Pathogenesis of Subacute Spongiform Encephalopathies

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ABSTRACT

The subacute spongiform encephalopathies include scrapie of sheep, transmissible mink encephalopathy, and kuru and Creutzfeldt-Jakob disease of man. These diseases are caused by filterable infectious agents with unique physical properties. The usual sources of infection in nature are not completely known. Epidemiological evidence suggests that the agents may enter the body through breaks in the skin and mucous membranes.

Experimental studies of scrapie after subcutaneous inoculation demonstrated early replication of the agent in lymphoid tissues and later appearance in other organs; as the amount of agent in the central nervous system (CNS) increased, it decreased in or disappeared from lymphoid tissues. In preliminary studies of kuru and Creutzfeldt-Jakob disease, the infectious agents were regularly recovered from the brains of clinically-ill patients and experimental animals but only occasionally from organs outside the CNS. It remains to be seen if early events in the pathogenesis of the two human diseases, before the appearance of clinical signs, are similar to those in scrapie.

Introduction

Extensive vacuolation of the cerebral cortical gray matter, or status spongiosus, was noted by Alfonso Jakob in one of his patients with presenile dementia, and later authorities have used this histopathological finding as one criterion for classifying patients with degenerative brain diseases. The term “subacute spongiform encephalopathy” was introduced by Nevin et al to describe 23 patients with a syndrome of rapidly progressive dementia, motor paralysis and focal signs of degeneration of cerebral cortex and frequently of cerebellum and other areas of the brain. The brains of these patients showed variable status spongiosus, neuronal degeneration and proliferation of astrocytes throughout the cerebral cortical gray matter and basal ganglia, and sometimes neuronal degeneration in the cerebellum. Although Nevin believed subacute spongiform encephalopathy to be a distinct disease entity, others consider it a variant of the Creutzfeldt-Jakob syndrome. Gibbs and Gajdusek suggested the name subacute spongiform virus encephalopathy as a generic term to encompass not only the spongiform encephalopathy or Creutzfeldt-Jakob syndrome itself, but also another
human disease, kuru, and two animal diseases, scrapie and transmissible mink encephalopathy. These four diseases share several clinical and histopathological features, and each is an infection caused by a transmissible, filterable, self-replicating agent. Each disease fulfills the general criteria of a slow infection; a long asymptomatic incubation period lasting months or years; a protracted clinical illness ending fatally; pathology restricted to one organ system, the central nervous system; and an etiologic agent transmissible to a limited range of susceptible hosts (table I).

The epidemiology, clinical picture and histopathology of these diseases will be reviewed as well as some of the biological and physical properties of their agents and the limited information available on their pathogenesis.

**Kuru**

Kuru was first described in 1957 among the inhabitants of part of the Eastern Highlands of Papua New Guinea; these are a Melanesian people who had, until recently, a neolithic culture in which ritual cannibalism of dead family members was practiced. Kuru, which means shivering in local languages, was very common and was responsible for over half the total mortality after infancy in some parts of the region. Children of both sexes older than four years were affected equally, but adult women with the disease greatly outnumbered men. The incidence of kuru has recently decreased markedly and the disease is no longer seen among children. This dramatic change suggests that transmission of the disease was associated with ritual cannibalism, which stopped by 1959.

Kuru is a stereotyped clinical illness. Patients are first aware of impaired walking and later of general motor incoordination which becomes progressively more severe. There is a typical shivering tremor which gives kuru its name. Most striking on physical examination are signs of cerebellar ataxia. Changes in affect are common, with euphoria, or less often depression; these may progress to mild dementia, but patients remain alert throughout the illness. Patients become dysarthric, later dysphagic, and strabismus is frequent. Extrapyramidal signs may be present. Convulsions have not been observed. Fever, the usual hallmark of serious systemic infections, occurs only with the secondary bronchopneumonia and decubitus ulcers which herald the end of the illness, usually within a year of onset. Hematological and blood chemical studies are normal. The cerebrospinal fluid has normal cell content and normal or mildly elevated protein. The EEG is normal early in kuru, with diffuse low-voltage slow activity in about two-thirds of patients later in the course of illness. Histopathologic changes are found only in the central nervous system, distributed throughout gray matter, and are most severe in the cerebellum and in the nuclei with which it connects. The triad of neuronal vacuolation and status spongiosus, neuronal degeneration and loss, and hypertrophy and proliferation of fibrous astrocytes is always present. There are also variable histopathologic changes including mild degeneration of myelin thought to be secondary to neuronal damage, reactive proliferation of microglia and binucleated neurons. At least half the brains studied had in the cerebellum, and less often in the pallium, amorphous plaques which stained by the periodic acid-Schiff reaction. Although occasional perivascular accumulations of round cells have been observed, these are uncommon. In general, there are none of the histologic findings of inflammation. Ultrastructural changes in kuru and the other subacute spongiform encephalopathies are reviewed.

**Creutzfeldt-Jakob Disease**

In contrast to kuru, the syndrome called Creutzfeldt-Jakob disease (CJD) or spongio-
form encephalopathy occurs throughout the world; it is uncommon, but not rare. Kirschbaum reviewed 150 cases reported between 1920 and 1966; over 100 histologically documented cases have been brought to our attention since 1969. An exhaustive survey of various population groups in Israel by Kahana et al revealed an incidence between 0.4 and 1.9 cases per million people except among Libyan Jews who constitute a focus of CJD with an incidence of 31.3 cases per million. CJD is a disease of the middle years with peak incidence in the sixth decade, although ages of patients have ranged from early 20's to late 70's, and the disease may well be unrecognized in older people. Males and females are affected in equal numbers.

It is not known how the infectious agent of CJD is transmitted to patients, but several case histories suggest possible mechanisms. One patient was probably infected iatrogenically when she received the transplant of a cornea obtained from the autopsy of a demented man who was later learned to have died of CJD; she developed signs of the disease herself 18 months later. Although such unfortunate accidents must be rare, three other patients with spongiform encephalopathy had histories of brain surgery 15 to 17 months before the onset of illness, suggesting incubation periods resembling those of Duffy's patient and of chimpanzees inoculated with the infectious agent (table II). It has been speculated that the infectious agent of CJD was somehow introduced into the brain at craniotomy or that surgery served to activate an otherwise silent infection that was already present. Another disturbing case is that of a neurosurgeon who had a systemic vasculitis diagnosed as papulosis atrophicans maligna (Köhlmeier-Degos' disease). He was thought to have the diffuse central nervous system involvement sometimes seen with that rare disease, but changes of both vasculitis and CJD were found in the central nervous system at autopsy. His brain contained an agent which elicited typical experimental CJD in primates. Because the patient was a neurosurgeon, the possibility of transmission of CJD by actual contact with infected brain tissue has been raised. Jellinger et al reported simultaneous onset of CJD in a married couple, which suggests either person-to-person transmission or perhaps simultaneous exposure to a common source. A recent epidemiological survey of 38 patients with CJD compared with a control cohort of spouses and close childhood friends showed a surprisingly frequent history of consumption of animal brains by both CJD and control groups, but no significant difference between them.

Perhaps the most intriguing epidemiological peculiarity of CJD is its frequent occurrence in families. About 10 percent of

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**TABLE I**

Spongiform Encephalopathies Demonstrated Range of Susceptible Experimental Hosts

| I. KURU | Primates
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Great apes (chimpanzee, gibbon)</td>
<td>Old World monkeys (macaques, mangabey)</td>
</tr>
<tr>
<td>New World monkeys (capuchin, woolly, spider, squirrel, marmoset)</td>
<td>Nonprimates</td>
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<td>None</td>
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| II. CREUTZFELDT-JAKOB DISEASE | Primates
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<tbody>
<tr>
<td>Great apes (chimpanzee)</td>
<td>Old World monkeys (macaques, vervet, mangabey, bushbaby, baboon)</td>
</tr>
<tr>
<td>New World monkeys (capuchin, woolly, spider, squirrel, marmoset)</td>
<td>Nonprimates</td>
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<tr>
<td>Cat</td>
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| III. SCRAPIE | Primates
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<tr>
<td>Old World monkeys (macaques)</td>
<td>New World monkeys (capuchin, spider, squirrel)</td>
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<tr>
<td>Nonprimates</td>
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<td>Ruminants (sheep, goat)</td>
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<tr>
<td>Cricetid rodents (mouse, rat, golden hamster, Chinese hamster, gerbil, vole)</td>
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<tr>
<td>Weasel family (mink)</td>
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| IV. TRANSMISSIBLE MINK ENCEPHALOPATHY | Primates
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<tbody>
<tr>
<td>Old World monkeys (macaques)</td>
<td>New World monkeys (squirrel)</td>
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<tr>
<td>Nonprimates</td>
<td></td>
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<tr>
<td>Weasel family (mink, ferret, skunk)</td>
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<tr>
<td>Ruminants (sheep, goat)</td>
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<tr>
<td>Cricetid rodents (golden hamster)</td>
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<td>Racoon</td>
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TABLE II

Experimental Kuru and Creutzfeldt-Jakob Disease (CJD): Length of Incubation Periods (Months)

<table>
<thead>
<tr>
<th></th>
<th>Primary Inoculation</th>
<th>Serial Passage</th>
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<tbody>
<tr>
<td></td>
<td>Shortest</td>
<td>Longest</td>
</tr>
<tr>
<td>Primates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kuru (6 serial passages)</td>
<td>10†</td>
<td>101§</td>
</tr>
<tr>
<td>CJD (5 serial passages)</td>
<td>10*</td>
<td>71*</td>
</tr>
<tr>
<td>Cat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CJD (1 passage)</td>
<td>30</td>
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</tbody>
</table>

Includes only animals inoculated intracerebrally with brain tissue. *Chimpanzee †Capuchin
$Rhesus macaque ‡Spider monkey †Squirrel monkey ‡Stumptail monkey

reported cases have a family history of presenile dementia. In some families, without
known consanguinity, CJD has occurred in
several generations, affecting more than 25
percent of siblings of both sexes, a pattern
consistent with autosomal dominant inheri­
tance. However brains of patients from
four such families have transmitted typical
CJD to animals.54,180 Although there may be
some hereditary susceptibility to CJD, an in­
fec­tious etiologic agent is present even in fa­
milial cases.

The clinical picture of CJD is much more
variable than that of kuru.132,150,180 Dementia
is required to make the diagnosis. Patients
usually come to medical attention with
vague sensory complaints, confusion or
depression. Dementia becomes more severe,
progressing to stupor and coma, sometimes
within a few weeks of onset. Signs of focal
cortical and upper motor neuron distur­
bances are usually present, frequently with
myoclonic jerks and sometimes with
generalized seizures. Cerebellar ataxia is
often found early in the course of illness.

The nosology of the presenile dementias is
controversial, but it is generally agreed that
there is an amyotrophic form of the disease
which tends to strike younger people and to
run a longer course with prominent signs of
lower motor neuron disturbance and little or
no status spongiosus. Nevin157 and others21
considered this amyotrophic syndrome to be
the classical form of CJD described by
Creutzfeldt and Jakob. May150 believed that
spongiform encephalopathy was described
by Jakob and is more properly considered
classical CJD, but he agreed that the
amyotrophic syndrome may constitute a
separate disease entity.151

Clinical hematology and blood chemis­
tries are generally normal in CJD except for
frequent mildly deranged liver function
studies, the reasons for which are not
clear.170 Cells in the CSF are normal, and
protein content is normal or slightly ele­
vated. The pneumoencephalogram often
shows cerebral and cerebellar atrophy, and
the electroencephalogram shows periodic
high-voltage complexes on a low back­
ground.

Histopathologic changes are found
throughout the gray matter of the brain.
They are most severe in the cerebral cortex
but affect the basal ganglia, cerebellum and
spinal grey as well. The triad of neuronal
vacuolation and status spongiosus, neuronal
degeneration and loss, and astrocytic pro­
liferation and hypertrophy are present.

Plaques similar to those in kuru were first
described in a case of CJD by Chou and
Martin34 and plaques have since been
reported in several other patients.1,110,113,135
These histopathological changes are almost
identical to those seen in kuru, differing only
in relative intensity, with cortex and striatum
more severely affected in CJD, and cerebellum in kuru.

Scrapie and Transmissible Mink Encephalopathy

Scrapie is a natural disease of sheep, and occasionally of goats, which has progressive ataxia, wasting and frequently severe pruritis. The general clinical picture and histopathological findings of scrapie closely resemble those of kuru, which prompted Hadlow\textsuperscript{101} to suggest that both diseases might also have similar etiologies. Scrapie was the first of the spongiform encephalopathies transmitted experimentally to susceptible animals (table I).\textsuperscript{30-40,41,92} Although scrapie has been studied longer and more intensively than the other spongiform encephalopathies, the mechanism of its spread in nature remains in doubt. Scrapie has been observed to spread by contact from naturally-infected sheep to uninfected sheep and goats,\textsuperscript{23,114} although apparently not from experimentally-infected sheep or from goats infected either naturally or experimentally. Both sheep and goats have been infected experimentally by oral feeding of large doses of scrapie agent\textsuperscript{166} although such transmission has not always been successful.\textsuperscript{177} Scrapie has long been thought to pass from ewes to lambs,\textsuperscript{140} and contact of a lamb with an infected ewe at the time of birth, even without suckling, may be sufficient to transmit infection.\textsuperscript{53,178} The placenta itself may be infectious.\textsuperscript{165} Some older sheep may be infected after contact with diseased animals, but such contact apparently must continue for a long time, perhaps for years. Exposure of susceptible sheep to pasture previously occupied by diseased sheep has been reported to transmit disease.\textsuperscript{96}

Scrapie has also been observed to pass from experimentally-infected mice to susceptible mice by contact.\textsuperscript{49,153,185} However, this appears to be an infrequent occurrence which might be due to inoculation through wounds.\textsuperscript{49,68} Mice have been infected by forced feeding,\textsuperscript{31,49,185} and ingestion of scrapie-contaminated materials by susceptible mice might also explain occasional occurrence of contact infection. Apparent "vertical" transmission of scrapie in mice, from diseased mothers to offspring, has been observed.\textsuperscript{59,152} Subsequent attempts to confirm this finding have not been successful,\textsuperscript{38} suggesting that such transmission might also result from occasional oral or cutaneous inoculation of susceptible animals with infectious material.

There appear to be differences, assumed to be genetic, in susceptibility to natural and experimental scrapie among different breeds of sheep. Parry\textsuperscript{164} concluded from field studies of sheep that all natural disease occurred in animals homozygous for an autosomal recessive allele. Dickinson et al\textsuperscript{164} reviewed Parry's and their own data and concluded that they were not consistent with genetic inheritance of scrapie but were better explained by vertical transmission from mother and possibly less often from father\textsuperscript{178} to offspring, as well as by later contact transmission.\textsuperscript{44} Goats and mice seem to be universally susceptible to experimental scrapie. In mice there is evidence for an autosomal gene that controls the length of incubation period of disease as well as anatomic distribution of histological lesions, although both these properties appear to depend on the strain of scrapie agent as well.\textsuperscript{45,50,51,82,71}

Transmissible mink encephalopathy (TME) is very similar to scrapie in clinical picture and histopathological findings,\textsuperscript{25,108} and indeed mink inoculated with sheep scrapie agent get a disease indistinguishable from natural mink encephalopathy.\textsuperscript{107} Mink encephalopathy, like scrapie, has been transmitted by the oral route,\textsuperscript{26,143} and it has been suggested that the disease may be a variant of scrapie introduced to mink in contaminated feed. There is no evidence for transplacental transmission of TME.\textsuperscript{108} All
varieties of mink tested have been susceptible to mink encephalopathy.

Experimental Transmission of Spongiform Encephalopathies

All four diseases have been transmitted to a variety of experimental animals (table I). Kuru was first transmitted to chimpanzees which developed an illness clinically resembling kuru in man. Chimpanzees had similar histopathological changes in the central nervous system differing from those of human kuru mainly in distribution of lesions, more severe in the cerebral cortex and less in the cerebellum, and in the absence of amyloid plaques. Kuru was later transmitted to spider monkeys and, subsequently, to four other genera of New World, two genera of Old World monkeys and another great ape, the gibbon.

The transmission of kuru to chimpanzees stimulated a successful attempt to transmit CJD from the brain of a patient to a chimpanzee. Primary transmission of CJD was confirmed by repeated transmission from the same tissue, serial passage of the infection in chimpanzees, and transmission of disease from a second patient. The clinical illness in chimpanzees with experimental CJD resembled human CJD (and differed from kuru) in the presence of marked spastic paralysis and myoclonic jerks. The histopathologic findings also resembled those of natural CJD. CJD has been transmitted to five genera of Old World, five of New World monkeys and the domestic cat. Transmission of CJD to the chimpanzee and the squirrel monkey has recently been confirmed in other laboratories. (Kuru had not been studied there, thus contamination of CJD experiments with the kuru agent is excluded.)

Scrapie and mink encephalopathy have even wider ranges of susceptible hosts. Both diseases have been experimentally transmitted to healthy sheep, goats and mink to cricetid (rat-like) rodents and to monkeys of both Old World and New World genera. It is anticipated that other animals susceptible to these infections will be found.

Although there are differences in the clinical and histopathological pictures of the spongiform encephalopathies in their natural and experimental hosts, the general similarity of the four diseases, especially in histopathological lesions, and their partially coextensive host range (each is transmissible to macaques and to squirrel monkeys) suggest that their agents might be variants of the same original agent, somewhat altered in biological properties. (Many conventional viruses undergo changes in biological properties, such as host range, incubation period and clinical illness elicited, after serial passages in different species of animals.) It is interesting that, when introduced into primates and mink, the scrapie agent, although serially transmissible in its new hosts, appears to have lost its ability to replicate readily in mice.

In table II are summarized incubation periods of experimental kuru and CJD in susceptible animals inoculated intracerebrally. Neither experimental disease has been detected clinically prior to ten months after inoculation. Animals infected with brain of kuru patients have required as long as 8.5 years before showing first signs of disease and on serial (animal-to-animal) passage as long as two years. The mean incubation period for chimpanzees infected with kuru drops significantly with the first serial passage, but not on subsequent passages.

The longest incubation period for successfully transmitted CJD has been almost six years. Mean incubation period after inoculation of chimpanzees (and probably other primates) inoculated with human CJD tissue has been shorter than that of chimpanzees with kuru but there has been no reduction in mean incubation period of experimental CJD on serial passage.
The infectious agents of all four diseases persist in tissue cultures prepared from the brains of infected animals. Actual replication of the scrapie and TME agents have been demonstrated in serial subcultures of brain tissues from infected animals and similar studies are in progress with kuru and CJD. When normal tissues were incubated with suspensions of infectious scrapie agent there was no evidence of propagation. A spontaneously transformed culture of CJD brain tissue has been studied but the transformed cells did not appear to contain the infectious CJD agent. Although differences in cellular morphology have been described between normal cell cultures and those infected with scrapie and CJD agents, none of these has provided a reliable assay method.

Physical Properties of the Agents of Spongiform Encephalopathies

Until there is a satisfactory assay system for the infectious agents of the spongiform encephalopathies in vitro, all studies of the physical properties of the agents and of the pathogenesis of the diseases will require bioassays. In such an assay, an animal of a species known to be susceptible is inoculated with material and observed for a long time. If the animal gets a typical clinical illness with histopathological findings of spongiform encephalopathy, it is concluded that infectious agent was present in the inoculum. Such experiments are very time-consuming and expensive.

Fortunately, the incubation periods of strains of scrapie agent adapted to mice are relatively short; clinical signs of scrapie may appear less than six months after intracerebral inoculation. The mouse has been used to study the size, sedimentation behavior and subcellular location of the scrapie agent and its inactivation by chemical and physical treatments. The physical properties of the scrapie agent suggest that it must have a structure which differs markedly from those of known microbial pathogens of animals.

By membrane filtration, the size of the scrapie agent has been estimated to be between 50 and 27 nm and by gel filtration at least 5 x 10^6 daltons. Membrane filtration of the agent of mink encephalopathy indicated that it also passed through pores of 50 nm average diameter. In preliminary studies the infectious agent of kuru failed to pass through a membrane filter of 100 nm average pore diameter. Additional membrane filtration studies of both CJD and kuru agents, now in progress, have not altered this estimate.

Particles of such size are small, but they should be visible by electron microscopy if present in sufficient quantity. No such particles have been seen in partially-purified preparations containing more than 10^6 infectious doses (lethal doses for mice) of the scrapie agent per ml. However, vesicular structures of about the right size have been described inside intact brain cells of animals with scrapie. Similar particulate structures have been described in brain cells of chimpanzees with CJD; larger virus-like structures have also been reported in brain cells of patients with CJD. It is not clear that any of these structures are particles of the infectious agents.

The scrapie agent is highly resistant to ionizing radiation, consistent with much smaller ”target” size than that estimated by filtration (perhaps only 2 x 10^5 daltons). It is also more resistant to ultraviolet light than any other known pathogen.

The agents of scrapie, TME and kuru are highly resistant to heat, even withstanding 30 minutes of boiling, although there is rapid loss of infectivity at temperatures above 87.5°, and inactivation is increased by fluorocarbon treatment and filtration. The scrapie agent is also relatively resistant to treatment with formalin, to a pH range of 2.5 to 10.5 and to betapropiolactone (which inactivate conven-
tional viruses) but sensitive to treatment with periodate, phenol, urea, 2-chloro-ethanol and some detergents and lipid solvents. The agent of mink encephalopathy behaves similarly. When homogenates of scrapie-infected tissues are fractionated, infectivity appears to be associated with preparations rich in fragments of membranes which are probably plasma membranes and perhaps endoplasmic reticulum as identified by the activities of enzymes typical of those structures.

It appears, then, that infectious preparations of the scrapie agent always contain fragments of membranes of the host cell, and that infectivity of scrapie is destroyed by reagents which dissociate membrane structures (as well as by several reagents which inactivate other cell components), all suggesting a close association with and location in host cell membranes. The resistance of the agent to ionizing radiation suggests that its replicating portion is probably very small (much smaller than the overall size of the particle in which it is contained), and its resistance to ultraviolet means that if the replicating moiety is nucleic acid, it is probably much smaller than the nucleic acids of conventional viruses.

Some have concluded that these properties of the scrapie agent are inconsistent with replication based on nucleic acid, and ingenious alternative coding mechanisms, such as self-replication of abnormal membranes, have been postulated. However, it may be premature to dismiss nucleic acid as the genetic material of these agents. Recent studies of plant diseases have uncovered a class of virus-like pathogens which appear to be naked molecules of ribonucleic acid (RNA) of very small size, about $8 \times 10^4$ daltons, which should be able to code for only about 60 amino acids. These plant "viroids" resemble the scrapie agent in their resistance to heat and in their close association with components of host cells. Because viroids differ markedly from the scrapie agent in their resistance to treatment with phenol, urea and lipid solvents, and are thought to be associated with nuclear RNA rather than cytoplasmic membrane components, it is also premature to conclude that scrapie is a viroid. However the plant viroids serve to demonstrate that a self-replicating nucleic acid of a very small size can still carry sufficient information to elicit disease. It might be that the agents of the spongiform encephalopathies contain tiny pieces of nucleic acid tightly bound to fragments of plasma membranes.

Pathogenesis of the Spongiform Encephalopathies

The term pathogenesis is loosely used to describe various interactions between an infectious agent and its host. Most studies of the pathogenesis of viral infections have been concerned with the spread of the infectious agent through the body of its host, with the mechanisms by which the agent injures the host and with the mechanisms by which the host defends itself.

HOST DEFENSES AGAINST INFECTION

Evidence has already been alluded to for one possible host defense against infection with these agents—genetic resistance. Limited evidence also indicates that age may also play a role; it has been reported that some newborn mice survived intraperitoneal inoculation with a dose of scrapie agent sufficient to kill 100 percent of older mice. In general, the spongiform encephalopathies are remarkable in the absence of detectable defense responses by the host.

The agents of the spongiform encephalopathies appear to be totally lacking in antigenicity for their hosts. All attempts to detect serum antibodies and delayed hypersensitivity to the agents have been without success. Sera of animals with scrapie contained no detectable antibodies.
to the infectious agent as measured by complement fixation, immunoprecipitation, indirect hemagglutination, passive-cutaneous and systemic anaphylaxis,\textsuperscript{29} direct and indirect immunofluorescence,\textsuperscript{154,168} and both conventional and antiglobulin-potentiated neutralization tests.\textsuperscript{93,95,167,168} No antigen-antibody complexes have been found to explain that absence of antibodies.\textsuperscript{93,168} The basic humoral immune mechanisms of mice with scrapie are intact; antibodies are produced normally in response to immunization with other particulate and soluble antigens.\textsuperscript{85,93}

As might have been predicted from the lack of inflammation in the central nervous system in spongiform encephalopathies, there is no detectable cell-mediated immunity. Splenic lymphocytes of scrapie-affected mice failed to transform to blast cells in suspensions of the infectious agent\textsuperscript{4} although the cellular immune mechanism in scrapie-affected mice is of normal competence as assessed by histology of lymphoid organs, number of T cells (lymphocytes bearing theta antigen),\textsuperscript{141} blastogenesis in the presence of phytohemagglutinin\textsuperscript{4} and allograft rejection.\textsuperscript{141} Limited studies of mink encephalopathy\textsuperscript{148} and of kuru\textsuperscript{77} indicate that in these diseases as well, there are neither detectable antibodies in serum nor antigen-antibody complexes in tissues, and, at least in kuru, no evidence of cellular immunity to the infectious agents.

Interferon has not been detected in blood, spleens or brains of mice with scrapie although the animals produce interferon normally when superinfected with conventional viruses or treated with synthetic nucleic acid.\textsuperscript{125,181} Slightly decreased replication of murine tumor viruses\textsuperscript{32} and Flaviviruses\textsuperscript{4,56} in scrapie-infected mice has been reported. Delayed onset of disease owing to competition between rapid and slow-acting strains of scrapie agent has also been noted.\textsuperscript{48} However, cell cultures prepared from animals with CJD supported normal replication of several conventional viral agents.\textsuperscript{87}

It is not surprising that attempts to immunize animals with inactivated preparations of the scrapie agent have failed.\textsuperscript{4,103} Immunosuppression with cyclophosphamide,\textsuperscript{182} antilymphocytic serum\textsuperscript{155} and thymectomy plus irradiation\textsuperscript{141} also had no effect on incubation period or course of disease in mice with scrapie. Repeated treatment with concentrated interferon\textsuperscript{97} and with interferon inducers\textsuperscript{69,181} gave mice no protection from scrapie (although there has been one preliminary report of successful prophylaxis with tilorone).\textsuperscript{39} Recent studies reported some protection against scrapie by steroids\textsuperscript{161} and even by peanut oil,\textsuperscript{162} and amantadine is claimed to have improved the clinical status of patients with CJD.\textsuperscript{172} These findings remain unconfirmed.

**SPREAD THROUGH THE BODY**

The paths by which the agents of spongiform encephalopathies spread through the body are probably influenced by the species and perhaps the breed of animal infected, the route by which infection occurs, and by the strain of agent and the infectious dose. This variability is similar to that encountered in pathogenesis of more conventional bacterial and viral infections. The importance of these variables in pathogenesis is illustrated by studies of experimental scrapie conducted by Hadlow and colleagues.\textsuperscript{103} When goats were inoculated intracerebrally (ic) or subcutaneously (sc) with the same concentration and volume of scrapie agent, all goats inoculated ic got scrapie, but only three of four goats inoculated sc got the disease, and they had incubation periods almost twice as long as those with ic inoculation. When tissues of diseased goats were inoculated into mice to test for the presence of infectious agent, different patterns of distribution were found in goats that had been inoculated by the two routes. Goats inoculated ic had scrapie agent throughout the central nervous system and in peripheral nerve, adrenal and pituitary,
parotid salivary gland, and in lymph nodes but not in other organs. Goats inoculated sc had the agent not only throughout the central nervous system and in all the same organs as the ic-inoculated animals (except in the parotid) but also in many other organs, including lymphoid organs (tonsils, spleen and thymus), liver and gastrointestinal tract, nasal mucosa, and in the CSF. After an even smaller dose of scrapie agent was injected into goats sc, the pattern of distribution of the agent in tissues was slightly different. It has been of greatest interest to study pathogenesis of experimental spongiform encephalopathies where conditions of infection resemble those in the natural disease, in so far as those conditions are known. Epidemiological and experimental evidence suggests that in human kuru the inoculum is actually infected tissue and the portal of infection is through breaks in skin or mucous membranes; a similar mechanism might be responsible for "contact" spread of scrapie in mice and, perhaps, in ungulates. Such considerations impelled Hadlow and his colleagues to study pathogenesis of experimental scrapie in mice and goats infected by the subcutaneous route. In both mice and goats, the scrapie agent seemed to spread through the body of the host in similar ways.

Mice were inoculated subcutaneously with a large dose of scrapie agent, were killed and their organs were tested at intervals after inoculation. One week after inoculation a small amount of agent, probably part of the original inoculum, was found in the spleen; however, at two weeks no agent could be detected anywhere in the bodies of the mice. Four weeks after inoculation the scrapie agent was again detected in spleen and lymph nodes. By eight weeks it was found throughout lymphoid tissues, including thymus, and in salivary glands in much larger amounts than those inoculated, indicating that multiplication had taken place. The agent was first detected in the spinal cord at 12 weeks and in the brain at 16 weeks, by which time it was distributed widely throughout the body in lymphoid tissues, salivary glands, lungs and intestines. Mice remained clinically well until 23 weeks after inoculation when some showed clinical signs of neurological dysfunction and histopathological evidence of proliferation and hypertrophy of astrocytes. During the final weeks of infection, the agent appeared in a few new organs (e.g., in bone marrow and kidney) and increased in amount in the central nervous system while decreasing in lymphoid tissues. This pattern of pathogenesis is similar to that seen in some conventional viral infections, only with a much slower time course. The inoculated agent is taken up by the spleen and probably by other lymphoid organs. It goes into "eclipse" during which early events of replication may take place before the appearance of complete infectious progeny. The agent then spreads to other lymphoid tissues, later to visceral organs and finally to the central nervous system. Replication of agent goes on within the central nervous system for several weeks before onset of overt disease. Finally there is a gradual decrease in the amount of agent within lymphoid tissues by the time of death.

When goats were inoculated subcutaneously with very small doses of scrapie agent (much lower than those in the mouse experiment) and the animals killed and examined at intervals, no infectious activity was detected in any organ tested for almost six months. The agent was then detected at 24 weeks in the regional lymph node draining the inoculation site of one goat. During the subsequent year and a half the agent was recovered from several goats in local lymph nodes and from other lymphoid tissues including distant nodes, tonsils and spleen, (and in one goat a very small amount from the intestine). After the middle of the third year (140 weeks), the agent was never again detected in any lymphoid tissue. Shortly before the middle of the third year
(128 weeks), the scrapie agent was first detected in the central nervous system of one asymptomatic goat, distributed through midbrain, medulla and spinal cord. At the end of the third year (150 weeks), one goat developed signs of scrapie and the agent was isolated from all areas of brain and cord as well as from the sciatic nerve and pituitary. During the final two years of the experiment, the agent was recovered from three more goats. In each, it was distributed throughout the central nervous system (though absent from cerebral cortex of one animal) and was found in the sciatic nerve of two goats and in the pituitary and adrenal of one. The scrapie agent could not be found in any other tissues.103

In spite of the small infecting dose, this study of goats implies spread of the scrapie agent by paths generally similar to those in mice inoculated by the same route. The agent makes its way from the subcutaneous inoculated site to the local lymph node and, perhaps, if the inoculum contains enough agent, to other lymphoid organs as well; it then goes into eclipse and replicates. There is secondary spread to other organs, including lymphoid organs, and further multiplication. At about the time that the agent is increasing in amounts in the central nervous system, several months before the onset of clinical illness, it disappears from lymphoid tissues.

One striking difference was noted in the two experimental infections. In mice high titers of scrapie agent were found in salivary glands and intestines, whereas in goats the nasal mucosa, salivary glands, saliva and intestines contained little or none. Hadlow103 speculated that infectious saliva or intestinal secretions might serve as a source of the contact infections seen in mice, while the absence of infectious secretions in goats might explain the fact that goats with scrapie do not appear to be contagious.

It must not be concluded that the paths of spread of the scrapie agent through the body have been completely worked out, even in experimental infections of mice. Other routes of inoculation, not so well studied as the sc route, appear to give somewhat different patterns of pathogenesis. For example, in some studies of ic or intraperitoneal inoculation, no eclipse period was seen although there is evidence that in infections by both these routes, replication of agent may occur in spleen before brain.186 Congenital asplenia or splenectomy of mice before or during the first month after intraperitoneal (but not ic) inoculation with scrapie agent have been reported to prolong the incubation period47,72 and the same "sinc" gene that is believed to control the incubation period and anatomic distribution of lesions of scrapie in mice has also been interpreted as influencing the time course of multiplication of the scrapie agent in the spleen.46 The strain of agent and route of inoculation183 and even the species of animal through which the agent was previously passaged have been reported to affect distribution of lesions.187

According to the scheme postulated for sc infections, the scrapie agent should travel from the inoculation site to lymphoid organs and from there throughout the body in lymphatic and blood vessels. Hadlow was unable to demonstrate infectivity in the blood of any mice61 or goats103 although other investigators have reported finding the agent in blood of scrapie-infected rodents.35,65,85 One study gave histopathological evidence for preferential spread of the scrapie agent from the inoculation site to the corresponding region of spinal cord.66,70 Such spread does not seem consistent with the pattern discussed previously and would need a different pathway.

In experimental mink encephalopathy, the route of infection (with relatively large doses of agent) was found to affect the distribution of agent throughout the body,143 although highest titers were always found in the brain. In symptomatic mink, which had been infected by intramuscular inoculation, moderate amounts of agent were detected in
liver, spleen, kidney and bladder, and small amounts in lung and muscle. A small amount was also demonstrated in feces suggesting that, as postulated for scrapie in mice, this might be a source of contact infection. Although the agent was found in kidneys and bladder, it did not make its way into urine in detectable amounts. No infectivity was found in blood. Recently the corneal epithelium of hamsters with TME was found to contain infectious agent; the amount of agent dropped slightly when cells were passaged in culture, suggesting that the agent was associated with some non-replicating element in the cornea, perhaps nerve endings.146

A comparative study of scrapie and transmissible mink encephalopathy after ic inoculation of Syrian hamsters showed that both agents replicated to much higher titers in brain than in spleen, and that histological lesions appeared first near arachnoid and ventricular surfaces, suggesting spread of the inoculum through CSF.147 Serial spread of TME agent after subcutaneous inoculation has not been studied.

Because of their inconvenient assays, even less is known of pathogenesis of kuru and CJD. The portals of natural infection (except in the corneal transplant patient) remain conjectural. Most experimental infections have been initiated by direct intracerebral inoculation although, as summarized in table III, other routes, such as intramuscular and combined intravenous-intraperitoneal, have also been effective. However, administration of large doses of infectious kuru agent by nasogastric tube failed in four attempts to transmit disease to chimpanzees observed for six years or longer. If these results in chimpanzees can be extrapolated to man, they suggest that breaks in skin or mucous membranes and not the intact gastrointestinal tract are the portal for the kuru agent in human cannibalism.

Serial studies of pathogenesis of kuru and CJD encephalopathies have not been performed, and information is available only on distribution of the agents in organs of clinically ill patients and animals.

Brain tissues of most patients with kuru and CJD tested in our laboratories (table IV) contained the infectious agents as evidenced by transmission of typical disease to primates. It may be significant that among the six CJD patients whose brains have not transmitted disease to primates three years or more after inoculation, two had the amyotrophic form of the disease, and no successful transmission has yet been made from that type of patient.180 The brains of animals with experimental kuru and CJD have always contained the infectious agents in amounts which, on the few occasions in

### TABLE III

<table>
<thead>
<tr>
<th>Effective Routes of Inoculation</th>
<th>Kuru</th>
<th>CJD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct intracerebral</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Combined intravenous, intraperitoneal, intramuscular, subcutaneous</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gastric*</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*Four chimpanzees, longest negative experiment > seven years

+Disease transmitted -Disease not transmitted

### TABLE IV

<table>
<thead>
<tr>
<th>Kuru and Creutzfeldt-Jakob Disease (CJD)</th>
<th>Agent in Brain (Isolation of Agent from Brains of Patients and Animals with Kuru and CJD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Isolations*</td>
<td>Number of Attempts</td>
</tr>
<tr>
<td>Kuru</td>
<td>Human 15/16+</td>
</tr>
<tr>
<td></td>
<td>Experimental 23/23</td>
</tr>
<tr>
<td>CJD</td>
<td>Human 46/59+</td>
</tr>
<tr>
<td></td>
<td>Experimental 9/9</td>
</tr>
</tbody>
</table>

*Isolation attempt: Inoculation of brain suspension into a susceptible primate. Positive: histological spongiform encephalopathy. Negative experiments in progress less than three years are not included.

*Includes as negative, the brain of one patient prepared as tissue culture only.
Kuru and Creutzfeldt-Jakob Disease (CJD): Agent in Pools of Organs Outside Central Nervous System
(Transmission of Disease from Pooled Viscera of Patients or Animals with Kuru and CJD to Animals)

<table>
<thead>
<tr>
<th>Positive Isolations</th>
<th>Organ Pool</th>
<th>Incubation (Months)</th>
<th>Longest Negative Experiment (Years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Attempts*</td>
<td>Kuru</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>Liver, kidney, spleen</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>Liver, kidney, spleen, node</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>CJD</td>
<td>Liver, kidney, spleen, node</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>Liver, kidney, spleen</td>
<td>--</td>
</tr>
</tbody>
</table>

*Each "isolation attempt" was an inoculation of suspension of pooled organs from one patient or experimental animal into a susceptible primate. Only experiments in progress for three years or more are included as negative attempts.

which they have been determined, appear to be high (at least $10^5$ infectious doses per gram of brain in kuru and $10^4$ in CJD).

The agents of kuru and CJD have also been detected in tissues outside the central nervous system, but much less regularly than in brain. When pooled visceral tissues (liver, kidney, spleen and sometimes lymph node) of patients and animals with symptomatic kuru and CJD were assayed for infectivity, only occasional pools were found to contain the agents (table V). Individual tissues and body fluids of patients and animals in late stages of the two diseases are still under study. Preliminary results (table V) show the kuru agent in one human lymph node and spleen as well as the CJD agent in one human lymph node, one kidney and three liver specimens. A smaller number of attempts to recover the agents from the same organs of primates remain negative. The amounts of the agents in these organs are not known. As shown in table VI, several other tissues and biological fluids (including blood) have also been tested without finding infectivity. Although these few observations hardly form the basis for a general scheme of pathogenesis of kuru and CJD, a certain similarity to experimental scrapie is apparent. During later stages of clinical kuru and CJD, both in naturally-infected people and in experimental primates inoculated by the ic route, the agent is only infrequently found outside the central nervous system both in lymphoid and other organs.

MECHANISMS OF CELLULAR DAMAGE

Another aspect of pathogenesis concerns the actual mechanisms by which the infection damages the cells of the host thus

## TABLE VI

<table>
<thead>
<tr>
<th>Kuru</th>
<th>CJD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Isolations</td>
<td>Positive Isolations</td>
</tr>
<tr>
<td>Number of Attempts*</td>
<td>Number of Attempts*</td>
</tr>
<tr>
<td>Patients</td>
<td>Animals</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Spleen</td>
<td>1/4</td>
</tr>
<tr>
<td>Lymph node</td>
<td>2/4</td>
</tr>
<tr>
<td>Liver</td>
<td>0/3</td>
</tr>
<tr>
<td>Kidney</td>
<td>0/10</td>
</tr>
<tr>
<td>Muscle</td>
<td>0/2</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td>Muscle-lung-heart</td>
<td></td>
</tr>
<tr>
<td>Placenta-amnion†</td>
<td>0/1</td>
</tr>
<tr>
<td>Serum/blood§</td>
<td>0/10</td>
</tr>
<tr>
<td>Cerebrospinal fluid§</td>
<td>0/1</td>
</tr>
<tr>
<td>Urine/</td>
<td>0/1</td>
</tr>
<tr>
<td>Milk§</td>
<td>0/1</td>
</tr>
</tbody>
</table>

*Includes only experiments in progress more than three years.
†One patient.
§Pool of three or four patients or animals.
/Pool of eight patients.
causing disease. Basic mechanisms of cellular damage are not understood, but observations of biochemical and ultrastructural changes in spongiform encephalopathies suggest avenues for further study. Kimberlin found an increase in deoxyribonucleic acid (DNA) synthesis in brains of mice with scrapie, mostly in ependymal and glial cells. Increased DNA synthesis was not seen in other organs in which the scrapie agent was present and was associated with nuclear fractions of cells which contained little infectivity. Autoradiographic tracing demonstrated that the distribution of cells in which increased DNA synthesis was taking place did not parallel histopathologic changes, nor did their number increase as disease progressed. Others have showed similar increased incorporation of precursors into DNA-polysaccharide complexes in cytoplasm and membrane fractions of brains and spleens of scrapie-infected mice. Again, however, these fractions were poor in infectivity. Therefore, it seems likely that the increased DNA synthesis observed is not a primary event either in replication of the scrapie agent or in evolution of the cellular lesions. Abnormalities in cerebral water, lipids and other substances are also probably secondary to neuronal degeneration, glial hypertrophy and proliferation.

Kimberlin and Marsh recently measured 15 different biochemical parameters in brains of hamsters with scrapie and TME and found essentially the same abnormalities in both diseases. Biochemical changes never preceded histopathological changes, and the pattern of abnormalities in hamsters differed from that in mice with scrapie. These observations further suggest that none of the biochemical abnormalities described so far are directly involved in the evolution of spongiform encephalopathy or the replication of the agents, and that all are secondary changes.

Lampert remarked on the great similarity of the electron microscopic lesions of scrapie, kuru and CJD. Status spongiosus by light microscopy seemed to correspond to areas of vacuolation and of “swelling” of the cytoplasm. Vacuoles were postulated to begin with focal clearing of cytoplasm in which normal organelles were replaced by fine granular material. Cleared areas were surrounded by plasma membrane, which was often found to have ruptured, with accumulations of curled fragments of membranes at the sites of rupture. Lampert found vacuoles in cytoplasm of neuronal axons, dendrites and perikarya. Swelling and clearing of adjacent areas of neighboring neurons led to their fusion. In astrocytes, besides nonspecific reactive changes, only swelling and herniation were observed, especially in areas adjacent to diseased neurons. An occasional astrocyte even seemed to have fused with a nerve cell. The frequent involvement of adjacent areas of cells by the disease process suggested to Lampert that the primary site of damage might be in the plasma membranes and that hypothesis is consistent with evidence linking the infectious agents with those membranes.

Bignami and Forno noted that swelling and vacuolation frequently distorted the preterminal axons of neurons. They proposed that such changes might disturb synapses, causing the markedly abnormal EEGs seen in CJD. Cathala et al observed the onset of abnormal EEG in a chimpanzee with CJD as early as five months after ic inoculation and eight months before the onset of overt clinical disease. In a recent study designed to examine early events in experimental kuru, morphological changes were found as early as four weeks after ic inoculation of spider monkeys with large doses of the kuru agent. There were membrane-bounded cytoplasmic vacuoles, similar to those described by Lampert only with their lining membranes intact, both in neurons and astrocytes. Large numbers of binucleate neurons were seen throughout limbic cortex, striatum and hypothalamus, and binucleate Purkinje cells were found in the cerebellum. (Purkinje cells are enclosed
in baskets and cannot fuse with neighboring cells, so their nuclei must have divided.) In the cerebellar nodulus, mostly in neuronal dendrites and perikarya, were peculiar concentric laminar arrays derived from endoplasmic reticulum. These changes clearly preceded the appearance of astrocytic hypertrophy and proliferation formerly thought to be the earliest cellular change in kuru. It remains to be seen if such morphological changes occur in kuru after other routes of infection and in the other spongiform encephalopathies.

Conclusion

The study of pathogenesis of spongiform encephalopathies has only begun. It is not known how the agents produce pathological changes, why they appear harmlessly in many organs while devastating the central nervous system, and why the body does not recognize them as alien and mount a defense response. There will probably be no answers to these questions until the structure and replication of the agents are understood.

References


87. Gibbs, C. J., Jr., Cornellus, R., and Gajdusek,


119. Hunter, G. D. and Millson, G. C.: Studies on the heat stability and chromatographic behavior of the


154. Moulton, J. E. and Palmer, A. C.: Attempts to


