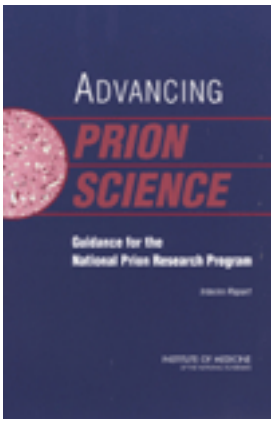

Advancing Prion Science: Guidance for the National Prion Research Program -- Interim Report

Rick Erdtmann and Laura Sivitz, Editors, Committee on Transmissible Spongiform Encephalopathies: Assessment of Relevant Science

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Advancing Prion Science

Guidance for the National Prion Research Program

Interim Report

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Committee on Transmissible Spongiform Encephalopathies:
Assessment of Relevant Science

Medical Follow-Up Agency

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THE 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 hemotoxylin (blue), the magnified tissue manifests microscopic holes (white circles). This report aims to guide scientists beyond histopathology toward new strategies to diagnose prion diseases non-invasively, 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

Richard T. Johnson, Chair, Distinguished Service Professor of Neurology, Microbiology, and Neuroscience, Johns Hopkins University School of Medicine

Harvey J. Alter, Chief of the Infectious Diseases Section and Associate Director for Research, Department of Transfusion Medicine, National Institutes of Health

Dean O. Cliver, Professor of Food Safety, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis

Roger Y. Dodd, Executive Director for Biomedical Safety, American Red Cross Holland Laboratory

Frederick A. Murphy, Professor and Dean Emeritus, Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis

Michael B.A. Oldstone, Professor, Department of Neuropharmacology, Division of Virology, The Scripps Research Institute

David Relman, Associate Professor of Medicine and of Microbiology and Immunology, Stanford University

Raymond P. Roos, Marjorie and Robert E. Straus Professor in Neurological Science, and Chairman, Department of Neurology, University of Chicago Medical Center

David M. Taylor, SEDECON 2000 and retired Senior Scientist, Neuropathogenesis Unit, Institute for Animal Health, Edinburgh

Reed B. Wickner, Chief, Laboratory of Biochemistry and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

Robert G. Will, Professor of Neurology, University of Edinburgh; Director, National Creutzfeldt-Jakob Disease Surveillance Unit; and Consultant Neurologist and Part-Time Senior Lecturer, Department of Neurosciences, Western General Hospital, Edinburgh

Consultants

Adriano Aguzzi, Professor and Associate Dean for Research, Department of Pathology, Institute of Neuropathology, Institute of Neuropathology, University Hospital at Zurich

David M. Asher, Chief, Laboratory of Bacterial, Parasitic, and Unconventional Agents, Division of Emerging and Transfusion Transmitted Diseases, Office of Blood Research and Review, Center for Biologics Research and Evaluation, Food and Drug Administration

Pierluigi Gambetti, Professor and Director, Division of Neuropathology, Case Western Reserve University, and Director, National Prion Disease Pathology Surveillance Center

David A. Harris, Professor, Department of Cell Biology and Physiology, Washington University School of Medicine

Stanley B. Prusiner, Director, Institute for Neurodegenerative Diseases, and Professor of Neurology, University of California, San Francisco

Elizabeth S. Williams, Professor, Department of Veterinary Science, University of Wyoming

Project Staff

Rick Erdtmann, Study Director, Medical Follow-up Agency
Laura B. Sivitz, Research Associate, Medical Follow-up Agency
Reine Y. Homawoo, Project Assistant, Medical Follow-up Agency
Karen Kazmerzak, Research Associate, Medical Follow-up Agency

Auxiliary Staff

Richard N. Miller, Director, Medical Follow-up Agency
Pamela Ramey-McCray, Administrative Assistant, Medical Follow-up Agency
Andrea Cohen, Financial Associate
Mary Poos, Senior Program Officer, Food and Nutrition Board
Tina Rouse, Program Officer, Board on Agriculture and Natural Resources, Division on Earth and Life Sciences

Reviewers

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:

Susan L. Lindquist, Professor of Biology, Massachusetts Institute of Technology, and Director, Whitehead Institute for Biomedical Research

Eugene O. Major, Senior Investigator, Molecular Medicine and Virology Section, Laboratory of Molecular Medicine and Neuroscience, National Institute of Neurological Disorders and Stroke**,** National Institutes of Health

Janice M. Miller, Veterinary Medical Officer**,** National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture

Neal Nathanson, Vice Provost for Research, University of Pennsylvania

Kenrad Nelson, Professor, Department of Medicine, Johns Hopkins School of Medicine, and Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health

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 **Morton N. Swartz,** Chief, Jackson Firm of Medical Service, Department of Medicine, and Chief Emeritus, Division of Infectious Diseases, Massachusetts General Hospital. Appointed by the Institute of Medicine, he was 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

This is the interim report of the Institute of Medicine's (IOM's) Committee on Transmissible Spongiform Encephalopathies: Assessment of Relevant Science, convened to provide advice to the U.S. Department of Defense (DOD) pertaining to the newly established National Prion Research Project (NPRP). Congressional leaders saw the health and economic catastrophes caused by prion diseases elsewhere on the globe and preemptively established a new research effort that would accelerate our national capability to prevent or ameliorate prion diseases. The U.S. Congress asked DOD to administer the program. DOD, in turn, asked IOM to assist it by assembling a group of experts to assess the state of prion science and recommend the areas in which prion disease research was most needed.

We can take great pride in the marvelous accomplishments achieved over the past century through the application of scientific knowledge and technology in combating diseases in humans and animals. Vaccines, improved diets, environmental engineering, and antibiotics, among other techniques and chemoprophylactic agents, have greatly reduced the rates of premature morbidity and mortality. Despite these major achievements, significant new health threats continue to emerge not only in the less well developed nations but in technically advanced societies as well. A perfect example is the topic of this report: prion disease. Prions are believed to be abnormally folded proteins that can replicate by converting adjacent normal prion proteins into the altered conformation associated with disease. This

transformation occurs in the absence of any currently detectable RNA or DNA. Every known animal and human variety of prion disease is transmissible and uniformly fatal.

A prion disease of sheep called scrapie has been clinically recognized for centuries, but a new variety that recently emerged in cows in the United Kingdom has spread to continental Europe and beyond. This bovine spongiform encephalopathy (BSE) resulted in a massive outbreak in the late 1980s and mid-1990s, infecting hundreds of thousands of cattle and causing billions of dollars in economic damage. The catastrophe was further exacerbated in the mid-1990s when it became apparent that human consumption of BSE-tainted food crossed the species barrier, creating a new form of disease in humans called variant Creutzfeldt-Jakob disease (vCJD). Because of the potentially long incubation period of vCJD, the extended period of human exposure to BSE in the United Kingdom, and the absence of information on the infectious dose of BSE for humans, it is not possible to accurately predict an epidemic curve for vCJD. The upper predictions of the numbers of people affected are in the tens of thousands. This has driven an intense effort to ensure that good surveillance systems are in place for animals and humans. Surveillance, in turn, requires that good tests be available to detect the causative agent and to diagnose new cases.

This is the launching point of the interim report. At present, our ability to accurately detect the putative agents that cause prion diseases is limited. In the United States, the Food and Drug Administration has not approved for use a single test that can be used to screen for the abnormal prion protein in people, although the U.S. Department of Agriculture has approved the use of a single test, from Bio-Rad Laboratories, for testing of animals. Moreover, because no validated test that can detect the abnormal prion protein in living human tissues, including blood, is available, persons who have possibly been exposed to BSE are deferred from donating blood on the basis of a theoretical risk.

Tests for the detection of prions are commercially available for postmortem testing of animals in Europe, and one for the detection of chronic wasting disease in mule deer has recently been licensed for use in the United States. In addition, we have in the United States postmortem and experimental antemortem tests for the detection of prions in people and animals. The progress in developing accurate, inexpensive, and sensitive diagnostics, however, has been slow and fraught with technical challenges in need of novel research strategies. A great amount of effort and funding, particularly in Europe, but also in

the United States, has resulted in incremental improvements. Following old patterns of progress may replicate old patterns of disappointment, however. We need strategies to leverage both new technology and new fundamental knowledge.

This IOM report is intended to suggest such strategies. This interim report focuses heavily, although not exclusively, on the research needed to improve diagnostics for prion diseases. The research recommended in this interim report was carefully timed to support the programmatic review by a DOD panel of experts who will determine which of the research proposals submitted most strongly supports DOD's prion research program.

This interim report briefly touches upon prion research infrastructure issues and unique risks from transmissible spongiform encephalopathies to the military. These areas and others, as noted in the committee's task statement, will be developed in even greater depth in the final report.

Richard T. Johnson, M.D.
Chair

Acknowledgments

The Committee on Transmissible Spongiform Encephalopathies thanks the many people who contributed to this interim report. We first thank the report's sponsor, 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 the expert technical advice that our six standing consultants have provided throughout the study. Their willingness to travel long distances to committee meetings without remuneration reflects their professionalism and their dedication to advancing prion science. In addition, one of the consultants, 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.

We also extend our appreciation to the many invited guests who attended our meetings to share their expertise through both formal presentations and participation in committee discussions. They

provided a significant body of information for us to draw upon as we formulated the recommendations in this report.

In addition, we thank our chair, Dr. Richard Johnson, for planning meetings with the study staff and for his effective guidance and direction.

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, Jennifer Bitticks' guidance on report production, and Michael Hayes' exceptionally detailed and thoughtful copyediting.

Finally, we thank our dedicated staff at the IOM. TSE Study Director Rick Erdtmann, Research Associate Laura Sivitz, and Project Assistant Reine Homawoo have done an outstanding job planning the committee meetings, providing us with background literature, keeping the study on track and editing this report. Their efforts were particularly impressive because this is the first IOM study they have facilitated.

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Abbreviations and Acronyms

AAFES	Army and Air Force Exchange Service
ASBP	Armed Services Blood Program
BSE	bovine spongiform encephalopathy
CDMRP	Congressionally Directed Medical Research Program
CJD	Creutzfeldt-Jakob disease
CNS	central nervous system
CSF	cerebrospinal fluid
CT	computed tomography
CWD	chronic wasting disease
DOD	U.S. Department of Defense
EEG	electroencephalography
ELISA	enzyme-linked immunosorbent assay
FCS	fluorescent correlation spectroscopy
FDA	Food and Drug Administration
FLAIR	fluid attenuated inversion recovery
GAO	General Accounting Office
ID_{50}	a dose that infects 50 percent of the population exposed to the infectious agent
IHC	immunohistochemistry
IOM	Institute of Medicine
IU	infectious unit
kDa	kilodaltons
LCGE	laser-assisted capillary gap electrophoresis
MRI	magnetic resonance imaging

MRMC	Medical Research and Materiel Command, U.S. Army
MUFS	multispectral ultraviolet fluorescence spectroscopy
NaPTA	sodium phosphotungstate
NIH	National Institutes of Health
NMR	nuclear magnetic resonance
NPRP	National Prion Research Program (DOD) and National Prion Research Project (congressional language)
nvCJD	new-variant Creutzfeldt-Jakob disease
PCR	polymerase chain reaction
PK	proteinase K, an enzyme that digests cellular PrP
PRNP	prion protein gene in humans
Prnp	prion protein gene in mice
PrP	prion protein
PrP^{C}	protease-sensitive cellular protein
PrP^{res}	protease-resistant protein associated with prion disease
PrP^{Sc}	protease-resistant protein associated with prion disease
RIA	radioimmunoassay
sCJD	sporadic Creutzfeldt-Jakob disease
TSE	transmissible spongiform encephalopathy
USAMRMC	U.S. Army Medical Research and Materiel Command
vCJD	variant Creutzfeldt-Jakob disease
WHO	World Health Organization

Executive Summary

HISTORICAL AND MEDICAL CONTEXT

Two New Prion Diseases Arise in the 1980s

The 1985 outbreak of mad cow disease in the United Kingdom generated global awareness of a previously obscure set of neurodegenerative diseases called transmissible spongiform encephalopathies (TSEs) (Table 2-1). Unlike all other known infectious diseases, the infectious agent of TSEs 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, ending in death within a period of months.

Mad cow disease, or bovine spongiform encephalopathy (BSE), became an epidemic that affected hundreds of thousands of animals in the United Kingdom and that severely harmed the country's cattle farmers and beef industry. Cases of BSE have also been reported in Europe, Israel, Japan, and elsewhere.

Human consumption of BSE-infected beef products gave rise to a fatal, human neurodegenerative disease called variant Creutzfeldt-Jakob disease (vCJD), which was identified in 1996 (Will et al., 1996). There were 129 definite or probable vCJD cases in the United Kingdom as of December 2, 2002 (Department of Health, United Kingdom, 2002), and a handful of cases in other countries. Estimates of the total number of people who will contract vCJD as a result of the BSE epidemic vary from hundreds to tens of thousands depending on

assumptions regarding the incubation period, individual susceptibility, and the level of exposure. The incubation period for another human prion disease, kuru, was 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, 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 poured millions of euros into research to develop better diagnostics for TSEs, especially BSE, with modest success. Some commercial diagnostic tests for postmortem BSE detection have been developed and are used throughout the United Kingdom and Europe. The tests cannot detect prions present at low levels, however. The lack of highly sensitive, accurate, and rapid tests has led to controls such 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 not received a request from any of the European companies that manufacture BSE-screening tests to approve them for human use in the United States, nor has any company based in the United States submitted any TSE screening test to FDA for approval for human use (Personal communication, D.M. Asher, FDA, July 18, 2002). However, the U.S. Department of Agriculture's Center for Veterinary Biologics has approved the use of one test produced by Bio-Rad Laboratories for the detection of chronic wasting disease (CWD) in mule deer.

Congress Creates the National Prion Research Project

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 (Senate Committee on Appropriations, 2001). NPRP will fund 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, and the department delegated it to the Army's Medical Research and Material 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.

To complement this rigorous research management process, MRMC requested that the Institute of Medicine (IOM) assist it (Department of Army Contract DAMD17-02-C-0094, May 2002). IOM was asked to produce a report that would advise the integration panel on the most pressing areas where TSE research is needed. This report would help guide the integration panel to recommend the highest-priority research for funding.

STUDY PROCESS AND INFORMATION SOURCES

In June 2002 IOM formed an 11-member committee supplemented by six consultants who are internationally recognized experts in prion research. The committee members were selected for their expertise in infectious disease, prion molecular biology, microbiology, neurology, epidemiology, blood banking, veterinary medicine, and food safety. The consultants provided essential technical insight.

The Committee on Transmissible Spongiform Encephalopathies: Assessment of Relevant Science and its panel of consultants convened three times between July and October 2002. The committee was charged with evaluating the state of prion science, especially as it relates to research needed in diagnostics. The members of the committee were asked to look at 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, with respect to their blood supply, or in any other way; and provide advice on public health policies or surveillance programs that require new research or that might affect the military. Finally, they were asked to recommend additional research to reduce or prevent TSEs (Box 1-1, p. 18).

The committee evaluated information from the sponsor, peer-reviewed journal articles provided by committee staff, and presentations by invited guests with expertise relevant to TSE diagnostics (Appendix).

As part of the contract, MRMC asked the committee to produce an interim report advising the integration panel on the most promising avenues of research for developing antemortem TSE diagnostic tests. That report, presented here, is intended to help the integration panel

prioritize submitted research proposals that pass peer review. Table ES-1, on pages 13 and 14, summarizes the committee's recommendations.

PRIONS

The protein playing a critical role in prion disease is called prion protein or PrP. It is encoded by the PRNP gene on chromosome 20 in humans. Like all proteins, PrP has a characteristic conformation, but under certain conditions it folds into an abnormal shape that causes fatal neurodegeneration after a long incubation period. In this report, the terms "prion" and "PrP^{Sc}" refer to the protease-resistant protein associated with prion disease.[1]

TSE DIAGNOSTICS

Obstacles to Developing Antemortem Diagnostics for TSEs

Conventional methods used to diagnose most infectious diseases, such as malaria, tuberculosis, hepatitis, and human immunodeficiency virus, fail to detect prion diseases for numerous reasons. 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. Since it lacks DNA and RNA, it cannot be identified by molecular methods such as polymerase chain reaction and other nucleic acid-based tests, nor can prions be identified by customary methods such as direct visualization under a light microscope, cultivation in the laboratory, or detection of specific antibodies or antigens by standard immunology methods. Prions are insoluble, distributed unevenly in body tissues, and found in a limited set of tissues by currently available tests. PrP^{Sc} is neurotropic, so ultimately it affects cells of nervous system tissues. 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.

[1] At times an additional term, PrP^{res}, is used synonymously with PrP^{Sc}. PrP^{res} is abnormally folded prion protein that is highly resistant to digestion by the enzyme proteinase K (PK) and that is strongly associated with prion disease. However, unlike PrP^{res}, 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.

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 strategy so far has been to mix the test material with the proteinase K enzyme, which digests normal prion protein but only a portion of the abnormally folded protein. Then, various techniques, described below, detect the residual PrP^{Sc} after digestion. This process inadvertently reduces the small amount of original PrP^{Sc} captured, making it less sensitive than experimental methods that do not rely on proteinase K 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. The tests must not only differentiate between normal and abnormal prion proteins, but also, for some purposes, discriminate 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. This then introduces the ultimate objective of a prion detection test: find a single infectious unit while avoiding a falsely 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 can be found. Standard histopathological and immunohistochemical techniques are used to view the tissue microscopically to see characteristic vacuoles, plaques, or other abnormal features and staining associated with prion diseases. The standard confirmatory test is the Western blot.

Attempts to develop accurate, rapid, and highly sensitive antemortem tests to date have largely failed, especially for detecting prions early in the course of infection. Also, most tests still involve proteinase K digestion. The specificities and sensitivities of tests that do not use proteinase K digestion must be demonstrated further. Newer tests have seemingly improved the limits of detection, but it will take improvements in sensitivity of several orders of magnitude to reliably detect an infectious unit of the prion particle. To date, all these newer detection methods are experimental and have not been independently verified and reported. New testing methods are critically needed. Researchers have attempted a variety of novel ways to improve the sensitivities of tests for TSEs (Table 3-4, p. 54).

Novel Approaches Will Achieve NPRP's Goals in Diagnostics

The committee concluded that it will take breakthroughs to achieve the levels of sensitivity and specificity needed to detect prions in live animal and human tissues. The integration panel should support proposals for research to develop assays capable of radically superceding the quality of existing tests, which are described in Chapter 3.

Recommendation: Focus funding for new assays on the proposals 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 IU of PrP^{Sc} per unit of ultimate product used (e.g., 1 liter of blood or 100 grams of beef).

Major improvements must await the availability of more novel testing techniques or reagents. These reagents include new types of antibodies, peptides, nucleic acids, synthetic derivatives, and chimeric molecules that can be designed to specifically target the PrP^{Sc} molecule.

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

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 perhaps 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 that use such surrogate markers. This strategy is successfully being used for the detection of certain cancers (Petricoin et al., 2002a,b), and the committee suggests that it be applied to TSEs.

Recommendation: Identify surrogate markers or signatures for the detection of prions or prion diseases.

The committee also sees promise in strategies for amplification of the 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.

Recommendation: Improve in vitro techniques that amplify small amounts of PrPSc to enhance the sensitivities of diagnostic tests.

Cell Culture Assays

In vitro culture systems have been used for prion detection with moderate success. Yet, the committee believes that these assays would hold great promise if a stable and robust cell culture assay were developed. Their speed and biological simplicity would make them very effective for testing for TSEs. The committee encourages NPRP to support the development of a cell culture assay.

Recommendation: 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.

Multiphoton microscopy uses near-infrared light, which penetrates more deeply than visible or ultraviolet light and which permits imaging of microscopic structures within the cortex of the living animal at an extraordinarily high resolution with no apparent deleterious effects. It has been used to characterize the natural history of senile plaques and to evaluate antiplaque therapy in mouse models of Alzheimer's disease (Bacskai et al., 2001, 2002; Christie et al., 2001; Klunk et al., 2002). Similar studies could be performed with transgenic mouse models of prion disease to characterize the progression of PrP^{Sc} accumulation and localization by repeatedly imaging the same diseased brain region over time.

Recommendation: 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.

BASIC RESEARCH IS ESSENTIAL TO DEVELOPING TSE DIAGNOSTICS

The committee has 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} (Chapter 4, pp. 71-78).

Recommendation: Fund basic research to elucidate the structural features of prions, the molecular mechanisms of prion replication, the mechanisms of TSE pathogenesis, the epidemiology and natural history of TSEs, and the physiologic function of PrP^{C}.

The committee believes that basic research in these five areas will supply the knowledge required to advance TSE diagnostics more quickly than applied research alone. Boxes 4-1 through 4-5 list the unanswered questions that the committee finds relevant to the development of diagnostic tools.

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.

It is believed that both the conversion of PrP^{C} to PrP^{Sc} and the accumulation of prions depend on the help of one or more molecules (Caughey, 2001), which 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 mysteries about the pathogenesis of prion disease prevent better characterization of diagnostic targets and strategies. Explanations for those mysteries will result in tests with greater sensitivities and specificities. In addition, isolating the multiprotein complexes that

contain prions might identify new cofactors important to the formation and stabilization of PrP^{Sc} and infectivity.

Understanding the normal role of PrP^{C} may also reveal associated molecules and pathways that are appropriate detection targets for TSE diagnostics. Investigators must clarify 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.

The committee concluded that focusing too much on applied, rather than basic, research accounts for the European Union's slow progress in TSE diagnostics.

RESEARCH INFRASTRUCTURE

The United States Must Improve Its Prion 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. prion research infrastructure, although of high quality, is small compared with the size of its European counterpart. Fewer than 20 principal investigators in the United States receive the full, annual allocation of funds from the National Institutes of Health (NIH) to conduct prion research. In fiscal year 2000, NIH spent only 0.16 percent ($23.86 million) of its $14.69 billion research budget on TSE research (Johnson, 2002; Kirschstein, 2001). Furthermore, 75 percent of the funds given out 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. They include the lack of available laboratory space and the high start-up costs associated with setting up a prion research laboratory. The 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. In addition, it often takes years to reach the 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.

For these reasons, the committee determined that programs funding TSE research should attract and train more investigators and should expand the granting periods for investigators conducting bioassay research to 5 to 7 years. The committee also encourages NPRP to provide funds to increase the capacities of animal facilities and containment laboratories (biosafety levels 2 and 3) for research on prions and TSEs.

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 prion laboratory has needed to produce its own reagents and materials. Consequently, standardization of these reagents between different laboratories or even at 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. Specifically, the NPRP should support the establishment of a collection of reference materials and genetically engineered animals (including transgenic mice), as well as reference repositories, useful for the development of TSE diagnostics and for TSE research. All investigators involved in prion research should have access to this collection. The committee believes it would be reasonable to include the collection in existing, high-quality repositories for similar standardized reagents.

The committee also recommends that the Food and Drug Administration (FDA) develop a standard set of panels of reagents that would be useful for validating the accuracies of new PrP detection tests. 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 leverage research opportunities to work with European investigators. The committee believes that 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 are not only feasible but also highly desirable.

THE RISK OF TSEs TO THE U.S. MILITARY

The committee considered the risk of prion infection for members of the U.S. military and their families who are or have been deployed to Europe. The deliberation focused mainly on the possibilities of the consumption of beef products or the 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 (PXs) in Europe were procured from suppliers in the United Kingdom and continental Europe that later reported cases of BSE among their livestock. Some therapeutic blood is also obtained from a host nation's medical facilities in exceptional circumstances.

The committee determined that the U.S. military was 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. The committee recommends that existing passive surveillance systems be used to monitor the incidence of Creutzfeldt-Jakob disease (CJD) among members of the U.S. military and veterans receiving health care through the DOD and the U.S. Department of Veterans Affairs health systems.

Research on TSEs in Blood

The committee also concluded that more research is needed to determine the risks of acquiring vCJD and sporadic Creutzfeldt-Jakob disease (sCJD) from blood products. This risk was judged to be small but unknown. Since accurate antemortem tests for the detection of prions in humans are not available, conservative health policies have been established. Because experimental studies have demonstrated that the blood of animals can transmit prion disease by blood transfusion (Hunter et al., 2002), caution dictates that individuals who have been exposed to BSE-tainted beef products be prevented from donating blood or organs. This has created hardship for blood-servicing organizations in the United States and abroad as well as anxiety in people who have been deferred from donating blood.

The committee recommends that the amount of prions in the blood of individuals with sCJD and vCJD be determined. Numerous

experimental studies with animals indicate that that prions theoretically could be present in the blood of humans who have sCJD or vCJD, although there is no evidence that the blood of TSE-infected people contains prions. The committee also recommends that NPRP fund research to estimate the amount of PrP^{Sc} that corresponds to 1 ID_{50} (a dose that infects 50 percent of the population exposed to the agent) of sCJD and vCJD in human blood. Knowing both the titer of prions in human blood and the estimated size of 1 ID_{50} would enable the determination of whether a blood product could transmit the TSE agent to another person.

Ultimately, it would be desirable to have a blood test whose PrP^{Sc} detection level was at or below the level needed to infect another person by use of a blood product. Individuals who are now deferred from donating blood simply because of their possible contact with BSE-tainted beef products might then be able to rejoin the donor pool.

THE RISK OF BSE IN THE UNITED STATES

Despite some assurance from a study commissioned by the U.S. Department of Agriculture that concluded that the United States is at very low risk of a BSE epidemic (Harvard Center for Risk Analysis, Harvard School of Public Health, Center for Computational Epidemiology, College of Veterinary Medicine, Tuskegee University, 2001), a recent General Accounting Office report cautioned that existing vulnerabilities could allow BSE to occur (GAO, 2002). This justifies careful attention and heightened efforts to control TSEs in the United States.

CONCLUSION

The recommendations in this report should equip the programmatic review panel of NPRP to prioritize the prion research proposals of scientific merit submitted in 2002. The final report of the IOM Committee on Transmissible Spongiform Encephalopathies: Assessment of Relevant Science will expand upon this report, addressing all of the committee's tasks in detail.

TABLE ES-1 Committee Recommendations

Recommendation	Chapter
Improving Diagnostics	
➢ Focus funding for new assays on the proposals 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 IU of PrP^{Sc} per unit of ultimate product used (e.g., 1 liter of blood or 100 grams of beef).	4
➢ Improve in vitro techniques that amplify small amounts of PrP^{Sc} to enhance the sensitivities of diagnostic tests.	4
➢ Develop novel methods and reagents that detect or bind to prions, including new antibodies, peptides, nucleic acids, synthetic derivatives, and chimeric molecules. This may lead not only to better diagnostics, but also to therapeutic and prophylactic strategies.	4
➢ Identify surrogate markers or signatures for the detection of prions or prion diseases.	4
➢ Improve techniques for propagating prions in cultured cells and develop new in vitro cell systems as a means to assay and study prions.	4
➢ 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.	4
Basic Research	
➢ Fund basic research to elucidate: • the structural features of prions • the molecular mechanisms of prion replication • the mechanisms of TSE pathogenesis • the epidemiology and natural history of TSEs • the physiologic function of PrP^{C}	4

TABLE ES-1 Continued

Recommendation	Chapter
Prion Research Infrastructure	
➢ Support programs that 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.	5
➢ Provide funds to increase the capacities of animal facilities and containment laboratories (biosafety levels 2 and 3) to conduct prion research.	5
➢ Provide funding for collaborative research and training with European investigators and facilities that provide unique opportunities for prion research.	5
➢ Establish a collection of reference materials and genetically engineered animals (including transgenic mice), as well as reference repositories, useful for the development of TSE diagnostics and for TSE research. All investigators involved in prion research must have access to this collection. It would be reasonable to include the collection in existing, high-quality repositories with similar standardized reagents.	5
➢ The Food and Drug Administration should have panels of reference reagents available to evaluate the performance characteristics of tests to detect the prion protein and infectivity.	5
Risks to the U.S. Military	
➢ 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 U.S. Department of Defense and the U.S. Department of Veterans Affairs health systems.	6
➢ Determine the amount of sCJD and vCJD prions in human blood and estimate the amount of PrP^{Sc} corresponding to one ID_{50} of sCJD and vCJD prions in human blood.	6

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1

Introduction

This chapter provides the context of the Institute of Medicine's (IOM's) role in support of the National Prion Research Project (NPRP). The 107th U.S. Congress established NPRP (Senate Committee on Appropriations, 2001), directing the U.S. Army Medical Research and Materiel Command (USAMRMC) to administer the program and allocating $50 million to fund it (Congress actually allocated $42.5 million). USAMRMC manages NPRP through the preexisting Congressionally Directed Medical Research Program.

DOD asked IOM to provide independent advice on the state of prion science and the field's most pressing research needs (Box 1-1). In June 2002, IOM formed an 11-member committee supplemented by six consultants who are internationally recognized experts in prion research. The committee members were selected for their expertise in 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 TSE diagnostics (Appendix).

The contract for this study, which lasts from May 1, 2002, until September 30, 2003, requests an interim report from IOM on or before January 15, 2003, to help guide the panel evaluating the programmatic merit of prion research proposals submitted to NPRP. This is that report.

NPRP issued a call for research proposals in August 2002 (DOD, 2002). To evaluate the proposals submitted, it is using the two-tiered approach that IOM recommended in 1993 (IOM, 1993). Proposals first undergo peer review for scientific merit. Those that pass that review then undergo another level of review evaluating how well the proposed research would support NPRP's objectives. Subject-matter experts, clinicians, and consumers chosen by DOD will conduct the programmatic review (DOD, 2002). This report is designed to guide them.

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 (TSE) and advise the Medical Research and Materiel Command (MRMC) and its Congressionally Directed Medical Research Program (CDMRP) 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.

The IOM committee's recommendations suggesting areas of prion research deemed most critical could be useful to the integration panel at this point in the research review process. The final committee report, which will be delivered in the fall of 2003, could also have great utility because it could help shape future DOD program objectives and research announcements if funding is continued.

This interim report concentrates on TSE diagnostics, 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—will support investigator-initiated research designed to do the following:

1. develop a rapid, sensitive, and reproducible test for the detection of prions suitable for use as an antemortem diagnostic test;
2. develop a rapid, sensitive, and reproducible test for the detection of prions suitable for use as a screening assay; and
3. study the prevention, transmission, inactivation, or pathogenesis of transmissible spongiform encephalopathies (TSEs), including chronic wasting disease (Table 1-1).

This report recommends research priorities in these three areas. The committee's final report will have a broader focus and will address each of the committee's subtasks in greater depth and detail.

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.

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2

Prion Diseases and Their Challenges

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 transmissible spongiform encephalopathies (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 sponge-like appearance at the microscopic level, hence the term "spongiform encephalopathy."

Ethnological and epidemiological studies indicated that kuru was transmitted during an endocannibalistic[1] 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

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

figures in recent years (Huillard d'Aignaux et al., 2002; Klitzman et al., 1984).

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 (Hadlow, 1959). He pointed out in a 1959 letter to *The Lancet* that the brains of people 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" (p. 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 was 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" (p. 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 scrapie. 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.

Similarities in the neuropathology of kuru and a rare, fatal condition called Creutzfeldt-Jakob disease (CJD) led investigators to attempt experimental transmission of CJD to nonhuman primates. They successfully transmitted the disease 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. Many hypotheses on the nature of the agent 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.

In 1982, neurologist Stanley Prusiner asserted that the infectious agent in 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, *pro*teinaceous *in*fectious particles that are resistant to inactivation by most procedures that modify nucleic acids" (Prusiner, 1982, p. 141).

At the time, replication of microorganisms and viruses was thought to require nucleic acids. Several investigators had 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 other investigator had provided compelling data to support his hypothesis.

Applying advanced biochemical techniques, Prusiner generated a purified infectious scrapie preparation that yielded a peptide fragment. By determining the nucleic acid sequence that encoded the peptide, he located the gene where the sequence for the peptide was embedded, allowing him to decipher the full-length protein PrP (the prion protein) (Brown and Bradley, 1998). He then demonstrated that the scrapie agent resisted six different procedures known to attack nucleic acids and was susceptible to six methods of protein inactivation (Prusiner, 1982). (It was later established that prions and PrP^{Sc} [a protease-resistant protein associated with prion disease] were resistant to limited digestion by one of those methods, proteinase K digestion.) Like some investigators whose theoretical work preceded him, Prusiner correctly suggested that a prion might act as "an inducer or template for its own synthesis" (Prusiner, 1982, p. 139).

Two decades of research have borne out the prion hypothesis, leading most TSE experts to accept this theory. Prusiner won the Nobel Prize in Physiology or Medicine in 1997 for his groundbreaking work. But because Koch's postulates[2] have not been demonstrated for prions,

[2] Koch's postulates: 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 organ-

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 DISEASES

The protein that plays a critical role in prion disease is called PrP and is encoded by the gene PRNP on chromosome 20 in humans. Like all proteins, PrP has a characteristic conformation, but under certain conditions it folds into an abnormal shape that is associated with fatal neurodegeneration after a long incubation period. In this report, the terms "prion" and "PrP^{Sc}" refer to the protease-resistant protein associated with prion disease.[3]

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

Unlike PrP^{C}, the aberrantly folded PrP^{Sc} is aggregated and insoluble, resists complete digestion by proteinase K, and contains a higher proportion of flat peptide regions called β-sheets (Prusiner, 2001). To date it appears that the immune system does not recognize and destroy PrP^{Sc}, presumably because it has the same primary structure[4] as the normal isoform, despite its distinct conformation. This lack of an immune response makes it difficult to develop diagnostic tools based on antibodies, since there is no confirmed[5] antibody that binds exclusively to PrP^{Sc}.

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ism 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, p. 19)

[3] PrP^{res} is abnormally folded prion protein that is highly resistant to proteinase K digestion and that is strongly associated with prion disease. It is sometimes used synonymously with PrP^{Sc}. However, unlike PrP^{res}, PrP^{Sc} demonstrates a gradient of resistance to proteinase K. PrP^{Sc} is associated with infectious potential and with prion disease even in circumstances where it may be sensitive to proteinase K digestion.

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

[5] Korth reported in 1997 that he had identified a monoclonal antibody that bound to PrP^{Sc} but not to PrP^{C} (Korth, 1997). His results have not been validated by other investigators to date.

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	1999
Familial Creutzfeldt-Jakob disease		Genetic	1924[a]
Fatal familial insomnia (FFI)		Genetic	1986[b]
Gerstmann-Straussler-Scheinker disease		Genetic	1936[c]
Kuru		Exposure to contaminated human tissues during endocannibalistic rituals	1957
Iatrogenic Creutzfeldt-Jakob disease		CJD-infected surgical equipment or tissue transplants	1974[d]
Variant Creutzfeldt-Jakob disease (vCJD)		Eating BSE-infected tissue; other modes[e]	1996

TABLE 2-1 Classification of TSEs (continued)

Type of TSE	Affected Mammals	Modes of Natural Transmission	Date First Recognized
In animals			
Scrapie	Sheep, goats	Contact with infected placenta; possibly oral exposure to environmental contamination	18th century
Transmissible mink encephalopathy	Mink	Eating infected tissue	1947
Chronic wasting disease	Deer, elk	Unknown; likely oral	1967
Bovine spongiform encephalopathy (BSE)	Cattle	Eating TSE-infected tissue	1986
	Nyala, gemsbok, Arabian oryx, eland, kudu, scimtar-horned oryx, puma, cheetah, ocelot, tiger[f]	Eating BSE-infected tissue	Mid-1980s
	Domestic cat[g]	Eating BSE-infected tissue	1990

[a] Gambetti et al. (1999).
[b] Lugaresi et al. (1986).
[c] Kretzschmar et al. (1991).
[d] Duffy et al. (1974).
[e] It is unknown whether the disease is transmissible by transfusion or transplantation.
[f] The TSE affecting these animals was called "exotic ungulate encephalopathy" until it was discovered to be BSE. These animals living in zoos became infected by eating BSE-contaminated feed.
[g] The TSE affecting domestic cats was called "feline spongiform encephalopathy" until it was discovered to be BSE. The animals became infected by eating BSE-contaminated feed.
SOURCES: Godon and Honstead (1998), Johnson and Gibbs (1998), Haywood (1997), Prusiner (1995) and E. Williams, University of Wyoming, December 2002.

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). By a poorly understood mechanism, prions convert PrP^{C} into the abnormally folded conformation containing β-sheets. 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 pathogenic prions (Bueler et al., 1993). It is widely hypothesized that one or more so-called chaperone molecules also play an indispensable role in the conversion of PrP^{C} to PrP^{Sc} (Chernoff et al., 1995; Telling et al., 1995).

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). Prions multiply in the lymphoreticular system, but this activity is not essential for neuroinvasion. The misfolded proteins aggregate into rod-shaped fibrils, and 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, but inevitably, it seems to lead to death. Both the incubation period 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 observations 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 Fraser identified two different alleles called *sinc* (strain incubation) genes in inbred mice 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 (TME) in hamsters showed differences in clinical presentation and glycoform patterns by prion strain (Bessen and Marsh, 1992).

More 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 bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD) (Bruce et al., 1997); (Scott et al., 1999). There are about 20

phenotypically distinct, mouse-passaged strains of scrapie alone (Haywood, 1997).

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

THE EPIDEMIC OF BOVINE SPONGIFORM ENCEPHALOPATHY 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, rapidly progressive dementia, and death (Wells et al., 1987; Wilesmith et al., 1988). Neuropathological examinations 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 they named it "bovine spongiform encephalopathy" (BSE) (Wells et al., 1987). The outbreak appeared mainly in dairy cows rather than beef cattle (Anderson et al., 1996) because dairy cows much more commonly consumed meat and bonemeal that was the attributed source for BSE transmission.

BSE quickly ballooned into an epidemic that peaked at more than 37,000 annual cases in the United Kingdom in 1992 (Figure 2-1) (Department for Environment, Food and Rural Affairs, United Kingdom, 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, in 1990 the United Kingdom increased epidemiological surveillance of a rare, human spongiform encephalopathy called CJD. It was thought that changes in the pattern of CJD could signify a link to

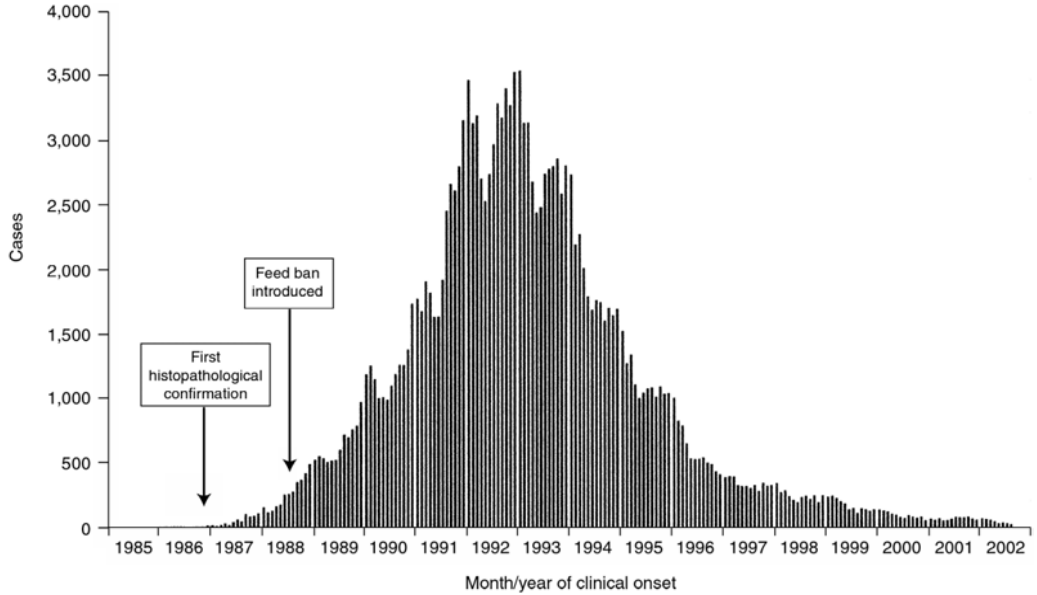

FIGURE 2-1 Confirmed cases of BSE by month and year of clinical onset.
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Online: http://www.defra.gov.uk/animalh/bse/bse-statistics/graphs/epidem.pdf

BSE. The neuropathological profiles and age distribution of 10 of the 207 patients with CJD examined between 1990 and 1997 differed markedly from those that are 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.

Glycosylation studies revealed that the new variant, called vCJD, involved the same prion strain involved in BSE (Collinge et al., 1996), and transmission studies with inbred mice confirmed this (Bruce et al., 1997; Collinge et al., 1996).

There is evidence of genetic susceptibility to vCJD. A 1997 study found that a specific genotype of codon 129 in PRNP 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). However, it is possible that individuals with the other genotypes are susceptible but that the incubation periods in these individuals 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.

The fear of vCJD devastated the United Kingdom's beef industry. Hundreds of thousands of cattle have died of BSE or have been culled. Many other countries worldwide unknowingly imported BSE-infected cattle and contaminated meat and bonemeal from the United Kingdom and have also suffered outbreaks of BSE, public panic, financial losses, and political repercussions.

The Risk of BSE in the United States

Two recent reports suggest that the federal government should strengthen its policies designed to avert BSE and vCJD , although the first study concluded that the risks of a BSE outbreak in the United States are minimal (GAO, 2002; Harvard Center for Risk Analysis, Harvard School of Public Health, Center for Computational Epidemiology, College of Veterinary Medicine, Tuskegee University, 2001).

The 2002 report by the General Accounting Office (GAO) identified a number of holes in Food and Drug Administration (FDA) and U.S. Department of Agriculture (USDA) policies that could allow BSE to slip into the United States without prompt detection, spread to other cattle, contaminate meat in the food supply, and be consumed. The report asserts that the United States lacks sufficient capacity to

inspect all cattle imports and that mandated animal testing excludes some high-risk animals: those that die on farms. FDA does not know the full extent of industry compliance because its inspection data are "severely flawed," according to GAO (p. 3). Moreover, GAO asserts that FDA has been slow or has neglected to enforce the animal feed ban even in firms found to be noncompliant. Finally, cattle brains and other central nervous system tissue may be sold as human food without being labeled as such.

In a report commissioned by USDA, researchers at the Harvard School of Public Health and Tuskegee University determined that USDA and FDA policies prevent BSE from taking root in the United States. These have included a ban on the import of live ruminants and ruminant meat and bonemeal from the United Kingdom since 1989 and from all of Europe since 1997, as well as a 1997 ban to prevent the recycling of potentially infectious cattle tissues by prohibiting the use of cattle tissue as animal feed. There is a 20 percent chance that 173 of the 334 cattle imported from the United Kingdom before 1989 exposed U.S. cattle to BSE, the Harvard study reported, and those cases were probably so few in number that they fell under the radar of U.S. disease surveillance. (The committee notes that there is no evidence that BSE is a contagious disease and that the greatest risk would have occurred if the carcasses of any of these animals had been used to manufacture meat and bonemeal.) However, even if U.S. cattle had been exposed to the BSE agent, present policies would keep the disease from becoming established in U.S. cattle herds, the study concluded, making the risk of vCJD in the U.S. human population extremely low.

THE SPREAD OF CHRONIC WASTING DISEASE IN THE UNITED STATES

Although the BSE epidemic that struck Europe has spared the United States so far, another TSE called 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 and died of emaciation, secondary complications, or euthanasia within 2 weeks to 8 months after the onset of clinical symptoms. The nature of these signs led biologists to name the illness "chronic wasting disease."

The most striking and consistent pathological features of CWD 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 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 related species either directly after exposure or indirectly from the pasture occupied by an infected 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 about how CWD spreads and whether it can cause disease in humans and cattle has lent urgency to research on CWD and the development of tools to detect prions in humans, animals, and the environment.

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 geographical 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 in the wild and on game farms that have existed for a decade or two—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 unclear how the disease arose in the first place.

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

Unique Challenges in Conducting Prion Research

Much about prion diseases remains unclear: how prions replicate, why they target neurons, and how prions kill neurons are a few examples. Prion 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 molecule, precluding the use of many tools designed for studying infectious diseases. 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 that are expensive to construct, and the United States has few such facilities for prion research. In addition, standardized reagents are hard to come by: there is only one U.S.-based repository for vCJD tissue and no repository for other reagents or transgenic animals. Until recently, the federal government's limited interest in prion diseases meant that it was relatively difficult to win research grants, 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. 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 reaching this important goal.

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3

Diagnostics for Transmissible Spongiform Encephalopathies

This chapter addresses diagnostics for transmissible spongiform encephalopathies (TSEs), the fundamental focus of this interim report. In the case of animals, the ability to diagnose or detect an infection drives food safety interventions, which can prevent the introduction of tainted 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 can 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 human immunodeficiency virus, can be diagnosed by conventional methods. This is not the case with TSEs. 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 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 stymie detection. They are 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) is neurotropic, so ultimately, it affects cells of the nervous system tissues. However, where and how PrP^{Sc} progresses through the body prior to its final assault on the nervous system is largely unclear, 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 so 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 inadvertently reduces the small amount of original PrP^{Sc} captured, this approach is inherently less sensitive than methods that do not rely on PK digestion.[1]

The fact that only small amounts of the infectious prion may be available for detection in accessible living tissues such as blood, urine, and cerebrospinal fluid (CSF) challenges the diagnostician to develop a sufficiently sensitive test. In addition, diagnostic tests must be of sufficient specificity to differentiate between normal and abnormal prion proteins and, for some purposes, to discriminate between one or more strains of PrP^{Sc}—a challenge resulting from basic deficiencies in understanding of prion strain diversity and the nature of strain variation.

This then introduces the ultimate objective of a prion detection test: find a single infectious unit while avoiding a falsely positive test result. Reaching this objective will be a complicated task, because a single infectious unit is a variable measure rather than a static one. For example, the size of a single infectious unit of prions injected intracerebrally would be different than the size of a single infectious unit of prions given parenterally, intravenously, or orally. The smallest amount of prions needed to cause an infection may also vary by the strain of prion involved, by the physical composition of aggregated prion molecules, by the type of source tissue, and by the genetic susceptibility of the host animal or person.

The quest for antemortem diagnostics will play a fundamental role in controlling the spread of TSEs, yet current tests are largely

[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.

unvalidated and not readily available. Although much effort has been made to improve the means of TSE detection, the ultimate objective is far from being achieved. This chapter describes the clinical and laboratory diagnostic tests in use as well as experimental ones under development. In Chapter 4, the committee recommends new approaches that are more likely to yield effective antemortem diagnostics in the foreseeable future.

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. However, in important circumstances, they give the clinician some clues that may help to 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 (Table 3-1). For example, vCJD, contrary to 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 longer than a year (WHO, 2001a). Spencer and colleagues recently reviewed the early psychiatric manifestations of vCJD (Spencer et al., 2002). They have described the clinical characteristics of the first 100 vCJD patients identified and concluded 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 variant Creutzfeldt-Jakob disease, particularly if this is combined with any suggestion of cognitive impairment" (Spencer et al., 2002, p. 1482). Despite these differences between vCJD and CJD, they are not sufficient to establish a definitive diagnosis.

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 (Table 3-2), the electroencephalographs of more than 80 percent of patients show distinctive changes (Parchi et al., 1999). The tracing shows biphasic and triphasic periodic complexes in the clinical course (Parchi et al., 1999). They are evident more than 90 percent of the time with repeated tracings (Chiofalo et al., 1980). These

TABLE 3-1 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
EEG	Bi- or triphasic periodic complexes	Nonspecific, slow
MRI	Increased signal in basal ganglia, caudate nucleus, and putamen	Hyper-intense signal in pulvinar region of the thalamus
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 disease
PrP^{Sc} isotype by Western blot	Type 1A	Type 2B

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 in patients with a protracted clinical course the CT scan may show atrophy (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). 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 in vivo test for the diagnosis of vCJD (WHO, 2001b).

CSF Protein

CSF contains a protein called 14-3-3 that is normally present in CSF and whose levels are elevated in both patients with sCJD and patients with vCJD, especially those with the typical sCJD subtype (Zerr et al., 2000). This test was 53 percent sensitive when it was used to diagnose vCJD (WHO, 2001a). The 14-3-3 protein has the same electrophoretic pattern in patients with vCJD and sCJD, so it cannot be used to differentiate the two diseases (WHO, 2001a). Other conditions, such as viral encephalitis and recent stroke, can also cause elevations in the levels of this protein (Johnson and Gibbs, 1998; WHO, 1998).

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 patients 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

TABLE 3-2 Classification of Sporadic Prion Diseases

Subtype[a]	Previous nomenclature	Percentage of cases	Onset (ave. 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 Familial Insomnia					
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: P. Gambetti, Case Western Reserve University, November 2002.

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 PrPSc. From that 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 early PrP^{Sc} accumulates in human tonsils or the human appendix before the onset of symptoms or whether all positive individuals will inevitably progress to the fatal central nervous system (CNS) disease. Nevertheless, studies of sheep naturally infected with the scrapie agent and of mice experimentally infected with it 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. 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, however. 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, so 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 familial 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 to19 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 different 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 the ratios of the three PrP^{Sc} glycoforms (Parchi et al., 1997) and on the basis of 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 also 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. 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 it is obtained by combining information from the clinical examination, ancillary clinical tests, and laboratory tests. Yet, even when all this information is combined, the present diagnostic tools lack sufficient sensitivity and specificity. The need to develop tests to improve the early diagnosis of human prion disease and to more reliably detect presymptomatic infections in human and animals is a major priority.

CURRENT TSE DIAGNOSTIC LABORATORY METHODS

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." The World Health Organization position is that "a definitive diagnosis of CJD including nvCJD [new variant CJD] is established only by neuropathological examination" (WHO, 1998, p.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 is generally augmented with immunohistochemical staining of the tissue, which uses a PrP antibody-tagged stain that affixes onto PrP. The stain would be abnormally dark or dense in areas where an abnormal amount of PrP was present. Electron microscopy can also be used to observe fibrils, called the scrapie-associated fibrils, in fresh postmortem tissue (Merz et al., 1983) as well as in autolytic tissue.

Immunochemical Detection Methods

Three standardized commercial screening tests (by Prionics, Enfer, and Commissariat à l'Energie Atomique [CEA]) have been approved by the European Commission for use in the direct and rapid detection of PrP^{Sc} (Moynagh and Schimmel, 1999). They were developed in Europe and are primarily used there. To date the U.S. Food and Drug Administration (FDA) has not received a request from any of the European companies that manufacture these tests to approve them for human use in the United States, nor has any company based in the United States submitted any TSE screening test to FDA for approval for human use (Personal communication, D.M. Asher, FDA, July 18, 2002). However, the U.S. Department of Agriculture's Center for Veterinary Biologics has approved the use of one test produced by Bio-Rad Laboratories for the detection of chronic wasting disease (CWD) in mule deer.

The rapid test most widely used to screen for bovine spongiform encephalopathy (BSE) in Europe is a rapid Western blotting test produced by Prionics AG in Switzerland called Prionics Check Western. Test kits are available for the diagnosis of both 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 is broken down by proteinase K.

The two other tests use slightly different mechanisms for detection. The Enfer test from Ireland is an enzyme-linked immunosorbent assay (ELISA). After digestion with PK, antibody is bound to the residual PrP^{Sc}. This material is then bound to a second antibody-enzyme complex that binds to the original PrP^{Sc}-antibody complex, activating an enzyme reaction that colors the substrate when PrP^{Sc} is present. The CEA test from 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 the three approved tests (Table 3-3).

TABLE 3-3 Estimated Detection Limits of 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 the CEA
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, p.105)

[b] Two samples rated inconclusive at this dilution.

SOURCE: Adapted from Moynagh and Schimmel, 1999.

The European Commission's laboratory has performed a second round of testing on five more candidate assays, all of which look promising (Schimmel et al., 2002). The results were under review as of November 2002.

The approved rapid tests are generally used to diagnose TSEs in animals after death. Postmortem, the level of accumulation of PrP^{Sc} has reached its peak and PrP^{Sc} is most concentrated in brain tissue. Testing for CWD by immunohistochemistry and ELISA of both CNS and peripheral lymphoid tissue samples can provide positive results fairly

early in the incubation period (Sigurdson et al., 1999). In BSE, however, the level of PrP^{Sc} in those tissues is too low to detect by immunohistochemistry and ELISA until later in the incubation period. Consequently, these approved rapid tests are sufficiently sensitive for the detection of BSE only when they are used to evaluate clinically sick cattle or cattle that are apparently healthy but late in the incubation period. This has given impetus to the development of newer, more sensitive test systems. The following sections review that progress.

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 that 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 assay available for the detection of prions in potentially infectious material, even though animal bioassays do not directly detect PrP^{Sc}.

The animals first used to successfully demonstrate infectivity were goats infected with sheep scrapie (Cuille and Chelle, 1939). Goats were used in experiments to study sheep scrapie because the goats became infected more consistently than sheep did (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 scrapie 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. These engineered murine models helped to "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, p.116).

Although mice are the predominant animal model used in bioassays for TSE research, nonhuman primates have been used in the past and continue to have importance. 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. Therefore, 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 the study of human prion disease. Yet, the cost and scarcity of nonhuman primates, the complexity of their PrP genotype, and the long incubation period associated with TSE infection in them make their use limited 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. A number of investigators have used in vitro cell culture systems to learn more about the biology of prions. The obvious advantage of a cell culture system would be to significantly shorten 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 makes it easier to interpret the molecular and biological effects due to any specific variable being studied. The space and personnel needed to maintain a cell culture system are significantly less than those needed 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 used 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 which has been used successfully to study the scrapie agent (Schatzl et al., 1997). The PC 12 cell line, which is derived from

rat pheochromocytoma cells, also has been used to study mouse prions (Rubenstein, 1984; Prusiner et al., 1999).

The main shortcomings of existing cell culture systems have been 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 cell culture systems for the detection of PrP^{Sc}.

NEWER LABORATORY DIAGNOSTIC TESTS

Various strategies have been adopted to increase the sensitivities of tests used to detect PrP^{Sc}. These include concentrating PrP^{Sc} within a given test sample, amplifying the initial amount of prions present in a sample, developing antibody tags that preferentially bind to various conformations of prion protein, electrophoretic separation techniques, and special spectroscopic methods (Table 3-4). 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 reported 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} (Safar et al., 1998). 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 selectively bind to PrP^{Sc} and thus concentrate the abnormal protein for further characterization (Harris, 2002).

Protein Misfolding Cyclic Amplification

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 periodically subjected to sonication, which breaks them up and turns them into several new templates for the further conversion of PrP^{C} to PrP^{Sc}. This technique is called protein misfolding cyclic amplification, and 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

TABLE 3-4 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
Unvalidated[b]			
PMCA	Incubation with substrate PrP^{C}; ultrasound sonication	Yes	10- to 100-fold more sensitive than Western blotting[c]
CDI	PrP^{Sc} absorption; anti-PrP antibody; anti-IgG enzyme-linked antibody; fluorescence	Yes	0.2–2 pM
CIE	Gel electrophoresis; Beckman capillary device	Yes	100-fold more sensitive than Western blotting[d]

FCS	Two fluorescent antibodies; confocal microscopy	No	2 pM
FTIR	FTIR spectroscopy; artificial neural networks	No	Not specified
MUFS	Ultraviolet light; fluorescence; multi-variate analysis	Yes	In the picomolar range

NOTE: PMCA = protein misfolding cyclic amplification; CDI = conformationally dependent immunoassay; 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 picomolar (pM) of PrP^{Sc}. One picomolar equals 10^{-12} M.

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

[c] Harris (2002).

[d] Schmerr et al. (1997).

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

ultimate amount generated. Therefore, the test generated an approximately 30-fold increase in the amount of PrP^{Sc}.

Conformation-Dependent Immunoassay

Another innovative approach has been to identify selective sites on the prion protein that are not visible to an antibody when the protein is in its PrP^{Sc} conformation, but that are visible to the antibody when the protein is in its native PrP^{C} configuration or when it is chemically denatured. In general, the less denaturable the protein is, the greater the amount of β-sheet structure present in the protein compared with the amount of α-helical structure present (Safar et al., 1998). By this technique, guanidine hydrochloride was used to denature PrP. When the ELISA feature of this test was used, the test could quantify the amount of PrP^{Sc} present by determining the ratio of the amount of native PrP to the amount of denatured PrP. Safar and colleagues used this 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 assay for strain characterization was based on calculation of the ratio of the amount of denatured PrP to the amount of native PrP as a function of the amount of PrP^{Sc} before and after limited digestion with PK (Safar et al., 1998).

Capillary Electrophoresis

At least one group of investigators has reported on the use of capillary electrophoresis to detect PrP^{Sc} (Schmerr et al., 1997). This method has been used to analyze other proteins in the past (Tsuji, 1994) and was further adapted to detect PrP^{Sc}. The technique involves ultracentrifugation and PK digestion of a sample containing PrP followed by resuspension in a sodium dodecyl sulfate-buffered gel. The migration times were calculated by using Ferguson plots, which in turn were used to estimate the molecular weights of the proteins in the test material. The investigators compared the sensitivity of this method to that of the Western blot method and claimed a 100-fold improvement in sensitivity for the detection of PrP^{Sc}.

Fluorescent Correlation Spectroscopy

Another approach to improve the sensitivity and specificity of TSE diagnostics is to use newer, more advanced biotechnology tools such as fluorescent correlation spectroscopy (FCS). One group of investigators tagged PrP-specific antibodies with florescent 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 florescence intensity distribution analysis system and confocal microscopy with a scanner. This method incorporates a technology involving the scanning of intensely florescent 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

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 Spectroscopy

A recently reported spectroscopic approach to the identification of prion-infected hosts used Fourier transform infrared spectroscopy, in combination with a highly sophisticated automated computer-assisted pattern recognition program referred to as artificial neural networks, to detect disease-associated differences in patterns of small molecules in serum (Schmitt et al., 2002). By this method the investigators correctly differentiated between blood taken 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 indicated that the test needed to be assessed with species other than hamsters, and they cautioned 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 Fourier transform infrared spectroscopy does not involve PK digestion.

Summary of Newer Laboratory Diagnostics

Despite recent improvements in the sensitivities of diagnostic tests for TSE, they 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 used on livestock in countries reporting BSE would be the disposal of perfectly good meat. However, in countries where a false-positive test result represents a sentinel BSE case, the economic, political, and societal consequences of that incorrect result would be monumental. The impact of a false-negative test result might allow a contaminated beef product to enter the food chain. A false-negative result by a test used 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 TSE are not available for general diagnostic use or for screening. They 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 and reagents. Circumstances beg for fresh ideas that leverage a broader array of new technologies.

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4

Research Recommendations for TSE Diagnostics

LEVERAGE NEW TECHNOLOGY

New antemortem laboratory tests for the detection of PrP^{Sc}, the protease-resistant protein associated with prion disease, are imperative. Research considerations in improving 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 that an ideal test would detect less than 1 infectious unit (IU) of prions in the relevant organism or sample. Prusiner and colleagues demonstrated that 1 IU equals approximately 10^5 PrP^{Sc} molecules in a purified prion preparation (Prusiner et al., 1982). However, it is possible that the size of an IU differs depending on the host, the strain, and the mode of transmission. 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 PrP^{Sc} infectious agent, albeit indirectly. Yet, these animal tests, such as the murine bioassay, are hampered by the species barrier. For example, conventional mice used to bioassay the infectivity of the agent of bovine spongiform encephalopathy (BSE) could detect only 1,000 IU per inoculum (Wells et al., 1998). The sensitivity of the bioassay is also limited by the small inoculum size that can be given to these mice intracerebrally (Wadsworth et al., 2001).

Recommendation: Focus funding for new assays on the proposals 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 IU of PrP^{Sc} per unit of ultimate product used (e.g., 1 liter of blood or 100 grams of beef).

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

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 and distinction among different allelic and strain variants of the prion protein. Such distinctions could be made, in principle, by 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 upon upconversion of phosphors with negligible natural background fluorescence or those mentioned below, a number of approaches offer significant potential. In general, researchers need to leverage 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 those that are specific for different alleles. In principle, these molecules could be antibodies, for example, 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). At present, prion detection methods are dependent on a few antibodies selected in vivo that offer a

limited, if any, ability to distinguish between infectious and noninfectious prion protein conformations.

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 (single-chain antibody variable region fragments [scFvs]), and derivatives that contain well-behaved constant regions (e.g., from mouse immunoglobulin G fetal calf serum [Fcs]) 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 further 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 readily be synthesized by expression in bacteria, yeast, or other cell-based systems.

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

Physics-Based Methods

Within the past two decades, numerous detection methods based on physical phenomena have also been devised. They include evanescent-wave methods (such as those based on surface plasmon resonance), methods that detect resonances in the microwave range, meth-

ods 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 last method, none of these are as sensitive as RIAs and ELISAs, but they offer advantages, as they can be used with underivatized recognition and target molecules and can be directly coupled to optical or electrical readouts. Evanescent-wave devices, which are used widely, 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, and these possibly have even greater sensitivities for the detection of prions. One of these is the protein complementation technique (Remy and Michnick, 1999). Another method couples recognition proteins with polymerase chain reaction (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, and the lack of an industrial-scale effort, among perhaps other reasons.

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 issues 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 may make their commercial application difficult until patent-sharing schemes can be devised. Nevertheless, neither technical nor legal barriers block governmental or philanthropic groups from funding the production of these reagents for use to detect prion particles.

In summary, a wealth of natural and engineered molecules along with a variety of detection technologies have been developed for recognition of biological targets and have been used for other applications. Now prion investigators must apply them to the development of selective, sensitive tools that 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: Develop novel methods and reagents that detect or bind to prions, including new antibodies, peptides, nucleic acids, synthetic derivatives, and chimeric molecules. This may lead not only to better diagnostics but also to better therapeutic and prophylactic strategies.

SURROGATE MARKERS AND SIGNATURES OF PRION DISEASE

Diagnostic approaches based on detection of indirect disease markers have a long and checkered history. In general, they have been stigmatized by their lack of specificity (e.g., tests for the erythrocyte sedimentation rate and C-reactive protein) or their dependence on the generation of a specific antibody—which is delayed in all disease processes and which is absent in some disease processes, including transmissible spongiform encephalopathies (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 tech-

niques, focus on complex biological patterns, and depend on pattern recognition algorithms.

All forms of mammalian pathophysiology and pathology are accompanied by sterotyped and highly choreographed intra- and extracellular changes in the diversity, abundance, and spatial distributions 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 different 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 or prognostic signatures can be defined by surveying the complex patterns of the abundance of biomolecules in different pathologies. Pattern recognition algorithms fall into two groups: those that discover classes of disease (or genes), such as clustering and self-organizing maps, and those that predict different classes of elements from predefined signatures, such as support vector machines and f-test algorithms (for example, diagonal linear discriminant analysis). Some of the most compelling examples of this approach concern efforts to classify cancer subtypes and predict survival and the 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 to whose diagnosis the investigators were blinded and correctly identify 177 of 228 patients without prostate cancer (Petricoin et al., 2002b).

In diagnosing prion infections, it seems 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 path is a blind search for such protein patterns by 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: Identify surrogate markers or signatures for the detection of prions or prion diseases.

CELL CULTURE SYSTEMS

Research gains leading toward better diagnostics would be accelerated if better cell culture systems were in place. These systems have significant advantages over animal bioassay systems, with the most important advantage 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 susceptible to prions in vitro and 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 will not only improve the possibility for the use of cultured cells to assay prions, but also will shed light on the cellular mechanisms underlying prion replication.

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

CLINICAL NEUROIMAGING

Recent improvements in clinical neuroimaging have shown increasing utility in clinical diagnostics for TSEs. Magnetic resonance imaging (MRI) is able to visualize the brain pathology of patients with Creutzfeldt-Jakob disease (CJD) and can even help in differentiating variant Creutzfeldt-Jakob disease (vCJD) from sporadic Creutzfeldt-

Jakob disease (sCJD), as mentioned 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 that has been described (Zeidler et al., 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 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 UV light and which 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 Alzheimer's disease, researchers used multiphoton microscopy with locally applied fluorescently labeled antibody against β-amyloid or systemically administered fluorescent derivatives of chemicals that bind to β-amyloid, such as of 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 similar kinds of β-amyloid-imaging approaches 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 antiprion therapies.

Recommendation: 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.

PRIORITIES FOR BASIC RESEARCH

The committee believes that the fastest way to improve diagnostic tools is not through applied research to improve existing detection methods but through basic research that fills in the gaps in the fundamental knowledge of prions and their disease-causing abilities. The European experience provides evidence that applied research alone is insufficient. The European Union has spent approximately 30 million euros (30.7 million dollars) over the past 10 years to attempt to develop satisfactory postmortem diagnostic tests for BSE, yet the Western blot test, which has been around for three decades, is the still most commonly used method (Personal communication to the committee, A. Aguzzi, University Hospital of Zurich, July 15, 2002).

The history of medicine also provides 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 human immunodeficiency virus, 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. They have collectively made great progress, yet many fundamental questions remain. Basic research on prions also will serve as a foundation for new and better evidence-based surveillance and public health policies.

The most critical areas of basic prion research include solving the structure of PrPSc and relating the structure to prion strain differences (Box 4-1); determining endogenous and exogenous mechanisms of prion replication (Box 4-2); elucidating prion epidemiology and natural history (Box 4-3); clarifying the pathways and pathogenic mechanisms used by prions (Box 4-4); and elucidating the physiologic function of PrP^{C} (Box 4-5).

BOX 4-1
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.

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 recommends that the National Prion Research Program (NPRP) of the U.S. Department of Defense support research to these ends.

Defining the structural differences between PrP isoforms might enable scientists to synthesize a PrP^{Sc}-specific antibody probe or aptamer, opening the door to a TSE diagnostic tool. Antibody probes are increasingly 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}. One group of investigators reported an antibody specific to PrP^{Sc} (Korth et al., 2001).

Many of the tests for detection of PrP^{Sc} use antibody probes, as described in Chapter 3. Most tests, however, must use proteinase K to distinguish 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 is 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 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 Wuthrich 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 PrP 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 depend on the help of one or more molecules (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 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

BOX 4-2
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 (e.g., yeast).
- 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.

could potentially amplify PrP^{Sc}, helping to overcome current prion detection limits.

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

To develop better diagnostics, much more understanding about the nature and dynamics of prion infection must be gained. Studying the pathogenesis of TSEs holds the keys to such understanding. Researchers, clinicians, and public health officials must know which tissues are infectious and when, the mechanisms by which 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), the mechanisms by which the toxic events in TSEs lead to cellular dysfunction and clinical symptomatology; how the infectious agent spreads from host to host, and host determinants of susceptibility to infection. Understanding these issues will lead to improved characterization of diagnostic targets, better diagnostic strategies, greater target discrimination, and improved diagnostic sensitivity and specificity.

BOX 4-3
Priority research on mechanisms of TSE pathogenesis

- Identify direct and indirect 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.
- Identify 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 symptomatologies.
- Determine whether different prion subtypes and strains exhibit distinct pathogenic mechanisms.
- Use alternative model systems (e.g., *Drosophila*) to study pathogenic mechanisms.

The answers to these questions may vary among strains and hosts. Therefore, all pathogenetic studies must be interpreted in their specific contexts, which will make 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.

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 (Masison & Wickner, 1995). Soon after, it was discovered that, as in mammals, multiple strains of yeast prions could 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 1997).

Later that year, King and Wuthrich showed that the prion domain of Sup35p could form amyloid in vitro (King 1997). Then Glover and Lindquist showed 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 extract of [psi-] cells (Glover 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 very 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 a second prion (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 successful. 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).

Drosophila and *Caenorhabditis elegans* have also proved to be superb models for the study of a variety of cellular and molecular processes 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.

Epidemiology and Natural History of TSEs

Despite the number of TSE transmission studies performed to date, many unanswered questions in addition to those discussed above

BOX 4-4
Priority research on epidemiology and natural history of TSEs

- Determine whether non-human TSEs, including chronic wasting disease (CWD), are transmissible to humans.
- Identify the determinants of interspecies transmissibility.
- Identify the determinants of host *population* susceptibility and resistance, including genetic factors.
- Identify mechanisms and routes of human exposure to TSEs and prions.
- Determine the national and international geographic distributions of CWD by well-designed surveillance methods.
- Determine the breadth of strain diversity for agents of known TSEs.
- Search for previously unidentified TSEs.
- Determine the risks of human exposure to BSE and CWD internationally.

remain. Is chronic wasting disease (CWD) transmissible to humans? Is it transmissible to cattle or sheep? How is CWD transmitted among cervids? Further epidemiological and natural history studies would help answer these questions and would help shape appropriate measures to protect the public's health.

Several transmission studies are underway in Europe and the United States. In an ongoing experiment, Nora Hunter and colleagues at the United Kingdom's Institute for Animal Health recently demonstrated for the first time that healthy sheep can become infected with prions through transfusion of blood from BSE agent-infected sheep (Hunter et al., 2002). 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 rhesus monkeys to understand the potential for prion infection from blood products (Personal communication, A. Aguzzi, University Hospital of Zurich, October 12, 2002).

Several additional transmission studies are planned. Scientists at the Rocky Mountain Laboratories of the National Institutes of Health expect to use squirrel monkeys to study prion infectivity in blood (Personal communication, R. T. Johnson, Johns Hopkins University, 2002). The Commissariat à l'Energie Atomique in Paris, France, plans

BOX 4-5
Priority research on the physiologic 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.

to build a large, new facility that would house 60 macaques for TSE-related studies, including the infectivity of different prion strains, such as those that cause vCJD (Deslys, 2002).

Physiologic 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 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: Fund basic research to elucidate the structural features of prions, the molecular mechanisms of prion replication, the mechanisms of TSE pathogenesis, the epidemiology and natural history of TSEs, and the physiologic function of PrP^C.

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5

Prion Research Infrastructure

The goals of research on transmissible spongiform encephalopathy (TSE) in the United States are broadly aimed at preventing a bovine spongiform encephalopathy outbreak in the United States; preventing further the spread of chronic wasting disease; reducing the incidence of Creutzfeldt-Jakob disease; and developing 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.

THE PRESENT U.S. INFRASTRUCTURE

The prion research infrastructure in the United States is small, aging, and inadequately funded. 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. The funding for TSE research is miniscule compared to the amount directed at other diseases. In fiscal year 2000, NIH spent $14.69 billion on research, but only $23.86 million—0.16 percent of the institute's research budget—was directed toward TSE research (Johnson, 2002). Furthermore, 75 percent of the funds provided for TSE research go to only two laboratories (Personal communication, R.T. Johnson, Johns Hopkins University, 2002).

This level of effort and funding is quite different from that supported by the European Commission. One recent Commission report listed 58 delegations of researchers conducting TSE research

(European Commission, 2001). It is estimated that the countries of the European Union annually invest manyfold larger amounts of money in TSE research.

The paucity of funding dedicated to prion research is not the only reason why so few investigators are involved in this field. First, the small number of prion 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 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 require relatively long timeframes for a single experiment. Furthermore, prion laboratory equipment must be dedicated solely to TSE research and cannot readily be shared for other research purposes because of decontamination difficulties.

The biohazardous nature of prions also leads to delays, frustrations, and extra costs related to adherence to safety regulations and compliance requirements. Prion research laboratories are extremely expensive to build or to expand because special safeguards are required to protect both the investigator and the public. U.S. Department of Agriculture and institutional rules require TSE laboratories to meet the biological safety standards at biosafety level 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 are just out of training and who want to start up their own laboratories must weigh the high financial start-up costs, the long-term investment, and the uncertain availability of funds for prion research compared with those for other types of research.

Recommendation: Support programs that 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: Provide funds to increase the capacities of animal facilities and containment laboratories (biosafety levels 2 and 3) to conduct prion research.

INTERNATIONAL COLLABORATION

A quick remedy to the shortage of U.S. laboratory space for prion investigators is unlikely. However, several large European laboratories conducting prion research might offer opportunities for collaboration with U.S. investigators and might even allow U.S. investigators to use their laboratory space. The French government already has set up 35 fellowships for foreign TSE researchers and is actively seeking U.S. applicants (Personal communication, R.T. Johnson, Johns Hopkins University, 2002). France has a biosafety level 3 facility with housing for 60 macaques, and the government is building a dedicated prion research facility that will house 120 monkeys and provide laboratories for visiting scientists (Johnson, personal communicaton, 2002). At a recent Institute of Medicine committee meeting, 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).

Recommendation: Provide funding for collaborative research and training with European investigators and facilities that provide unique opportunities for prion research.

STANDARDIZED REAGENTS AND MATERIALS

Few of the basic materials used in prion research have been standardized or commercialized, increasing the number of challenges for TSE investigators. The reagents used to conduct individual studies are often made by the laboratory conducting the study or are borrowed from a fellow investigator's laboratory. The consequent lack of standardization has hampered the ability to replicate the results of one laboratory by another laboratory, delaying opportunities to validate key discoveries.

This lack of standardization is also true of the animals used in prion 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 deletions of specific genetic coding areas (knockout mice). The processes and the level of quality control required to produce these engineered animals are not well established.

This issue regarding standardized materials is also a concern for researchers in Europe and elsewhere. In September 1999, the World

Health Organization (WHO) held a special consultation meeting to address this problem. The group at the meeting recommended that a working group be established (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 investigators to have 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, and then other organizations could be subcontracted to carry out the requirements of the contract. This mechanism has worked well with the Vaccine Development Program at the National Institute of Allergy and Infectious Disease (Personal communication, R.T. Johnson, 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, which is 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. Later, after the repositories have been established, the fees paid by investigators using the materials would cover the general operating costs.

Recommendation: Establish a collection of reference materials and genetically engineered animals (including transgenic mice), as well as reference repositories, useful for the development of TSE diagnostics and for TSE research. All investigators involved in prion research must have access to this collection. It would be reasonable to include the collection in existing, high-quality repositories with similar standardized reagents.

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. The 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). 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. FDA has developed such panels in the past for validating antibody-screening tests for human immunodeficiency virus and hepatitis C virus.

Recommendation: The Food and Drug Administration should have panels of reference reagents available to evaluate the performance characteristics of tests to detect the prion protein and infectivity.

The pace of progress in prion disease research 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 mentioned earlier in this chapter, the research capacity to conduct TSE research must be enhanced significantly. That 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 approved 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 take sustained attention, effort, and funding.

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6

Risks to the U.S. Military

U.S. forces are continually deploying around the globe. On December 31, 2001, 255,065 U.S. forces were deployed to 144 countries outside the United States (DOD Almanac, 2002), including several countries where bovine spongiform encephalopathy (BSE) has 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 non-combat missions over an extended period. As a result, U.S. military personnel and their families deployed to countries where BSE had been reported 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, how much risk of exposure to BSE do U.S. forces face? Under what circumstances would that risk occur? Additionally, if a member of the U.S. forces is being treated for trauma with a blood transfusion, how much risk is there that prions are in the transfused blood? This chapter explores both sets of conditions and comments on the nature and extent of the risks.

RISK OF EXPOSURE TO BSE-TAINTED BEEF PRODUCTS

DOD Military Food Supply System

To assess the military's risk of exposure to BSE-tainted beef products, 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.[1]

Commanders of U.S. military units have the authority to purchase food products locally, including beef. A commander might authorize this if his or her troops had been eating pre-prepared rations for an extended period of time to offer variety and to maintain high morale. In that circumstance, if local beef was purchased in a country where BSE had been reported, the troops would be at risk of exposure to BSE. This practice of procurement of local beef, however, 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, so long as the source is approved by the U.S. Department of Defense (DOD) Veterinary Services. Some of this 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, also have access to beef products through several other outlets. The first is a commissary system. These commissaries are military supermarkets stocked primarily with food products from the United States. U.S. producers generally supply all the beef sold in these commissaries. However, in some European countries where BSE had been reported, some beef sold in commissaries was locally procured for certain periods of time. From 1980 to 1989, the monthly foreign beef procurement from non-U.S.

[1] U.S. Army Regulation AR40-657 (1997); U.S. Navy Regulation NAVSUPINST4355.4F (1997) ; U.S. Marines Regulation MCOP10110.316 (1997).

suppliers averaged 2.5 million pounds. Of this amount, 35 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, The 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. This made it 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 beef) 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 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, was 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 Kingdom through 1989. From 1990 to March 2000, either U.S. beef was used or beef was ground in an AAFES-operated facility in Germany by using a combination of U.S. beef and beef from European countries other than the United Kingdom. Between March 1996 and March 2000, most 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 for 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 that they consume comes from either the system used to feed troops or the commissary system.

The greatest period of risk for exposure to tainted 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 iteratively put in place 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-tainted beef products (Table 6-1).

U.S. military members and their families who were living in the United Kingdom and Europe between 1980 and 1996 were at increased risk of exposure to infectious prions as a result of their consumption of locally procured beef or their consumption of beef in local eating establishments compared with the risk of their counterparts in the United States. This risk of acquiring vCJD is judged to have been relatively small compared with the local population's risk. Notification and 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.

TABLE 6-1 DOD Active Duty Personnel and Dependents in Europe

Period	No. of Active-Duty Personnel	No. of Dependents	Total No. 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).

Recommendation: 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 U.S. Department of Defense and the U.S. Department of Veterans Affairs health systems.

RISK OF TSE INFECTION FROM BLOOD PRODUCTS

Blood transfusions could also place deployed forces at risk of infection by the agent of BSE or of other transmissible spongiform encephalopathies (TSEs). 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 a TSE agent such as the infectious prion that causes vCJD?

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 deployed 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 potential risk that a service member would be transfused with blood from a donor who might have preclinical vCJD is considered quite remote.

Under some circumstances, blood products are supplied to U.S. facilities by the host nation. Examples of such blood products include platelets, which have a very short shelf life, and products whose supplies are exhausted or not available. Additionally, U.S. forces deployed overseas use local emergency rooms or hospitals when medical care is not available from the DOD health care system. In situations in which the U.S. service member has an injury serious enough to warrant a blood transfusion, there would be a theoretical risk of exposure to an infectious prion such as that which causes vCJD, but those situations are uncommon.

DOD Blood Donation Deferral Policies

Although the risk that a member of the deployed forces acquired a prion responsible for TSE 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 (Table 6-2).

This policy results in the deferral of 18 percent of the 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 9 percent, which has helped to offset the losses (Sparks, 2002).

If a screening blood test that was sensitive enough to detect infectious prions were available, 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. In addition, approximately half a million non-DOD individuals (5 percent of the national blood donor pool) who are deferred from donating blood could be returned to the blood donor pool (Sparks, 2002).

TABLE 6-2 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 to present
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)	Cumulative time ≥ 6 months 1980 to present (all of Europe, regardless of USDA rating)
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.	

NOTE: USDA = U.S. Department of Agriculture.
SOURCE: Sparks (2002).

The Need to Screen Blood for Prions

The need to return individuals to the blood donor pool is driving the blood-servicing industry to make a screening test available. However, scientific evidence showing that blood from one person with CJD or vCJD can transmit the infectious agent by blood or blood product transfusion is lacking. A recent attempt to infect mice with the buffy coat and plasma of blood from a vCJD patient produced negative results (Bruce et al., 2001). However, this lack of evidence may result from inadequate knowledge of the behavior of prions in human blood,

the detection limits of today's prion detection assays, the limitations of cross-species bioassays, and the long incubation period of vCJD.

Of special concern was the finding that vCJD is present in lymphoid organs. The recent report of the successful transmission of the agents of BSE and scrapie between sheep by blood transfusion (Hunter et al., 2002) buttressed the precautions already in place to protect the public from the potential blood-borne transmission of vCJD. In addition, PrP^{Sc} (the protease-resistant protein associated with prion disease) was detected in the blood of a patient with sporadic Creutzfeldt-Jakob disease (sCJD) (Aguzzi, 2002). These reports magnify the importance of determining the amount of PrP^{Sc} in the blood of both sCJD and vCJD patients. The titer of infectious prions present should also be determined by assays with appropriate host species. This will require improved methods for detection of PrP^{Sc} and suitable hosts for transmission studies.

Ethical considerations and the present inability to identify presymptomatic carriers with vCJD or sCJD preclude the conduct of experiments to determine precisely whether the blood in those carriers is infectious for other humans. Therefore, scientists must rely on animal studies to estimate both the amount of PrP^{Sc} corresponding to one ID_{50} (the dose needed to infect 50 percent of the population exposed to the agent) of vCJD or sCJD prions in human blood and the titer of those infectious proteins. Nonhuman primates are excellent animal models for these experiments. The National Prion Research Program of DOD should fund the infrastructure and research protocols for primate studies to determine both the amount of PrP^{Sc} corresponding to one ID_{50} of vCJD and sCJD prions in human blood and the titers of those infectious proteins in human blood.

Recommendation: Determine the amount of sCJD and vCJD prions in human blood and estimate the amount of PrP^{Sc} corresponding to one ID_{50} of sCJD and vCJD prions in human blood.

Until circulating PrP^{Sc} in blood can be detected with confidence, blood donation deferral policies must be based on worst-case assumptions, namely, that persons who are known to have been exposed to infectious prions and who are possibly incubating them should be deferred from the blood donor pool.

SUMMARY OF OVERALL RISK

This chapter has summarized the risks that deployed U.S. forces have of acquiring a TSE as a result of the consumption of a contaminated food product or the receipt of a tainted therapeutic blood product. Both risks are deemed small to nonexistent. Nevertheless, the risk is unknown, so the precaution of deferring individuals who were potentially exposed to BSE-contaminated meat from donating blood is justified. Research that can clarify the infectious potential of blood products as a vehicle for transmitting prions will help immensely.

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Appendix

Study Methods

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 I
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 II
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 p.m.	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 a.m.	Adjourn Open Session

Meeting III
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 IV and V

OPEN SESSION, OCTOBER 29, 2002

Mini-Symposium on TSE Surveillance

1:00 p.m. 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 p.m. Adjourn open session