to their food and blood supplies
- Clarify the study tasks and determine a strategy for accomplishing them
- Determine if the committee is lacking any area of needed expertise
- Determine the format and identify presenters who should be invited to address the committee at subsequent meetings
- Determine study milestones and subsequent meeting dates

OPEN SESSION, JULY 17, 2002

9:15 a.m.	Introductory remarks, introductions of committee and expert consultants, and review of charge *Richard T. Johnson, M.D., chair of the committee*
9:30	Sponsor presentation—DOD Congressionally Directed Medical Research Programs and DOD National Prion Research Program *COL Ken Bertram, Director, Congressional Directed Medical Research Programs, U.S. Army Medical Research and Materiel Command*
10:00	DOD stakeholder meeting summary *COL Ken Bertram*
10:30	Break
10:45	Protecting the DOD's food supply from TSEs *COL Scott Severin, Deputy Director, DOD Veterinary Service Activity, Office of the Army Surgeon General*
11:15	Protecting the DOD's blood supply from TSEs *CDR Rebecca Sparks, Deputy Director, Armed Services Blood Program*
11:45	Evidence for or against transmission of TSEs in blood *Roger Y. Dodd, Ph.D., committee member*
12:15 p.m.	Lunch
1:00	Surveillance of TSEs in animals and risks to human health in the United States *Elizabeth S. Williams, D.V.M., Ph.D., consultant to the committee*
1:30	Surveillance of TSEs in humans in the United States *Pierluigi Gambetti, M.D., consultant to the committee*
2:00	New detection methods for TSEs in living sheep *Katherine O'Rourke, D.V.M., Ph.D., Animal Disease Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Pullman, Washington*

2:30 Break

2:45 New techniques for detecting prions in animal tonsillar tissue
Mike Miller, D.V.M., Ph.D., Colorado Division of Wildlife, Wildlife Research Center, Fort Collins, Colorado

3:45 Adjourn open session

OPEN SESSION, JULY 18, 2002

8:30 a.m. Currently available assays and reagents for detecting prions
David Asher, M.D., Chief, Laboratory of Bacterial, Parasitic, and Unconventional Agents, Division of Emerging and Transfusion-Transmitted Diseases, Office of Blood Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration

9:15 Commercial diagnostic testing for TSEs in Europe—Prionics
Alex Raeber, Ph.D., Chief of Research, Prionics AG, Schlieren, Switzerland

10:00 Adjourn open session

Meeting 2
September 12–13, 2002
500 5th Street, NW, Room 203, Washington, DC

Meeting Objectives

- Review information about prion structure and methods to better define its structure
- Review concepts of prion conversion, pathogenesis, and detection
- Review current and newer techniques useful for TSE diagnostics
- Discuss the compositions of the interim report and the final study report
- Develop draft recommendations regarding the essential research that will:
  - ➤ lead to better TSE diagnostics
  - ➤ address animal models, bioassays, reagents, and the research infrastructure needed for TSE research
  - ➤ achieve critical breakthroughs to jump-start progress in prion-disease science

OPEN SESSION, SEPTEMBER 12, 2002

8:30 a.m. Introductory remarks
Richard T. Johnson, M.D., chair of the committee

- Introduction of members who were not at previous meeting
- Summary of the first meeting
- Goal of this meeting: produce draft recommendations for the interim report

9:00 Group discussion
- Does the outline of the final report reflect the proper direction of the study?
- Review emerging topics. What should be added?

10:00 Critical prion research requirements and research infrastructure
Stanley B. Prusiner, M.D., consultant to the committee

11:00 PrP conversion, mechanisms, pathogenesis, and future research needs
Byron Caughey, Ph.D., Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, NIH, Hamilton, Montana

12:00 p.m. Lunch

12:30 Mini-symposium: prion structure and structure-based detection

- Tool 1: Electron crystallography
Holger Wille, Ph.D., Institute for Neurodegenerative Diseases, University of California, San Francisco

- Tool 2: Nuclear magnetic resonance (NMR)
David E. Wemmer, Ph.D., Lawrence Berkeley National Laboratory and University of California, Berkeley

2:00 Proteomic tools to detect prions and surrogate markers
Ron Hendrickson, Ph.D., formerly of MDS Proteomics, Toronto

3:00 Break

3:15 Next-generation detection methods
David A. Harris, M.D., Ph.D., consultant to the committee

3:55 Discussion of interim report:
- Which diagnostic tools show the greatest potential for advancing prion detection, particularly antemortem detection?
- What research is needed to develop these tools?

5:00 Adjourn session

OPEN SESSION, SEPTEMBER 13, 2002

8:30 a.m. Extraneural pathogenesis of prion disease and research gaps
Adriano Aguzzi, M.D., Ph.D., consultant to the committee

9:30 PrP^{Sc} diagnostics; building research capacity; international collaboration
Jean-Philippe Deslys, M.D., Ph.D., Head of the Prion Group, Medical Research Department, Commissariat à l'Energie Atomique, Fontenay-aux-Roses, France

10:30 Adjourn Open Session

Meeting 3
October 29–30, 2002
Arnold & Mabel Beckman Center of The National Academies
Irvine, California

Meeting Objectives

- Review and discuss the critical study time lines and tasks
- Review and refine draft interim report and recommendations
- Receive briefings on Creutzfeldt-Jakob disease (CJD) and chronic wasting disease (CWD) surveillance systems
- Receive briefings on novel proteomic tools
- Discuss agendas of Meetings 4 and 5

OPEN SESSION, OCTOBER 29, 2002

1:00 p.m. Mini-Symposium on TSE Surveillance

Linking bovine spongiform encephalopathy (BSE) to variant CJD in the United Kingdom: lessons learned and applications to CWD in the United States
Robert G. Will, M.D., committee member

2:00 National surveillance of CWD in captive cervids
Lynn Creekmore, Staff Veterinarian/Wildlife Disease Liaison, National Animal Health Programs, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Fort Collins, Colorado

Break

3:00 CWD surveillance of cervids from a state's perspective
Sam D. Holland, D.V.M., South Dakota Animal Industry Board, Pierre, South Dakota

4:00 Novel techniques at the cutting edge of protein detection
Roger Brent, Ph.D., Associate Director of Research, The Molecular Sciences Institute Inc., Berkeley, California

5:00 Adjourn open session

Meeting 4
January 21–22, 2003
500 5th Street, NW, Room 201, Washington, DC

Meeting Objectives

- Determine the content of the final report
- Develop research recommendations for TSE surveillance and future research needs
- Plan the program for the final meeting

OPEN SESSION, JANUARY 21, 2003

Mini-Symposium: CJD Surveillance

9:45 a.m. CJD surveillance in Canada
Neil Cashman, M.D., Center for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Canada

10:30 Break

10:45 CJD surveillance in the United States
Ermias Belay, M.D., Centers for Disease Control and Prevention, Atlanta, Georgia

Mini-Symposium: Prion Inactivation and Therapeutics for TSE

11:30 Prion inactivation
David M. Taylor, Ph.D., M.B.E., Committee Member

12:15 p.m. Lunch

12:45 Antibody-based therapeutics for TSE
R. Anthony Williamson, Ph.D., Department of Immunology, The Scripps Research Institute, La Jolla, California

1:30 Gene therapy as a treatment for TSE
Howard Federoff, M.D., Ph.D., Department of Neurology, University of Rochester School of Medicine, Rochester, New York

2:15 Break

2:30 Noninflammatory spongiform neurodegeneration caused by a conventional virus
John L. Portis, M.D., Rocky Mountain Laboratories, National Institutes of Health (NIAID), Hamilton, Montana

OPEN SESSION, JANUARY 22, 2003

8:30 a.m. Intracellular trafficking of PrP and cytosol folding
Jiyan Ma, Ph.D., Department of Molecular and Cellular Biology, The Ohio State University, Columbus, Ohio

9:15 Use of neuroimaging to diagnose TSE
William E. Klunk, M.D., Ph.D., Laboratory of Molecular Pharmacology, Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

10:00 Adjourn open session.

Meeting 5
March 25–26, 2003
500 5th Street, NW, Room 101, Washington, DC

Meeting Objectives

- Develop research recommendations for TSE surveillance, epidemiology, and therapeutics, and for prion inactivation
- Establish a process for the committee to review drafts of the final report during the next several months

OPEN SESSION, MARCH 25, 2003

8:30 a.m. Opening comments, review of objectives, and general discussion.
Richard T. Johnson, M.D., Committee Chair

Mini-Symposium: Controlling BSE

9:00 Control points in the beef processing industry
Mr. Dave Harlan, Taylor Packing and Excel Food Solutions Company, Wyalusing, Pennsylvania

9:45 The role of the federal government in managing and controlling the risk of BSE in the United States
Linda Detwiler, D.V.M.; Veterinary Service, Animal and Plant Health Inspection Service, USDA, Robbinsville, New Jersey

10:30 Break

10:45 Prion research sponsored by the U.S. Department of Agriculture
Caird E. Rexroad, Jr., Ph.D.; Administrator's Council, Agricultural Research Service, USDA, Washington, D.C.

11:30 Oral prion neuroinvasion: the role of the tongue
Richard Bessen, Ph.D.; Department of Medical Microbiology and Immunology, Creighton University, Omaha, Nebraska

12:15 p.m. Lunch

1:00
- Transmission of the BSE agent to nonhuman primates
- Identification of a laminin receptor that is a cell-surface receptor for PrP^C

Corinne I. Lasmézas, D.V.M., Ph.D.; Laboratory for Prion Pathogenesis, Atomic Energy Commission, Service de Neurovirologie, Fontenay-aux-Roses, France

2:00 Adjourn open session

OPEN SESSION, MARCH 26, 2003

8:30 a.m. The processing of deer and elk meat in the United States and intersects with commercial beef processing
Warrie J. Means, Ph.D.; Department of Animal Science, College of Agriculture, University of Wyoming, Laramie, Wyoming

9:15 Dynamics of CWD in mule deer populations: studies of prevalence and transmission at multiple scales
N. Thompson Hobbs, Ph.D.; Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, Colorado

10:00 Break

10:30 The quest for an FDA-approved test to screen human blood and blood products for prions: regulatory, scientific, and commercial obstacles
Jay Epstein, M.D.; Director Office of Blood Research and Review, FDA, Rockville, Maryland and *David M. Asher, M.D., consultant to the committee*

11:30 Lunch in room 101

12:30 p.m. Adjourn open session

B Biographical Sketches

MEMBERS OF THE COMMITTEE

Richard T. Johnson, M.D., *Chair*, is Distinguished Service Professor of Neurology, Microbiology and Neuroscience at The Johns Hopkins University School of Medicine and Bloomberg School of Public Health. He also is the editor of Annals of Neurology. His clinical and research work during the past 30 years has focused on infections of the nervous system, particularly inflammatory and demyelinating neurodegenerative diseases. The author of more than 250 papers and the book *Viral Infections of the Nervous System,* Dr. Johnson is a special consultant to the National Institutes of Health and an elected member of the Institute of Medicine. He presently chairs the IOM Committee on Spinal Cord Injury: Strategies in a Search for a Cure.

Harvey Alter, M.D., is chief of the Infectious Diseases Section and associate director of research at the National Institutes of Health Department of Transfusion. He also is codiscoverer of the Australia antigen, a key to detecting hepatitis B virus, and has been instrumental in the near-elimination of transfusion-associated hepatitis in the United States. His clinical research currently focuses on uncovering the causes and reducing the risks of transfusion-associated hepatitis. Dr. Alter is a Lasker Award recipient and an elected member of both the National Academy of Sciences and the Institute of Medicine.

Dean O. Cliver, Ph.D., is a professor of food safety at the University of California, Davis, and a professor emeritus at the University of Wisconsin,

Madison. His research interests include disease transmission through food and water. His laboratory is the World Health Organization's Collaborating Center for Food Virology. He has served as a member of the Food and Drug Administration's Transmissible Spongiform Encephalopathies Advisory Committee and belongs to several scientific associations. A member of the editorial boards of various scholarly journals, Dr. Cliver has participated in several workshops and has published nearly 200 articles and books.

Roger Dodd, Ph.D., is executive director for Biomedical Safety and head of the Transmissible Diseases Department at the American Red Cross's Holland Laboratory. His research interests include the epidemiology of infectious disease, with special reference to blood transfusion. An adviser to the World Health Organization, he has coauthored numerous papers regarding laboratory testing for retrovirus infection, hepatitis, viral inactivation, and blood transfusion. Dr. Dodd was president of the American Association of Blood Banks during the 2002–2003 term and served on its board. In addition, he is a member of the American Association of Immunologists, Sigma Xi, the American Society of Microbiology, and the International Society for Blood Transfusion.

Frederick Murphy, D.V.M., Ph.D., is dean emeritus and professor at the School of Veterinary Medicine, University of California, Davis. Formerly, he directed the National Center for Infectious Diseases at the Centers for Disease Control and Prevention. He has been a leader in viral pathogenesis, viral characterization, and taxonomy. His interests include public health policy; vaccine development; and new, emerging, and reemerging diseases. A recipient of the Presidential Rank Award, he belongs to the German Academy of Natural Sciences and the National Academies' Institute of Medicine. Dr. Murphy cochaired the National Research Council's Committee on Occupational Health and Safety in the Care of Human Primates. He presently serves on two committees of the National Research Council: the Committee to Review Research Proposals from Former Soviet Biological Weapons Institutes and the Committee on Future Contributions of the Biosciences to Public Health, Agriculture, Basic Research, Counterterrorism, and Non-Proliferation Activities in Russia.

Michael Oldstone, M.D., is a professor in the Department of Neuropharmacology at The Scripps Research Institute, where he heads the virology division. His research interfaces with virology and immunology. He has authored numerous publications, was editor for the *Journal of Virology,* and is currently editor of *Virology* and consulting editor for the *Journal of Clinical Investigation, Immunity,* and *Journal of Experimental Medicine.* He is the author of the book *Viruses, Plagues, and History*. He has received

numerous awards, including the J. Allyn Taylor International Prize in Medicine for his work in virus–host interactions, the Cotzias Award for research excellence in nervous system disease, the Rous-Whipple Award for research excellence in investigative pathology and the Abraham Flexner Award for contributions in biomedical research. He is a member of several professional societies and has been a member of the Institute of Medicine of the National Academies since 1996. He has been a scientific councilor to the Institute of Allergy and Infectious Diseases, National Institutes of Health, and a consultant to the World Health Organization for the eradication of poliomyelitis and measles virus infections. His interest is primarily in the study of persistent virus infection; he has also studied the pathogenesis of scrapie disease.

David A. Relman, M.D., is associate professor of medicine and associate professor of microbiology and immunology at Stanford University. He is also chief of Infectious Diseases at the Veterans Affairs Palo Alto Health Care System. His research interests concern microbial pathogen discovery and human microbial ecology and have generated more than 100 publications. Among the many awards and honors he has received are the Senior Scholar Award in Global Infectious Disease from the Ellison Medical Foundation and the Squibb Award from the Infectious Diseases Society of America. Dr. Relman is a member of the Board of Scientific Counselors of the National Institute for Dental and Craniofacial Research, National Institutes of Health, and the Board of Directors of the Infectious Diseases Society of America. He currently serves on the National Academies' Committee on Advances in Technology and the Prevention of their Application to Next Generation Biowarfare Agents.

Raymond P. Roos, M.D., is Marjorie and Robert E. Straus Professor and chairman of the Department of Neurology at the University of Chicago. His main clinical and research interests are neurodegenerative disease (amyotrophic lateral sclerosis, prion disease); inflammatory nervous system infections (multiple sclerosis, viral infections); and neuropathy. A member of the Food and Drug Administration's Transmissible Spongiform Encephalopathies Advisory Committee from 1996 to 2001, Dr. Roos has published more than 200 papers in the fields of neurology and virology.

David M. Taylor, Ph.D., M.B.E., recently retired from his position as principal research scientist in the Neuropathogenesis Unit of the Institute for Animal Health in Edinburgh. He worked extensively on the problem of inactivating the infectious agents of transmissible spongiform encephalopathies (TSEs) and published numerous research papers and book chapters on the subject. The British monarchy named him a Member of the Order of the

British Empire (MBE) in 1999 for his contribution to TSE research. He also is a member of the United Kingdom Department of Health Creutzfeldt-Jakob Disease Incidents Panel, the United Kingdom Joint Advisory Committee on Dangerous Pathogens/Spongiform Encephalopathy Advisory Committee, and the European Commission Working Group on Safe Disposal and Recycling Methods for Animal By-Products Not Intended for Human Consumption.

Reed B. Wickner, M.D., is chief of the Laboratory of Biochemistry and Genetics at the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health. He has focused on nonchromosomal genetic elements of *Saccharomyces*. His studies of yeast dsRNA viruses have revealed many of their interactions with the host translation and posttranslation modification systems. In 1994 Dr. Wickner discovered that yeast harbors two prions (infectious proteins), called [*URE3*] and [*PSI*]. He is a member of the National Academy of Sciences.

Robert G. Will, M.D., established the National Creutzfeldt-Jakob Disease Surveillance Unit at Western General Hospital, University of Edinburgh in May 1990 and served as the unit's director until 2000. He will direct the unit once every 3 years and will continue to serve as its consultant neurologist in the interim. He also holds a personal chair in clinical neurology in the department of clinical neurosciences at the university. Dr. Will has been intimately involved with the identification of the clinical and epidemiological features of variant Creutzfeldt-Jakob disease (vCJD). He was the lead author of both the landmark 1996 *Lancet* paper reporting the emergence of vCJD and of the 2000 *Annals of Neurology* paper proposing diagnostic criteria for vCJD. In addition, he coauthored the first report to demonstrate that the infectious agent of mad cow disease could cause vCJD-like symptoms in mice. In recognition of his scientific and medical contributions, the British monarchy awarded him the title of Commander of the British Empire (CBE) in 2000. At present, Dr. Will advises and collaborates with his international counterparts on CJD surveillance, blood transfusion risk, and transmission studies.

Liaison from the Board of the Medical Follow-up Agency

Linda D. Cowan, Ph.D., is George Lynn Cross Research Professor, Epidemiology, in the department of biostatistics and epidemiology at the University of Oklahoma Health Sciences Center. The recipient of several teaching awards, she conducts epidemiological research on neurological disease in infants and children, and she is the coinvestigator of a study begun in 1987 into the mortality, morbidity, and risk factors for cardiovascular disease in

three American Indian populations. Dr. Cowan is an elected member of the American Epidemiological Society. At present, she serves on the Institute of Medicine's Board of the Medical Follow-up Agency and Advisory Panel for the Study of Long-Term Health Effects of Participation in Project SHAD (Shipboard Hazard and Defense). She is also a member of the National Research Council's Committee to Assess the Health Implications of Perchlorate Ingestion. Previously, she was a member of the Institute of Medicine's Committee to Assess the Safety and Efficacy of the Anthrax Vaccine, the Vaccine Safety Forum, and the Committee to Review the Adverse Consequences of Pertussis and Rubella Vaccines.

CONSULTANTS TO THE COMMITTEE

Adriano Aguzzi, M.D., Ph.D., is professor and associate dean for research at the University of Zurich Medical School. His research career has focused entirely on prions, exploring how they damage brain cells, why they accumulate in follicular dendritic cells, and how they reach the brain after entering the body from peripheral sites. The president of the Swiss Society of Neuropathology, Dr. Aguzzi also has developed and patented diagnostics and therapeutics for TSEs. He served as director of the Swiss National Reference Center for Prion Diseases from 1995 until 1997, the year he became a member of the United Kingdom's Spongiform Encephalopathy Advisory Committee. He sits on the editorial boards of numerous journals, including *Brain Pathology,* for which he was deputy editor from 1994 to 1995. Among other honors, he won the 2001 Interscience Conference on Antimicrobial Agents and Chemotherapy Award from the American Society for Microbiology.

David M. Asher, M.D., is chief and supervisory medical officer of the Laboratory of Bacterial, Parasitic and Unconventional Agents in the Division of Emerging and Transfusion-Transmitted Diseases at the U.S. Food and Drug Administration. Previously he was a senior research investigator at the Laboratory of Central Nervous System Studies in the National Institute of Neurological and Disorders and Stroke, National Institutes of Health. Dr. Asher has led and currently leads several national and international efforts to protect the public and animals from TSEs, including the World Health Organization's Biologicals Programme Consultation on TSE Standards and Diagnostics, the Food and Drug Administration's Conference on TSE Diagnostic Tests, and the Food and Drug Administration's Inter-Center TSE Decontamination Working Group. In November 2002, he became a consultant and rapporteur to the World Health Organization Consultation on Vaccines, Blood Products and Tissue in relation to TSEs. He recently developed a technique now used by the Food and Drug Administration to evalu-

ate decontamination regimens for freeing glass, metal, and plastic surfaces of TSE agents. He also has published more than 175 articles, abstracts, and book chapters.

Pierluigi Gambetti, M.D., founded and directs the National Prion Disease Pathology Surveillance Center at Case Western Reserve University in Cleveland. He also is a professor and the director of the division of neuropathology at Case Western Reserve University School of Medicine and University Hospitals of Cleveland. In 1990, he chaired the prion study group of the International Commission on the Taxonomy of Viruses. He now serves on the executive committee of the World Health Organization and the Food and Drug Administration's advisory committee for TSEs. Dr. Gambetti, who has devoted much of his research during the past 4 decades to Alzheimer's disease, also codirects the Alzheimer Disease Research Center of Cleveland. He has authored or coauthored more than 200 papers and 60 books, book chapters, and invited articles.

David A. Harris, M.D., Ph.D., is a professor in the department of cell biology and physiology at the Washington University School of Medicine in St. Louis, Missouri. There he is investigating the conversion of normal prions into their malignant form, the intracellular targeting and processing of prions, and their interactions with other proteins. He is also studying how prions cause the clinical and neuropathological abnormalities associated with disease. Among other honors, Dr. Harris has won two awards from the National Institutes of Health: the Individual National Research Service Award in 1983 and the Clinical Investigator Development Award in 1986. He now serves on the editorial board of the *Journal of Biological Chemistry*.

Stanley B. Prusiner, M.D., is a professor of neurology and the founding director of the Institute for Neurodegenerative Diseases at the University of California, San Francisco. Discovering prions earned him the 1997 Nobel Prize in physiology or medicine. His laboratory currently aims to elucidate the molecular events responsible for the metamorphosis of cellular PrP into PrP^{Sc}, to identify the molecule or molecules that mediate this change, and to uncover the mechanism of prion diversity. Dr. Prusiner has authored or coauthored 10 books and more than 300 journal articles and has won more than 70 awards and honors. Among other activities, he consults for the Food and Drug Administration's Center for Biologics Evaluation and Research and chairs the executive committee of the Agnes Ginges Center for Neurogenetics at Hadassah Medical School and Hospital in Jerusalem. He was a member of the Food and Drug Administration's Transmissible Spongiform Encephalopathies Advisory Committee from 1997 to 2001. In

the commercial sphere, he holds 24 prion-related patents, as well as directorships on the boards of KBC Pharma Inc. and InPro Biotechnology LLC. An elected member of the Institute of Medicine and the National Academy of Sciences, Dr. Prusiner sits on the editorial board of *Proceedings of the National Academy of Sciences*.

Elizabeth S. Williams, D.V.M., Ph.D., is a professor in the department of veterinary science at University of Wyoming and a veterinary pathologist at the Wyoming State Veterinary Laboratory. She characterized chronic wasting disease during her graduate studies in 1980 and has made it a focus of her professional work. Her other research interests include diseases of endangered species, such as the black-footed ferret, and the relationship between wildlife and domestic animal diseases. The Wildlife Disease Association honored her with its Distinguished Service Award in 1996. She has authored or coauthored more than 90 papers and 16 book chapters and has given more than 40 invited presentations. She currently edits the *Journal of Wildlife Diseases*.

Glossary

aptamer: A small piece of DNA or RNA or a small peptide selected from a large pool of random DNA, RNA, or peptides based on the piece's ability to bind to a specific target. Targets may be proteins, many other kinds of small molecules, or even whole microorganisms. An aptamer tagged with an immunological or biochemical marker and bound to a protein can be used to detect that specific protein. If an aptamer binds specifically to PrP^{Sc}, it may have diagnostic or preventive potential. If the bound aptamer interferes with conversion of PrP^{C} to PrP^{Sc}, it may have therapeutic potential.

bovine spongiform encephalopathy (BSE): A *TSE* that primarily affects cattle and that develops when cattle eat feed contaminated with the infectious agent of either *scrapie or BSE*. First identified in 1986 in the United Kingdom, BSE became an epidemic affecting hundreds of thousands of cattle in both the United Kingdom and Europe. It appears that some humans who ate beef or beef products containing the BSE agent have contracted *variant Creutzfeldt-Jakob disease*, identified in 1996. Zoo animals fed beef or feed containing the BSE agent also have developed TSE.

BSE: See *bovine spongiform encephalopathy*.

buffy coat: The layer of white blood cells that lies at the top of the solid portion of centrifuged whole blood.

cervid: A member of the Cervidae family of mammals. Males have solid, deciduous antlers. The only animals known to naturally contract *chronic*

wasting disease are three cervid species: elk (*Cervus elaphus*), mule deer (*Odocoileus hemonius*), and white-tailed deer (*Odocoileus virginianus*).

chaotrope: A substance that can disrupt the structure of water to make nonpolar substances water-soluble and that can denature proteins as a result. Chaotropes are used to study protein folding and the interactions of proteins with other molecules.

chronic wasting disease (CWD): A *TSE* contracted by three North American *cervid* species: white-tailed deer, mule deer, and elk. Identified in the early 1980s; origin unknown. Transmitted among the three species by an unknown mechanism suspected to involve material shed from infected animals (e.g., urine, feces, saliva, hair, etc.). There is no documented evidence that the disease is transmissible to humans, but it has been transmitted experimentally to cattle by intracerebral inoculation. Its name derives from the emaciated appearance of animals in the late stage of this disease.

CJD: See *Creutzfeldt-Jakob disease*

classical Creutzfeldt-Jakob disease: Sometimes used when referring to s*poradic Creutzfeldt-Jakob disease.*

codon: A sequence of three nucleotides on messenger RNA (mRNA) that code for an amino acid or a stop or start signal. The codon number relates to its position in the gene's nucleotide sequence or the position of the amino acid on the encoded protein.

Creutzfeldt-Jakob disease (CJD): A fatal neurodegenerative prion disease in humans that is distinct from *kuru*. Some cases have an infectious or genetic origin, but most are of undetermined cause. There are several related diseases:

- **cCJD:** See *classical Creutzfeldt-Jakob disease.*
- **fCJD:** See *familial Creutzfeldt-Jakob disease.*
- **iCJD:** See *iatrogenic Creutzfeldt-Jakob disease.*
- **nvCJD:** See *new variant Creutzfeldt-Jakob disease.*
- **sCJD:** See *sporadic Creutzfeldt-Jakob disease.*
- **vCJD:** See *variant Creutzfeldt-Jakob disease.*

C-terminus: The carboxyl end of a protein. In *PrP*, the C-terminus is attached to a cell membrane by a glycosyl phosphatidylinositol (GPI) moiety known as the GPI anchor. This anchor is added to the molecule when amino acids 231 through 253, known as the GPI signal sequence, are removed

during the process called transamidation. Two cystine amino acid residues form a tight bond in the C-terminus region. In addition various complex carbohydrates attach to asparagine amino acids in this region.

epitope: A site on a large molecule to which a homologous antibody will bind.

familial Creutzfeldt-Jakob disease (fCJD): A variety of *CJD* in which cases cluster within families and are associated with mutations at one of several loci along the *PRNP* gene sequence. The phenotypic expression of fCJD depends on the mutation. Some forms of fCJD have been transmitted experimentally to animals by intracerebral inoculation, demonstrating that these forms are *prion diseases*.

fatal familial insomnia (FFI): A human *transmissible spongiform encephalopathy (TSE)* due to a mutation in which the amino acid aspartic acid is substituted by the amino acid asparagine at codon 178 on the *PRNP* gene, and this mutation acts in concert with the expression of the amino acid methionine at polymorphic codon 129. This condition results in both neurological signs and autonomic dysfunction, including insomnia.

FFI: See *fatal familial insomnia.*

genotype: With respect to PrP, relates to the composition of the *PRNP* gene.

Gerstmann-Sträussler-Scheinker disease (GSS): A human *TSE* due to genetic mutation involving one of several loci along the *PRNP* gene in concert with the polymorphic expression of methionine or valine at codon 129. Although the clinical characteristics vary depending on the *haplotype,* this condition has a lengthier clinical course than most human TSEs, generally lasting several years.

GSS: see *Gerstmann-Sträussler-Scheinker disease.*

haplotype: In general, refers to two sites, usually in proximity, along the same chromosome strand that are linked in terms of the *phenotype* they produce. In *prion* science, the term refers to the phenotypic effects of a mutation along the *PRNP* gene linked with a designated amino acid expressed by a polymorphic *codon*. For example, the haplotype D178N-129M signifies that at codon 178, one amino acid, aspartic acid (D), is substituted by another, asparagine (N), in combination with methionine (M) expressed at codon 129. A different haplotype would be D178N-129V.

iatrogenic Creutzfeldt-Jakob disease (iCJD): A form of *CJD* that is transmitted through medical mishaps. Known cases of iCJD were caused by the transplantation of *prion*-contaminated human dura mater, injection of prion-contaminated human growth hormone from pituitary extracts, and reuse of surgical instruments and devices contaminated by prior use in a patient with CJD.

ID_{50}: The dose of an infectious agent sufficient to infect 50 percent of exposed animals or people. The size of an ID_{50} of a single infectious agent may vary depending on several factors, including the route of transmission. For example, the ID_{50} of *prions* necessary to cause infection by the oral route would be higher than the ID_{50} delivered directly into the brain by intracerebral inoculation. Since *TSEs* are fatal, the ID_{50} equals the LD_{50} (L means lethal). The ID_{50} would not be equivalent to the LD_{50} if an asymptomatic carrier state existed.

infectious unit (IU): The smallest amount of infectious agent leading to an infection in a single person or animal exposed. The single infectious unit is not necessarily equivalent to a single aggregate of PrP^{Sc} molecules. One IU likely consists of many aggregates of misfolded PrP^{Sc}. It is not known at present how the combination of number and size of aggregates influences infectivity. For example 400 aggregates each containing 1000 PrP^{Sc} molecules (400,000 molecules) may confer more infectivity than 1000 aggregates each containing 500 PrP^{Sc} molecules (500,000 molecules). In addition to considering the physical composition of aggregated *prion* molecules, the amount of prions required to infect an animal or person will vary depending on the route of transmission, the species of origin of the prion, and the genetic susceptibility of the host animal or person. In the absence of the ability to precisely count and measure the size of prions, specified lab animals are used to control the variability mentioned. For example, if several groups of 20 mice each were experimentally exposed to prions by the intracerebral route (the most sensitive route), using a standard volume of material that was diluted 10-fold in each group of 20 mice, the highest dilution titer that causes a single case among the 20 mice contains one IU. The dilution titer that causes 10 of 20 of mice to become infected contains an ID_{50} which is equivalent to the LD_{50}. Consequently, an IU is at least 10-fold less than an ID_{50}, by convention. Several IUs may be required to infect that same mouse strain by the intraperitoneal route and even more by the oral route.

isoform: A protein molecule having the same primary structure (same amino acid sequence) as its counterpart, but a different three-dimensional shape.

isotype: A classification scheme for human *CJD* based on the mobility of fragments of the PrP^{Sc} molecule following cleavage with *proteinase K* using gel electrophoresis and *Western blot* techniques.

IU: See *infectious unit*.

kuru: A *TSE* contracted by members of the Fore Tribe of Papua New Guinea, as well as a Fore word meaning "to tremble," one of the clinical signs of neurological degeneration among affected individuals. The infectious agent incubates for 4 to 40 years. Ethnological and epidemiological studies indicate that the infectious agent was transmitted during endocannibalistic funeral rituals in which women and children ate the brains of deceased relatives and spread the brain tissue on their bodies. Since this ritual was banned in 1957, the number of kuru cases has declined to a handful.

LD_{50}: A dose of an infectious agent that is lethal to 50 percent of the exposed population. See ID_{50}.

new variant Creutzfeldt-Jakob disease (nvCJD): The original name of the human disease caused by the infectious agent of *bovine spongiform encephalopathy (BSE)*. It was described as a "new variant" of a known malady, Creutzfeldt-Jakob disease. The disease is now called simply *variant Creutzfeldt-Jakob disease,* or *vCJD*.

N-terminus: The unattached amino-terminal end of a protein molecule. In *PrP*, the N-terminus contains the signal peptide and five repeating octapeptides, which have preferential binding affinity to copper.

offal: The parts of a butchered animal not processed into human food, generally including blood, internal organs, legs, head, and spinal cord.

phenotype: With respect to *PrP*, relates to the metabolic, physiologic, and physical characteristics, both normal and pathological, exhibited by a host as a result of its *PRNP* gene expression interacting with the environment inside and outside the organism.

polymorphism: Possession of two or more alleles of a gene that code for different amino acids at the same site along a protein sequence. The frequency of the alleles is greater than can be explained by naturally recurrent mutations. Polymorphisms appear to improve a host's resistance to infectious agents and to noxious environmental effects.

prion: An acronym for "proteinaceous infectious particle." All known prions are misfolded isomers of a normal cellular protein coded by the *Prnp* gene. Aggregates of the misfolded protein of sufficient quantity and size are associated with *TSE* infectivity and neurodegenerative diseases in both animals and humans. In mammals, prions are, at the present time, found primarily in nerve cells and lymphoreticular cells. Whether formed endogenously or introduced from an external source, prions accumulate in cells by influencing the normal, cellular *prion protein* to assume the disease-associated *isoform*, which has limited resistance to digestion by *proteinase K*. Most but not all TSE experts favor the so-called protein-only theory, which contends that prions transmit TSE infections without accompanying nucleic acid. The preponderance of evidence suggests that prions may be the infectious agent of TSEs. However, a minority of respected TSE experts believe that the protein-only theory has not been proven beyond question.

prion disease: A fatal, transmissible neurodegenerative disease associated with aggregates of PrP^{Sc}, an abnormally folded *isoform* of the cellular protein *PrP* encoded by the *Prnp* gene. Often used synonymously with the term *transmissible spongiform encephalopathy (TSE)*. See *prion*.

prion protein: The normal *isoform* of a protein found mainly in the body's nerve cells. Its metabolic pathway and physiological function are currently unknown. This protein is sensitive to digestion by *proteinase K*.

prion strain: A *TSE* isolate or source of infection that consistently matches agreed criteria or characteristics. These characteristics have included incubation period and the patterns of distribution and relative severity of the spongiform changes in the brain (the lesion profile). For *scrapie*, strain characterization has been defined mostly in terms of the reproducibility and stability of the disease *phenotype* on serial transmission in a specified inbred mouse genotype. Newer criteria may include molecular conformation of the *prion*.

***PRNP*:** The gene that codes for *prion protein* in humans. All letters are capitalized and in italics.

Prnp: The gene that codes for *prion protein* in mice and other animals. Only the first letter is capitalized but all letters are in italics.

proteinase K: A protease enzyme that is used to cleave proteins in vitro. It is obtained from the fungus *Tritirachium album*, which derives its carbon and nitrogen from keratin, from which the letter *K* derives. Often represented by the abbreviation *PK*.

PrP: *Prion protein*. In the absence of a superscript, it generally refers to the normal cellular prion protein, but in some cases it may refer to either the normal prion protein or its abnormal *isoform*. For example, antibodies to PrP may bind to both PrP^{C} and PrP^{Sc}.

$PrP^{0/0}$: Indicates the inability of the host animal to produce *prion protein*. Usually this refers to experimental knockout mice that have been genetically manipulated to delete the *Prnp* gene from both alleles on the autosomal chromosome that codes for prion protein.

PrP 27–30: The fragment of the *prion* molecule that is most resistant to digestion by *proteinase K*. The numbers correspond to the molecular mass (in kilodaltons) of this prion fragment. See *Western blot*.

PrP^{C}: Normal cellular *prion protein* digestible by *proteinase K*.

PrP^{CWD}: Abnormally folded prion protein associated with a *TSE* called *chronic wasting disease (CWD)*, which is known to occur in elk, mule deer, and white-tailed deer. The disease's name derives from the observation that infected animals at the clinical stage lose muscle mass and appear wasted.

PrP^{res}: Abnormally folded *prion protein* that is highly resistant to *proteinase K* digestion and is strongly associated with *prion disease*. It is sometimes used synonymously with PrP^{Sc}.

PrP^{Sc}: Abnormally folded *prion protein* that has a gradient of resistance to *proteinase K* digestion. It is associated with infectious potential and with *prion disease* even in circumstances where it may be sensitive to *proteinase K* digestion.

PrP^{sen}: P*rion protein* that is sensitive to *proteinase K* digestion. Sometimes used synonymously with PrP^{C}.

rational drug design: The development of a drug based on foreknowledge of the three-dimensional structure and behavior of a specific molecule involved in a disease process. With this knowledge, drug developers can target a specific binding site on the molecule to disrupt, enhance, or redirect its normal activity, thus interrupting the disease process. By contrast, traditional drug development typically begins with a range of potentially therapeutic chemical compounds that are narrowed down to the best candidates through empirical observation of their effects. Applying rational drug design to *transmissible spongiform encephalopathies (TSEs)* would begin with the knowledge of differences between the tertiary structures of *cellular prion*

protein (PrP^C) and its misfolded *isoform (PrP^{Sc})* as well as the identification of one or more *epitopes* on these proteins. Blocking or activating the epitope(s) could potentially disrupt an essential step in TSE pathogenesis, such as the conversion of PrP^C to PrP^{Sc}.

scrapie: A *TSE* of sheep and goats. The TSE was first described by Scottish veterinarians in the 1700s, centuries before prions were first recognized. The modes of transmission are thought to be contact with infected sheep or goats or their placentas, contact with a scrapie-contaminated environment, or oral intake of scrapie agent-contaminated material. The "Sc" in the term PrP^{Sc} refers to scrapie. PrP^{Sc} is used to refer to the abnormal *isoform* of PrP^C associated with TSEs.

species barrier: The genetic, metabolic, physiological, and physical differences among species that result in variable susceptibility to an infectious agent.

sporadic Creutzfeldt-Jakob disease (sCJD): The most common variety of *CJD*. The cause is unknown. sCJD appears to occur worldwide at a rate of approximately one case per 1 million population. Most cases involve older adults.

transgene: A gene from one organism that has been transferred and integrated into the DNA of another organism of the same or different species such that the transferred gene is expressed in the host organism. Investigators conducting *prion* transmission studies use transgenes to convey unnatural molecular characteristics to experimental animals so as to circumvent the *species barrier*. For example, it is easier to transmit *BSE* to a transgenic mouse with a bovine transgene than to a normal mouse. These experiments are further enhanced when the mouse's own *PrP* gene expression is knocked out ($PrP^{0/0}$).

transmissible spongiform encephalopathy (TSE): A general term that refers to all diseases associated with the presence of *prions* in vacuolated central nervous system tissue. Prions from TSE-affected brain tissue are believed to transmit the neurodegenerative disease state from the affected animal to another host. A synonym for *prion disease*.

TSE: See *transmissible spongiform encephalopathy*.

variant Creutzfeldt-Jakob disease (vCJD): A clinical type of *CJD* first identified in 1996 and believed to result from the ingestion of beef products

containing the infectious agent of *bovine spongiform encephalopathy (BSE)*. The majority of vCJD cases occur in young adults.

Western blot: A technique used in molecular biology to detect and identify proteins in a test sample; also known as an immunoblot. A mixture of proteins is embedded in a slab of polyacrylamide gel and subjected to electrophoresis, during which an applied voltage causes the proteins to travel linearly toward the opposite end of the acrylamide slab at rates dependent on each protein's mass (measured in kilodaltons [kDa]) and charge. The pattern on the gel is transferred (blotted) to nitrocellulose paper or a nylon membrane. This paper is then probed with detector antibodies. This protein-antibody complex is then bound with a labeled antiglobulin that visualizes a pattern of dark bands that vary in intensity depending upon the amount of protein in the sample. The test is usually run simultaneously on the same gel and paper with appropriate control specimens.