

# UNCONVENTIONAL VIRUSES AND THE ORIGIN AND DISAPPEARANCE OF KURU

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Kuru was the first chronic degenerative disease of man shown to be a slow virus infection, with incubation periods measured in years and with a progressive accumulative pathology always leading to death. This established that virus infections of man could, after long delay, produce chronic degenerative disease and disease with apparent heredofamilial patterns of occurrence, and with none of the inflammatory responses regularly associated with viral infections. Soon thereafter, several other progressive degenerative diseases of the brain were likewise attributed to slow virus infections (see Tables 1 and 2). These include delayed and slow measles encephalitis, now usually called subacute sclerosing panencephalitis (SSPE), progressive multifocal leukoencephalopathy (PML), and transmissible virus dementias usually of the Creutzfeldt-Jakob disease (CJD) type. Thus, slow virus infections, first recognized in animals, became recognized as a real problem in human medicine.

Kuru has led us, however, to a more exciting frontier in microbiology than only the demonstration of a new mechanism of pathogenesis of infectious disease, namely the recognition of a new group of viruses possessing unconventional physical and chemical properties and biological behavior far different from those of any other group of microorganisms. However, these viruses still demonstrate sufficiently classical behavior of other infectious microbial agents for us to retain, perhaps with misgivings, the title of "viruses". It is about these unconventional viruses that I would further elaborate.

The group consists of viruses causing four known natural diseases: two of man, kuru and CJD, and two of animals, scrapie in sheep and goats, and transmissible mink encephalopathy (TME) (Table 1). The remarkable unconventional properties of these viruses are summarized in Tables 3 and 4. Because only primate hosts have been available as indicators for the viruses

Table 1. Naturally-occurring slow virus infections caused by unconventional viruses (subacute spongiform virus encephalopathies)

<i>In man :</i>	<i>In animals :</i>
Kuru	Scrapie
Transmissible virus dementia	In sheep
Creutzfeldt-Jakob disease	In goats
Sporadic	Transmissible mink encephalopathy
Familial	
Familial Alzheimer's disease	

Table 2. Slow infections of man caused by conventional viruses

<i>Disease</i>	<i>Virus</i>
Subacute post-measles leukoencephalitis	Paramyxovirus—defective measles
Subacute sclerosing panencephalitis (SSPE)	Paramyxovirus—defective measles
Subacute encephalitis	Herpetovirus—Herpes-simplex
	Adenovirus—Adeno-types 7 and 32
Progressive congenital rubella	Togavirus—rubella
Progressive panencephalitis as a late sequela following congenital rubella	Togavirus—defective rubella
Progressive multifocal leukoencephalopathy (PML)	Papovavirus—JC; SV-40
Cytomegalovirus brain infection	Herpetovirus—cytomegalovirus
Epilepsia partialis continua (Kozhevnikov's epilepsy) and progressive bulbar palsy in USSR	Togavirus—RSSE and other tick-borne encephalitis viruses
Chronic meningoencephalitis in immunodeficient patients	Picornaviruses—poliomyelitis, ECHO virus
Crohn's disease	Unclassified—RNA virus
Homologous serum jaundice	Unclassified—Hepatitis B, Dane particle
Infectious hepatitis	Parvovirus—Hepatitis A
	Unclassified—Hepatitis B, Dane particle
	Unclassified—Hepatitis C

Table 3. Atypical physical and chemical properties of the unconventional viruses

1. Resistant to formaldehyde
2. Resistant to  $\beta$ -propiolactone
3. Resistant to ethylenediamine tetraacetic acid (EDTA)
4. Resistant to proteases (trypsin, pepsin)
5. Resistant to nucleases (RNase A and III, DNase I)
6. Resistant to heat (80° C); incompletely inactivated at 100° C
7. Resistant to ultraviolet radiation: 2540 Å
8. Resistant to ionizing radiation ( $\gamma$  rays): equivalent target 150,000 daltons
9. Resistant to ultrasonic energy
10. Atypical UV action spectrum: 2370 Å inactivation =  $6 \times 2540$  Å inactivation
11. Invisible as recognizable virion by electron microscopy (only plasma membranes, no core and coat)
12. No non-host proteins demonstrated

causing human disease (or, more recently, cats (24) and guinea pigs (48) for CJD and mink for kuru (24), but with long incubation periods), it has been impossible to characterize these agents well; knowledge of the properties of unconventional viruses is based mostly on the study of the scrapie virus adapted to mice (39, 60) and hamsters (42, 49, 57). The unusual resistance of the viruses to various chemical and physical agents (items 1 to 9 in Table 3), separate this group of viruses from all other microorganisms. In fact, their resistance to ultraviolet (UV) and ionizing radiation, the atypical UV action spectrum for inactivation, and the failure to contain any demonstrable non-

host protein, make these infectious particles unique in the biology of replicating infectious agents, and it is only to the newly-described viroids causing six natural plant diseases [potato spindle tuber disease (7-10, 34), chrysanthemum stunt disease, citrus exocortis disease (57, 58), Cadang-Cadang disease of coconut palms (55), cherry chlorotic mottle, and cucumber pale fruit disease] that we must turn for analogy (see Figs. 1a, 1b).

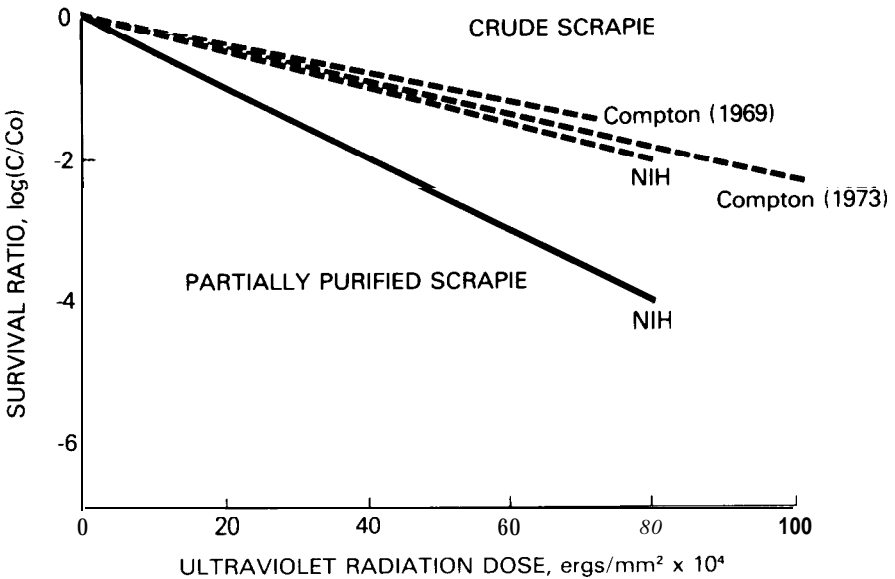


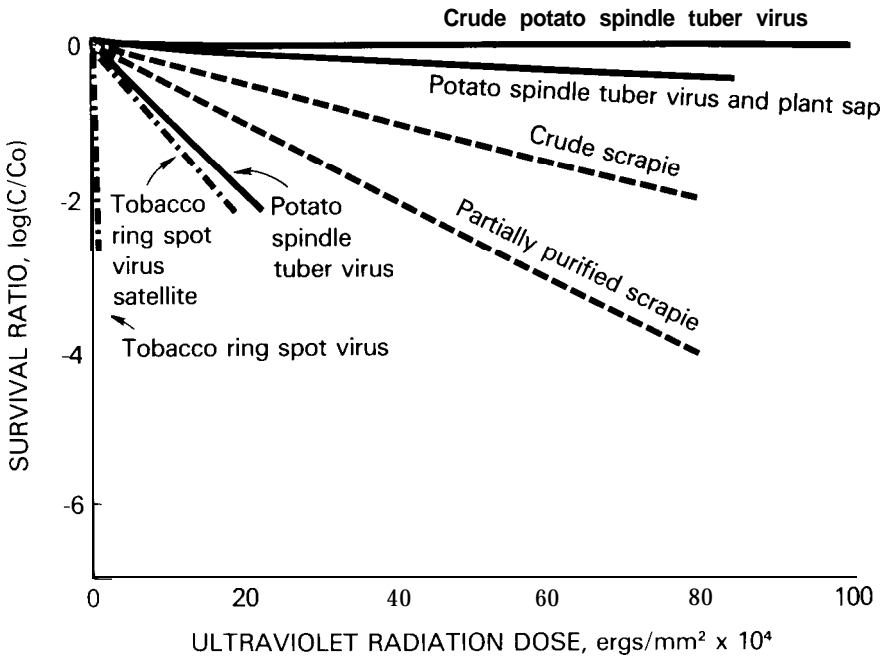
Figure 1. Scrapie virus is unusually resistant to UV inactivation at 2534 to 2540 Å (45,46). This has been interpreted as an indication that it contains no nucleic acid. Recent data from Diener (7,8), however, indicate that the smallest plant virus (potato spindle tuber viroid: PSTV), which is a naked single-stranded RNA of 120,000 daltons, is 90 times more resistant to such UV inactivation than are conventional plant viruses. Since the small infectious nucleic acid of tobacco ring spot virus satellite virus (single-stranded RNA of 75,000 daltons) is 70 times as resistant as are conventional viruses, this high resistance of the two plant viruses is probably because of their small size. The small RNA of PSTV is apparently single-stranded with a circular structure and of such small size that it could code for only about 25 amino acids. Inactivation of scrapie virus by ionizing radiation yields a target size for inactivation equivalent to molecular weight of 150,000 (45). These data, taken with the association of scrapie virus with smooth vesicular membrane during purification and the absence of recognizable virions on electron microscopic study of highly infectious preparations, suggest that the virus is a replicating membrane subunit. It may contain its genetic information in a small nucleic acid moiety incorporated into the plasma membrane. The membrane appears to be the host membrane without altered antigenicity.

1a. Scrapie virus in crude suspensions of mouse brain has been very resistant to UV inactivation at 2540 Å (36,45,46). These three experiments with crude scrapie are in close agreement: NIH (45); Compton A (36); Compton B (45).

Survival ratio is calculated as  $\log C/C_0$ :

$$\log_{10} \frac{\text{Infectivity titer after irradiation}}{\text{Infectivity titer before irradiation}}$$

Partially purified scrapie (suspension of scrapie mouse brain clarified by two treatments with Genetron in the cold) is somewhat less resistant to UV inactivation, but still much more resistant than other conventional viruses.



1b Scrapie inactivation by UV irradiation is compared with that of a conventional plant virus, tobacco ring spot virus, and with the tobacco ring spot virus satellite and potato spindle tuber viroid, both of which contain nucleic acid of molecular weights under 100,000 daltons (7,8). PSTV, as a highly purified nucleic acid, becomes almost totally resistant to UV inactivation (2540 Å) when mixed with clarified normal plant sap, while other viruses placed in this sap are not rendered so resistant. In the crude extract from infected plants the PSTV is almost totally resistant to UV inactivation (7,8). In a second such experiment a different Genetron treated scrapie preparation showed less reduction in UV resistance (45).

## UNCONVENTIONAL VIRUSES AS A CAUSE OF THE SUBACUTE SPONGIFORM VIRUS ENCEPHALOPATHIES

Kuru and the transmissible virus dementias have been classified in a group of virus-induced slow infections that we have described as subacute spongiform virus encephalopathies because of the strikingly similar histopathological lesions they induce; and, scrapie and mink encephalopathy appear, both from their histopathology, pathogenesis, and the similarities of their infectious agents, to belong to the same group (Table 1). The basic neurocytological lesion in all of these diseases is a progressive vacuolation in the dendritic and axonal processes and cell bodies of neurons and, to a lesser extent, in astrocytes and oligodendrocytes; an extensive astroglial hypertrophy and proliferation; and, finally, spongiform change or status spongiosus of grey matter (1, 2, 41, 43, 44). These atypical infections differ from other diseases of the human brain which have been subsequently demonstrated to be slow virus infections (Table 2) in that they do not evoke a virus-associated inflammatory response in the brain; they usually show no pleocytosis nor marked rise in protein in the cerebrospinal fluid throughout the course of infection; furthermore, they show

no evidence of an immune response to the causative virus and, unlike the situation in the other virus diseases, there are no recognizable virions in electron-microscopic sections of the brain (Table 4).

There are other slow-infections of the central nervous systems which are caused by rumbling nonproductive, even defective, more conventional viruses including measles virus, papovaviruses (JC and SV-40-PML), rubella virus, cytomegalovirus, herpes-simplex virus, adenovirus types 7 and 32, and probably RSSE virus (Table 2). However, unlike these "conventional" viruses the "unconventional" viruses of the spongiform encephalopathies have unusual resistance to ultraviolet radiation and to ionizing radiation (45), to ultrasonication, to heat, proteases and nucleases, and to formaldehyde, P-propiolactone, ethylenediamine tetraacetic acid (EDTA), and sodium desoxycholate (Table 4). They are moderately sensitive to most membrane-disrupting agents such as phenol (90%), chloroform, ether, urea (6 M), periodate (0.01 M), 2-chloroethanol, alcoholic iodine, acetone, chloroform-butanol, and hypochlorite (0.5-5.0%) (Table 5). Virions are not recognized on electron microscopic study of infected cells *in vivo* or *in vitro*, nor in highly infectious preparations of virus concentrated by density-gradient banding in the zonal rotor (60). This has led to the speculation that the infectious agents lack a nucleic acid, perhaps are even a self-replicating membrane fragment.

Table 4. Atypical biological properties of the unconventional viruses

- 
1. Long incubation period (months to years; decades)
  2. No inflammatory response
  3. Chronic progressive pathology (slow infection)
  4. No remissions or recoveries: always fatal
  5. "Degenerative" histopathology: amyloid plaques, gliosis
  6. No visible virion-like structures by electron microscopy
  7. No inclusion bodies
  8. No interferon production or interference with interferon production by other viruses
  9. No interferon sensitivity
  10. No virus interference (with over 30 different viruses)
  11. No infectious nucleic acid demonstrable
  12. No antigenicity
  13. No alteration in pathogenesis (incubation period, duration, course) by immunosuppression or immunopotentialization:
    - (a) ACTH, cortisone
    - (b) cyclophosphamide
    - (c) X-ray
    - (d) antilymphocytic serum
    - (e) thymectomy/splenectomy
    - (f) "nude" athymic mice
    - (g) adjuvants
  14. Immune "B" cell and "T" cell function intact *in vivo* and *in vitro*
  15. No cytopathic effect in infected cells *in vitro*
  16. Varying individual susceptibility to high infecting dose in some host species (as with scrapie in sheep)
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Table. 5. Methods of inactivating unconventional viruses

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1. Autoclaving (121° C at 20 p.s.i.; 30 min.)
  2. Hypochlorite ("Clorox"); 0.5-5.0 %
  3. Phenol (90%)
  4. Alcoholic iodine solution and organic iodine disinfectants
  5. Ether
  6. Acetone
  7. Chloroform or chloroform-butanol
  8. Strong detergents
  9. Periodate (0.01 M)
  10. 2-chloroethanol
  11. Urea (6 M)
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A major effort in my laboratory has been and is now being directed toward the molecular biological elucidation of the nature and structure of this group of atypical viruses.

The scrapie virus has been partially purified by fluorocarbon precipitation of proteins and density-gradient banding by zonal rotor ultracentrifugation (60). Other semipurified preparations have been made using ultrafiltration and repeated complete sedimentation and washing of the scrapie virus by means of ultrasonication for resuspension of the virus-containing pellets; such resuspended and washed virus has been banded into peaks of high infectivity using cesium chloride, sucrose, and metrizamide density gradients in the ultracentrifuge by Dr. Paul Brown in my laboratory. Sucrose-saline density-gradient banding of scrapie virus in mouse brains produced wide peaks of scrapie infectivity at densities of 1.14 to 1.23. A second smaller peak of high infectivity at density of 1.26 to 1.28 disappeared on filtration of the crude suspension through 200 nm Nucleopore membranes. On electron microscopic examination, fractions of high infectivity ( $10^7$  to  $10^8$  LD<sub>50</sub>/ml) revealed only smooth vesicular membranes with mitochondrial and ribosomal debris and no structures resembling recognizable virions. Lysosomal hydrolases (n-acetyl-P-D-glucosaminidase;  $\beta$ -galactosidase; acid phosphatase) and mitochondrial marker enzyme (INT-succinate reductase) showed most of their activity in fractions of lower density than in the fractions having high scrapie infectivity (60).

We have confirmed the previously noted resistance of scrapie virus to UV inactivation at 254 nm and UV inactivation action spectrum with a six-fold increased sensitivity at 237 nm over that at 254 or 280 nm (45). This should not be taken as proof that no genetic information exists in the scrapie virus as nucleic acid molecules, since work with the smallest RNA viruses, called viroids, indicates a similar resistance to UV inactivation in crude infected plant-sap preparations. Ultraviolet sensitivity also depends greatly on small RNA size, as has been shown by the high resistance of the purified very small tobacco ring spot satellite virus RNA (about 80,000 daltons) (7, 8). Partial purification of scrapie by fluorocarbon only slightly increases UV sensitivity

**at 254 nm (Figs. 1a, 1b) (7, 8, 45). Fluorocarbon-purified scrapie was neither inactivated by RNase A nor III nor by DNase I.**

On the other hand, the unconventional viruses possess numerous properties in which they resemble classical viruses, and some of these properties suggest far more complex genetic interaction between virus and host than one might expect for genomes with a molecular weight of only  $10^5$  daltons (Table 6). They are, moreover, not totally resistant to inactivation nor so dangerous that we cannot work safely with them by using appropriate inactivating agents (Table 5). In spite of very unusual resistance to heat, they are rapidly **inactivated by temperatures over 85° C. Autoclaving (120° C/20 p.s.i./45 minutes) completely inactivated scrapie virus in suspensions of mouse brain.**

Table 6. Classical virus properties of unconventional viruses

- 
1. Filterable to 25 nm average pore diameter (a.p.d.) (Scrapie, TME); 100 nm p.s.i. (kuru, CJD)
  2. 'Titrate "cleanly" (all individuals succumb to high LD<sub>50</sub> in most species)
  3. Replicate to titers of  $10^6$ /g to  $10^{12}$ /g in brain
  4. Eclipse phase
  5. Pathogenesis: first replicate in spleen and elsewhere in the reticuloendothelial system, later in brain
  6. Specificity of host range
  7. "Adaptation" to new host (shortened incubation period)
  8. Genetic control of susceptibility in some species (sheep and mice for scrapie)
  9. Strains of varying virulence and pathogenicity
  10. Clonal (limiting dilution) selection of strains from "wild stock"
  11. Interference of slow-growing strain of scrapie with replication of fast-growing strain in mice
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## CONVENTIONAL VIRUSES CAUSING CHRONIC DISEASE BY DEFECTIVE OR NON-DEFECTIVE REPLICATION

The other chronic diseases of man which have been shown to be slow virus infections are all caused by conventional viruses which in no way tax our imagination (Table 2). They comprise a wide spectrum of chronic and so-called degenerative diseases. Within this group of slow virus infections we find diverse mechanisms of viral replication, various modes of pathogenesis, and different kinds of involvement of the immune system.

In SSPE, the offending measles virus is apparently not present as a fully infectious virion, but instead asynchronous synthesis of virus subunits with defective or incomplete virion assembly occurs; only a portion of the virus genome is expressed, and replication is defective (6, 38, 54, 59). In the case of PML, on the other hand, fully assembled and infectious virus particles are produced (52, 64, 66). In fact, electron microscopically monitored suspensions of the virus particles of the JC papovavirus, density banded from human PML brain, shows that fewer defective particles are being produced than in any known *in vitro* system for cultivating papovaviruses, including the SV-40 virus. Thus, these ordinary viruses are causing slow infections by very different

mechanisms. In some cases, as with PML, an immune defect is demonstrated in association with the disease: in this case severe immunosuppression, either from natural primary disease (leukemia, lymphoma, sarcoid, etc.), or an iatrogenic immune suppression, as for renal transplantation or cancer chemotherapy.

The Russian Spring-Summer, or tick-borne encephalitis virus in cases of Kozhevnikov's epilepsy (epilepsia partialis continua) in the Soviet Union, Japan and India, and the rubella virus in adolescents with recrudescence of their congenital rubella infection (60a, 63a) appear also to be proceeding with defective virus replication. In chronic recurrent ECHO virus infection of the central nervous system in children with genetic immune defects, and in subacute brain infection with adenovirus types 7 (47) or 32 (55a), wholly infectious virus, as in the case of PML, seems to be produced.

Kuru and CJD, however, belong to a very different category of virus infections in which no involvement of the immune system has been demonstrable, in which there is no inflammatory response (no pleocytosis in the cerebrospinal fluid and no alteration in CSF protein), and in which the causative virus has defied all conventional attempts at virus taxonomy.

In recent years many other slow virus infections causing chronic diseases in animals have been used as models for various human diseases. Some of these are tabulated in Table 7. In these examples, as for the human diseases, many different mechanisms of virus replication or partial replication are involved in the persistent, latent, chronic, recurrent or slow virus infections. In some of these diseases the host genetic composition is crucial to the type of

Table 7. Slow infections of animals caused by conventional viruses

<i>Disease</i>	<i>Virus</i>
Visna	Retrovirus—Visna
Maedi (Zoegerziekte)	Retrovirus—Maedi
Progressive pneumonia of sheep (PPS; Montana sheep disease)	Retrovirus—PPS-Visna and Maedi related
Motor neurone disease of mice (mouse ALS)	Retrovirus—type "C"
Lymphocytic choriomeningitis	Arenavirus—LCM
Aleutian mink disease	Parvovirus
Hard-pad disease (old-dog distemper)	Paramyxovirus—distemper
Chronic tick-borne encephalitis (RSSE)	Togavirus—RSSE
Pulmonary adenomatosis of sheep (Jaagsiekte)	Unclassified
Mouse cataract disease	Unclassified—mouse cataract virus
Lactic dehydrogenase elevating virus of mice	?Togavirus—LDV
Equine infectious anemia	Unclassified—EIA virus
Rabies	Rhabdovirus—rabies
NZB mouse hemolytic anemia	Retrovirus
Chronic hydrocephalus in hamsters	Paramyxovirus—mumps
	Orthomyxovirus—influenza
Spontaneous progressive multifocal leukoencephalopathy in rhesus monkeys	Papovavirus—SV-40



pathogenesis that occurs, as is the age of the host at the time of infection, and the immune system may be involved in different ways; immune complex formation is important in some cases and not in others.

The suspicion has been awakened that many other chronic diseases of man may be slow virus infections (see Table 8). Data have gradually accumulated both from the virus laboratory and from epidemiological studies, which suggest that multiple sclerosis and Parkinson's disease, disseminated lupus erythematosus and juvenile diabetes, polymyositis and some forms of chronic arthritis may be slow infections with a masked and possible defective virus as their causes. The study of kuru was carried on simultaneously with a parallel attack on multiple sclerosis, amyotrophic lateral sclerosis, and Parkinson's disease; in addition, other degenerative dementias such as Alzheimer's disease, Pick's disease, Huntington's chorea and parkinsonism-dementia were also studied. Chronic encephalitis, epilepsia partialis continua, progressive supranuclear palsy, and degenerative reactions to schizophrenia are among the other diseases under investigation (16, 22, 25, 62). Our attempts at transmission of these diseases to subhuman primate and non-primate laboratory animals have been unsuccessful; no virus has been unmasked from *in vitro* cultivated tissues from the patients, and no virus etiology has been demonstrated for any of these diseases.

Table 8. Chronic diseases of man of suspected slow virus etiology

Multiple sclerosis	Carcinomatous cerebellar degeneration
Neuromyelitis optica—Devic's syndrome	Tuberous sclerosis
Parkinson's disease	Ataxia telangiectasia
Amyotrophic lateral sclerosis	Progyria
Progressive supranuclear palsy	Schizophrenic dementia
Chronic encephalitis with focal epilepsy	Neurofibromatosis
Alzheimer's disease	Disseminated lupus erythematosus
Pick's disease	Chronic arthritis
Huntington's chorea	Dermatomyositis
Parkinsonism-dementia	Scleroderma
Syringomyelia	Ulcerative colitis
Alper's disease	Juvenile diabetes
Polymyositis	Beget's disease
Papulosis atrophicans maligna (Köhlmeier-Degos)	Sjögren's disease

## KURU

Kuru is characterized by cerebellar ataxia and a shivering-like tremor that progresses to complete motor incapacity and death in less than one year from onset. It is confined to a number of adjacent valleys in the mountainous interior of New Guinea and occurs in 160 villages with a total population of just over 35,000 (Figs. 2-4). *Kuru* means shivering or trembling **in the Fore language. In the Fore culture and linguistic group, among whom over 80 % of the cases occur, it had a yearly incidence rate and prevalence ratio of**

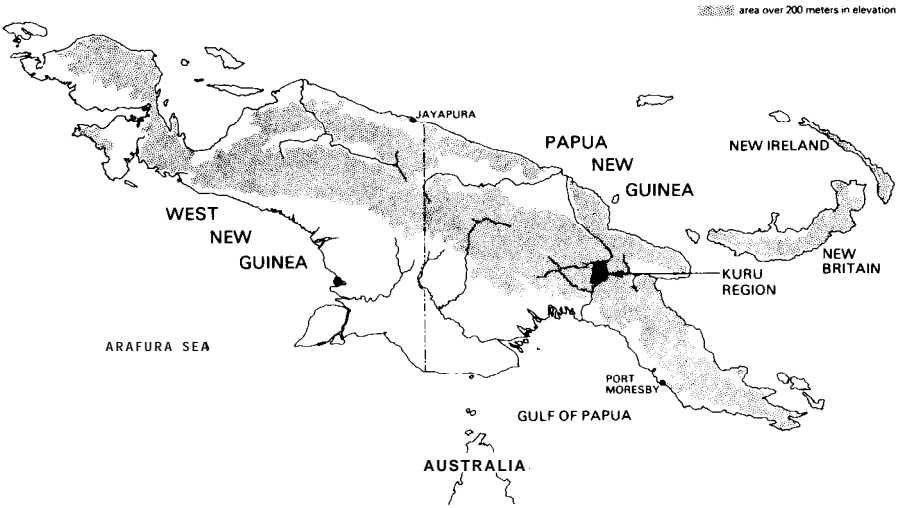


Figure 2. The region in New Guinea from which all kuru patients have come is shown by the irregular black area in the Eastern Highlands Province on the eastern side of the island in Papua New Guinea. It contains more than 35,000 people living in 160 villages (census units) that have experienced kuru. All kuru-affected hamlets lie nestled among rain forest covered mountains from 1,000 to 2,500 m. above sea level.

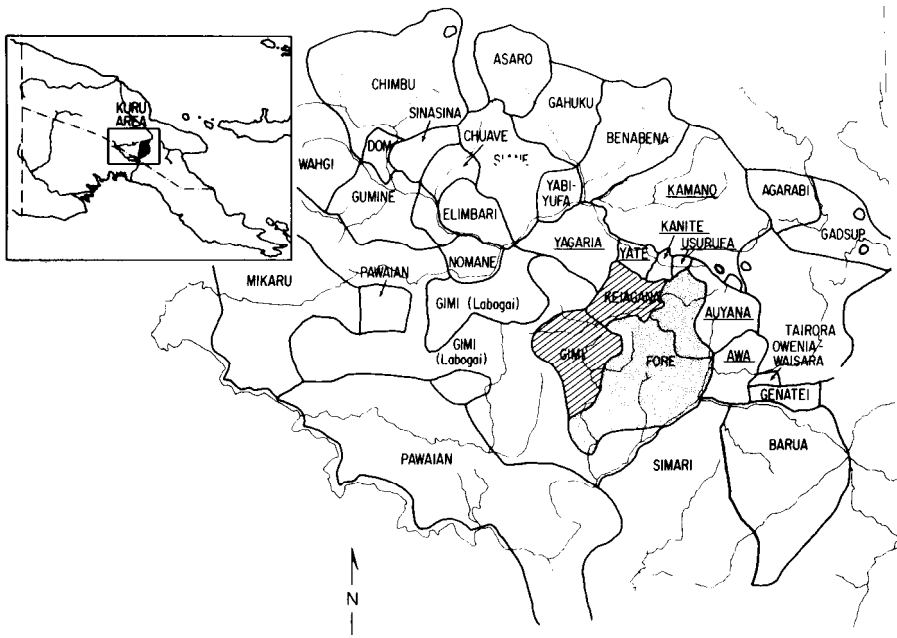


Figure 3. The kuru region in the Eastern Highlands Province of Papua New Guinea showing the cultural and linguistic groups in and surrounding the kuru affected populations. *Inset*, upper left: Eastern half of the island of New Guinea showing, in rectangle, area included in the map of larger scale.

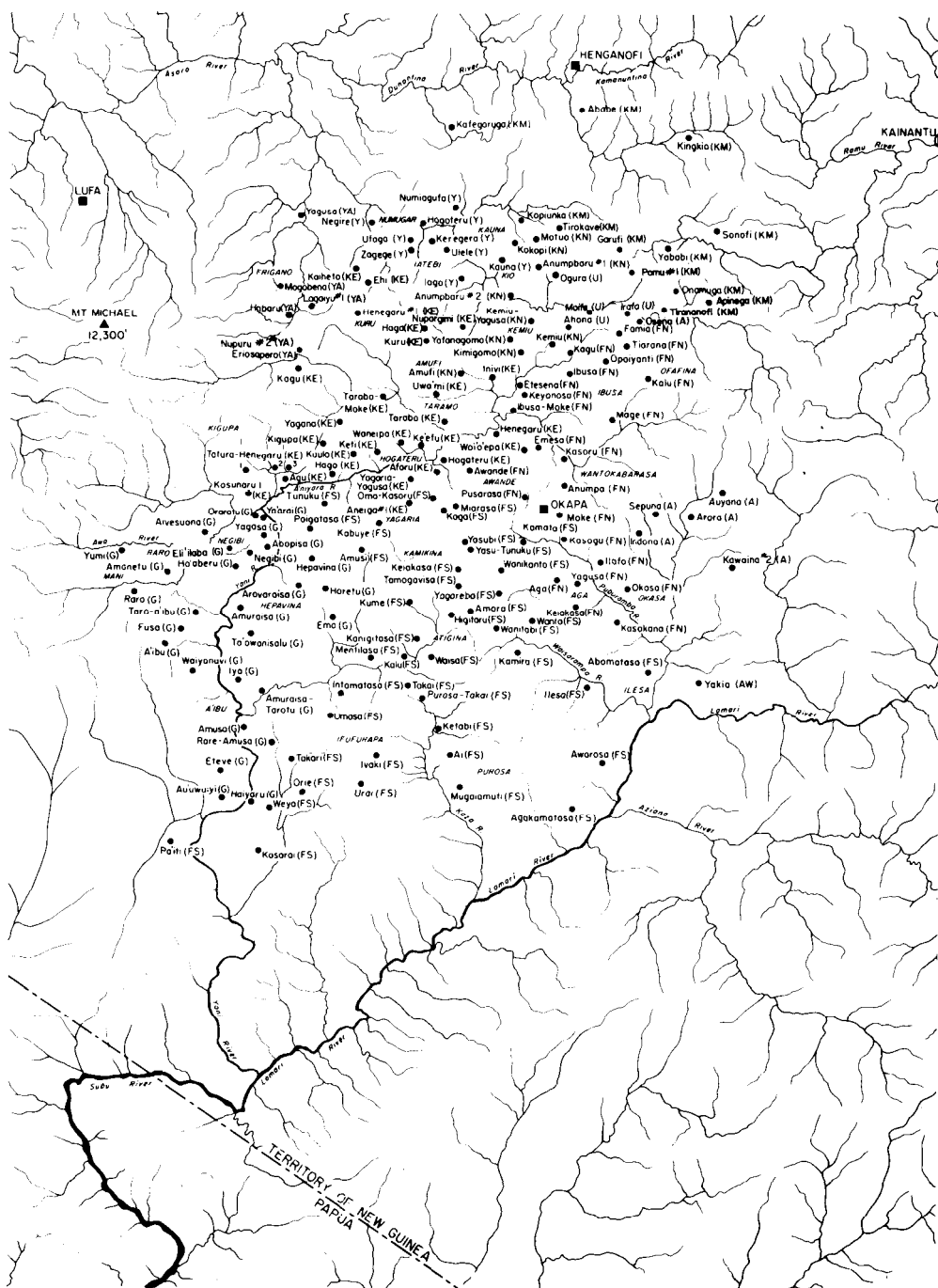


Figure 4. River drainages of the kuru region with superimposed locations of the 160 villages (census units) in which kuru has ever occurred. The cultural and linguistic group of each village is indicated: A Auyana, AW Awa, FN North Fore, FS South Fore, G Gimi, KE Keiagana, KM Kamano, KN Kanite, U Usurufa, Y Yate, YA Yagaria.

about 1 % of the population (Figs. 5a, b). During the early years of investigation, after the first description by Gajdusek and Zigas in 1957 (28), it was found to affect all ages beyond infants and toddlers; it was common in male and female children and in adult females, but rare in adult males (Fig. 6). This marked excess of deaths of adult females over males has led to a male-to-female ratio of over 3:1 in some villages, and of 2:1 for the whole South Fore group (17, 28, 29, 65).

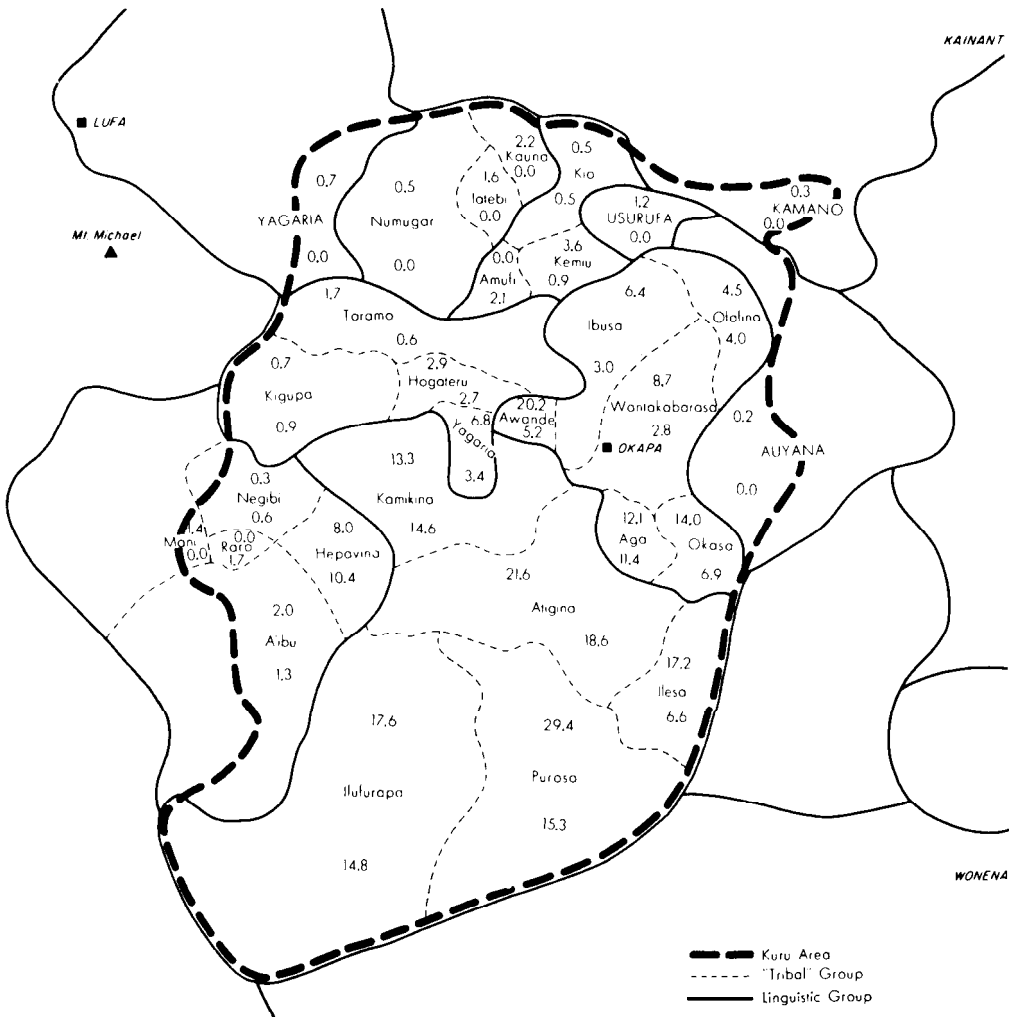
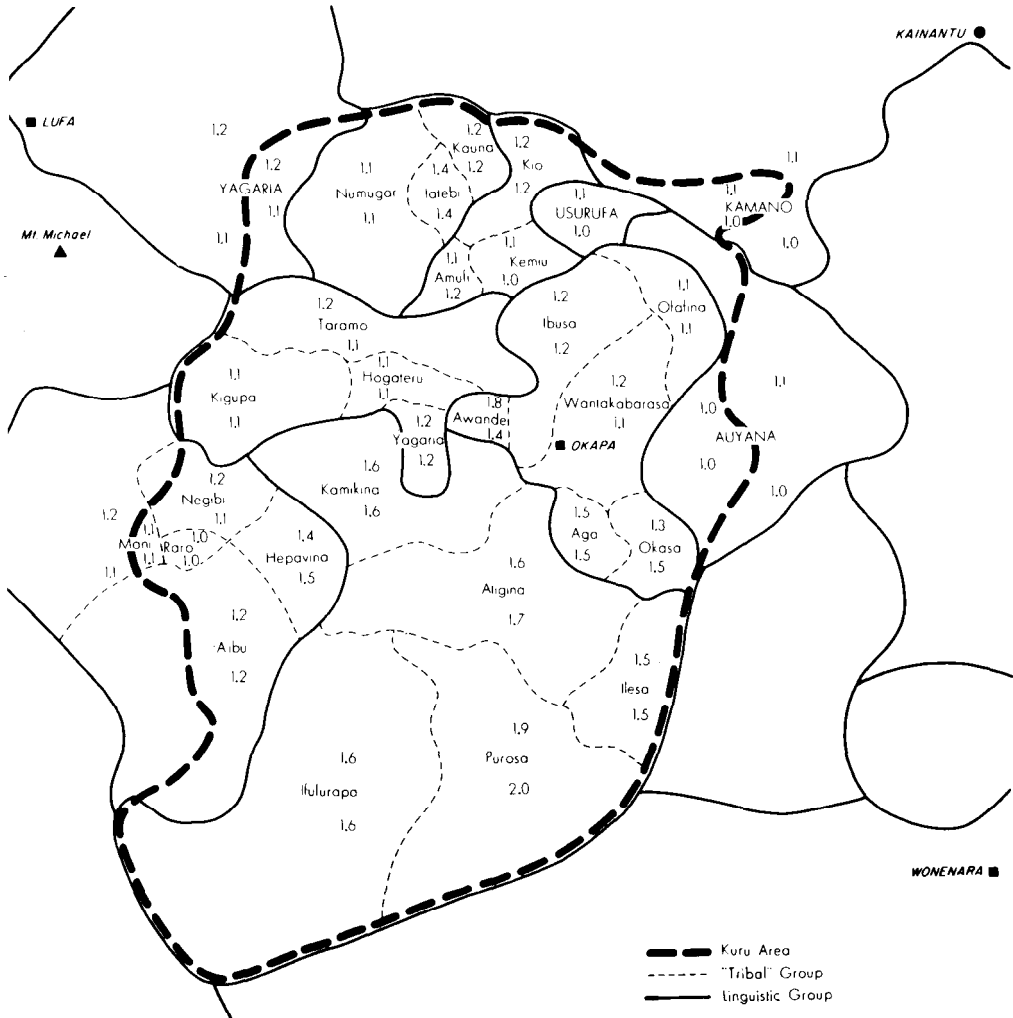


Figure 5. The discovery of kuru coincided with the height of the “epidemic”.

5a. Guru mortality rate in deaths per thousand population per annum in cnch-“tribal” group of thr kuru region. 1957-59 and 1961-1963. The numerators of the rates are obtained from the deaths which occurred in the two 3-year periods. the denominators are the populations for 1958 and 1962, respectively. The rates above each name refer to 1957-59, those below to 1961-63.



5b. Male: female population ratio in each "tribal" group of the kuru region, 1958 and 1962. The two sets of figures for peripheral groups refer to their portions within and without the kuru region. The ratios above each name refer to 1958, those below to 1962. In these early years of kuru investigation the disease, affecting predominantly females, was causing increasing distortion of the sex ratio.

Kuru has been disappearing gradually during the past 15 years (Fig. 7). The incidence of the disease in children has decreased during the past decade, and the disease is no longer seen in either children or adolescents (Figs. 8 and 9.) This change in occurrence of kuru appears to result from the cessation of the practice of ritual cannibalism as a rite of mourning and respect for dead kinsmen, with its resulting conjunctival, nasal, and skin contamination with highly infectious brain tissue mostly among women and small children (17).

AGE AND SEX DISTRIBUTION OF 1276 STUDIED CASES OF KURU

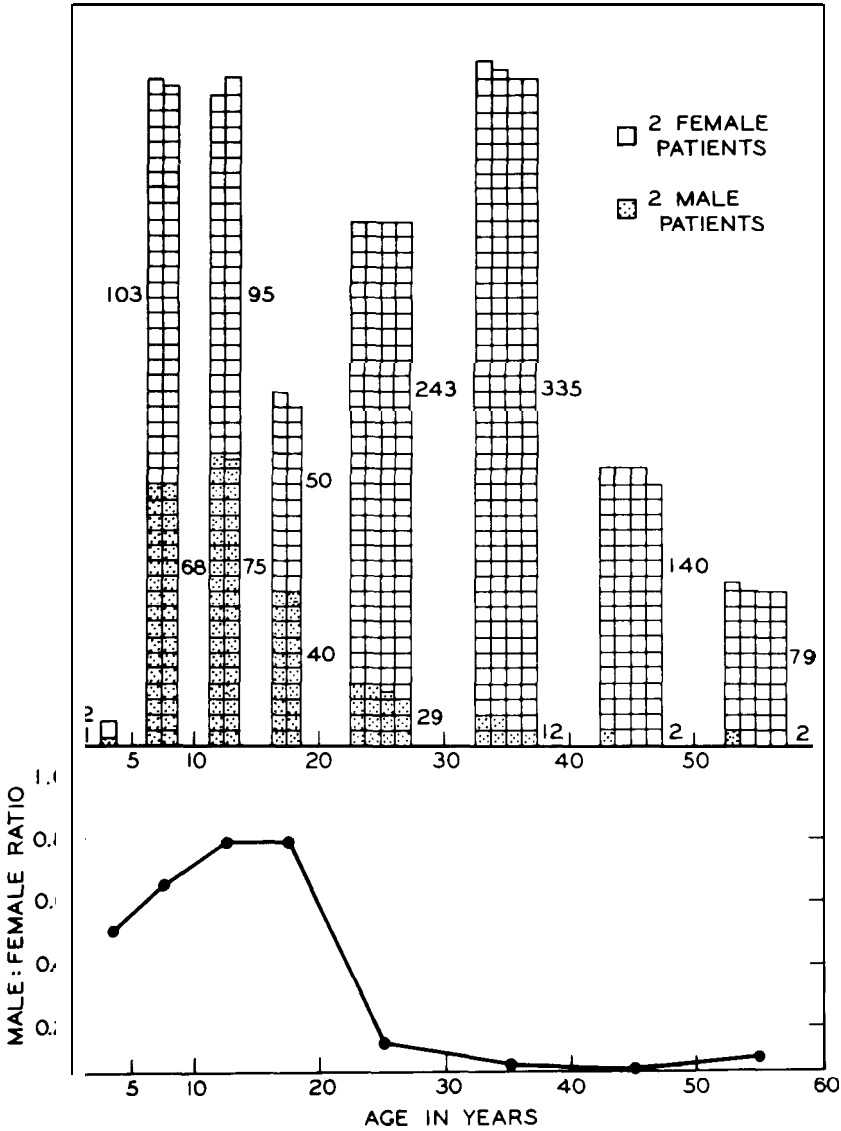


Figure 6. Age and sex distribution of the first 1276 kuru patients studied in the early years of kuru investigations. The youngest patient had onset at 4 years of age, died at 5 years of age.

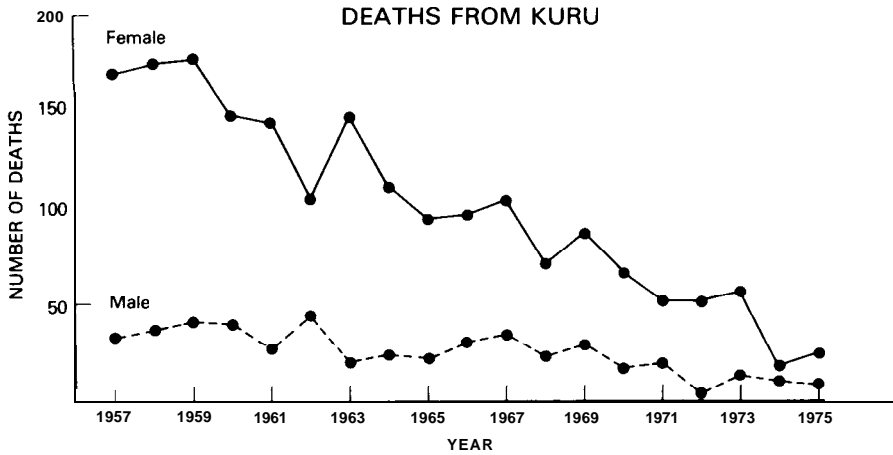


Figure 7. The overall incidence of kuru deaths in male and female patients by year since its discovery in 1957 through 1975. More than 2,500 patients died of kuru in this 17 year period of surveillance, and there has been a slow, irregular decline in the number of patients to one-fifth the number seen in the early years of kuru investigation. The incidence in males has declined significantly only in the last few years, whereas in females it started to decline over a decade earlier. This decline in incidence has occurred during the period of acculturation from a stone age culture in which endocannibalistic consumption of dead kinsmen was practiced as a rite of mourning, to a modern coffee planting society practicing cash economy. Because the brain tissue with which the officiating women contaminated both themselves and all their infants and toddlers contained over 1,000,000 infectious doses per gram, self-inoculation through the eyes, nose, and skin, as well as by mouth, was a certainty whenever a kuru victim was eaten. The decline in incidence of the disease has followed the cessation of cannibalism, which occurred between 1957 and 1962 in various villages.

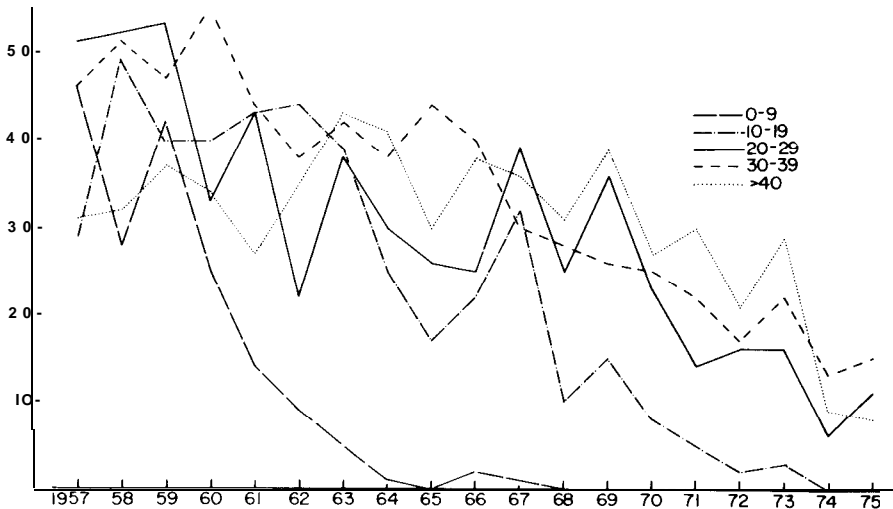


Figure 8. Kuru deaths by age group from 1957 through 1975. The disease has disappeared from the youngest age group (4-9 years) about 5 years before it disappeared in the 10 to 14 year olds, and now it has disappeared in the 15 to 19 year olds. The number of adult patients has declined to less than one-fifth since the early years of investigation. These changes in the pattern of kuru incidence can be explained by the cessation of cannibalism in the late 1950's. No child born since cannibalism ceased in this area has developed the disease.

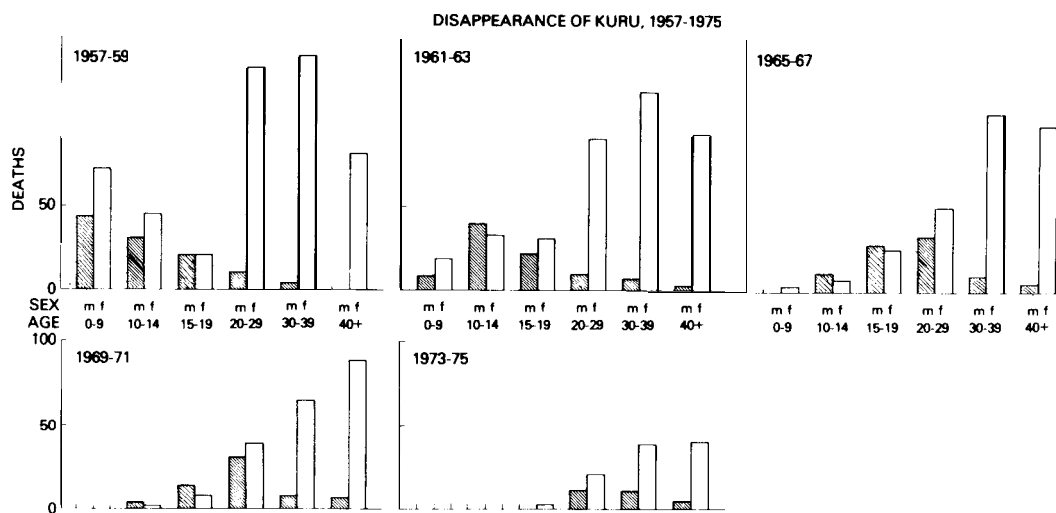


Figure 9. Kuru deaths by age and sex for the years 1957 through 1975 are plotted in 3 year periods, with the exception of those dying in the 1 year intervals between each plot, namely 1960, 1964, 1968, and 1972. These years have been omitted because of irregularities which may have occurred in arbitrarily assigning exact dates of death at the end of the year when dates were not known precisely. The disappearance first in the 4 to 9 year old patients (there were no cases in children under 4 years of age), then in the 10 to 14 year group, and, finally, in the 15 to 19 year group, is clearly shown. No patient under 22 years has died since 1973, and the youngest still-living patient is 24 years old.

The clinical course of kuru is remarkably uniform with cerebellar symptomatology progressing to total incapacitation and death, usually within three to nine months. It starts insidiously without antecedent acute illness and is conveniently divided into three stages: ambulant, sedentary and terminal (Figs. 10-15).

For several years all work on the kuru virus was done using chimpanzees, the first species to which the disease was transmitted (Figs. 16-18) (22, 25). Eventually, other species of nonhuman primates developed the disease: first, several species of New World monkeys with longer incubation periods than in the chimpanzee; and later, several species of Old World monkeys with yet longer incubation periods (Tables 9 and 10) (23, 32). Very recently, we have transmitted kuru to the mink and ferret, the first nonprimate hosts that have proved to be susceptible, although dozens of other species of laboratory, domestic and wild nonprimate and avian hosts have been inoculated without developing disease after many years of observation. We have now extended the nonprimate host range for the subacute spongiform virus encephalopathies, as shown in Table 11.

The virus has been regularly isolated from the brain tissue of kuru patients. It attains high titers of more than  $10^8$  infectious doses per gram. In peripheral tissue, namely liver and spleen, it has been found only rarely at the time of death, and in much lower titers. Blood, urine, leukocytes, cerebrospinal fluid, and placenta and embryonal membranes of patients with kuru have not yielded the virus.





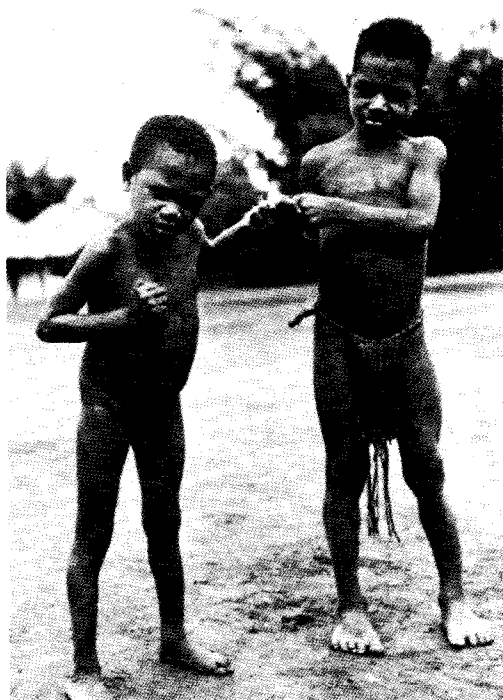
Figure 10a. Nine victims of kuru who were assembled one afternoon in 1957 from several villages in the Purosa valley (total population about 600) of the South Fore region. The victims included six adult women, one adolescent girl, one adolescent boy, and a prepubertal boy. All died of their disease within 1 year after this photograph was taken.



10b. Five women and one girl, all victims of kuru, who were still ambulatory, assembled in 1957 in the South Fore village of Pa'iti. The girl shows the spastic strabismus, often transitory, which most children with kuru developed early in the course of the disease. Every patient required support from the others in order to stand without the aid of the sticks they had been asked to discard for the photograph.



Figure 11. Six women with kuru so advanced that they require the use of one or two sticks for support, but are still able to go to garden work on their own. In all cases their disease progressed rapidly to death within less than a year from onset.



12a



2b



12c

Figure 12. Three Fore boys with kuru in 1957; all three were still ambulatory

12a. The youngest patient with kuru, from Mage village, North Fore, who self-diagnosed the insidious onset of clumsiness in his gait as kuru at 4 years of age, and died at 5 years of age, several years before his mother developed kuru herself.

12b. A South Fore boy from Agakamatasa village, about 8 years of age, who was caught by the camera in an athetoid movement while trying to stand without support, in the early stage of kuru.

12c. A mid-adolescent youth from Anumpa village, North Fore, who demonstrates the difficulty in standing on one foot associated with the early ambulatory stage of kuru.



13a



13b

Figure 13. Two Fore children with advanced kuru in 1957. Both had been sedentary for several months and were reaching the terminal stage of the disease.

13a. A girl, about 8 years old, who was no longer able to speak, but who was still alert and intelligent.

13b. A boy, about 8 years of age, who was similarly incapacitated after only 3 months of illness.



Figure 14. Four preadolescent children, totally incapacitated by kuru in 1957. All had such severe dysarthria that they could no longer communicate by word, but all were still intelligent and alert. All had spastic strabismus. None could stand, sit without support, or even roll over; none had been ill for over six months, and all died within a few months of the time of photography.



15a



15b



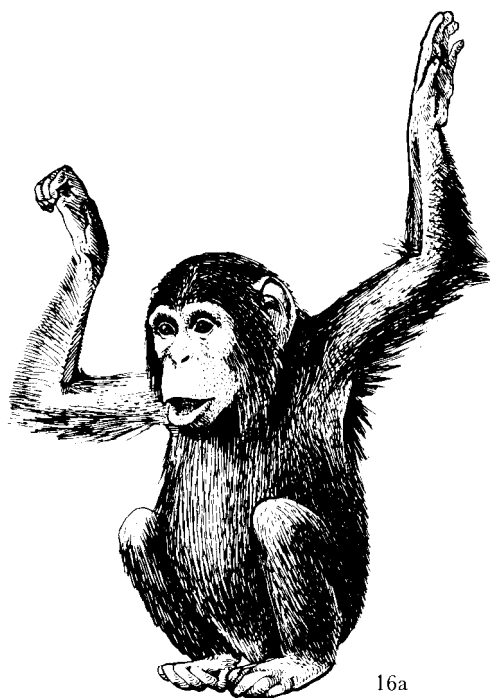
15c

Figures 15. Groups of kuru patients in 1957 at the Kuru Research Hospital in Okapa, New Guinea. All died within 1 year of photography. The pictures show many preadolescent child victims of kuru, an age group in which kuru has not occurred in recent years.

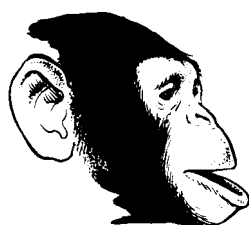
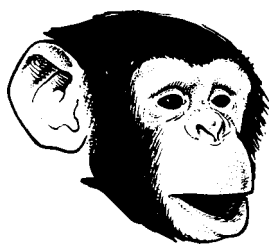
15a. Eight kuru patients in the first, or ambulatory, stage of the disease. Five adult women are holding sticks to maintain their balance. Three girls who are still able to walk without the aid of a stick, but with severe ataxia, sit in front of the women.

15b. Eight preadolescent children, four boys and four girls, with kuru. The girl at the far left, in her father's lap, is the same child as that on the left in (a), but is seen 2 months later in the secondary, or sedentary, stage of the disease.

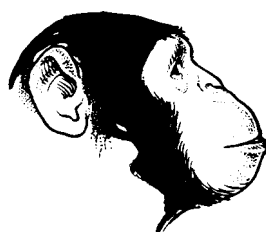
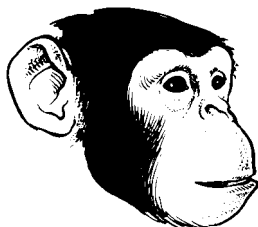
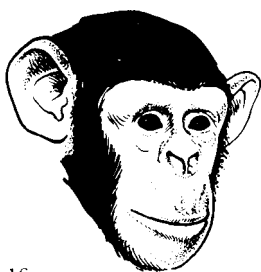
15c. Five children with kuru, two boys in the center, a girl on each side: the adolescent boy supporting the girl on the right is a kuru victim himself, but he is in an earlier stage of the disease. The 4 children requiring support are just passing from the first, or ambulatory, to the second, or sedentary, stage of the disease.



16a



16b



16c

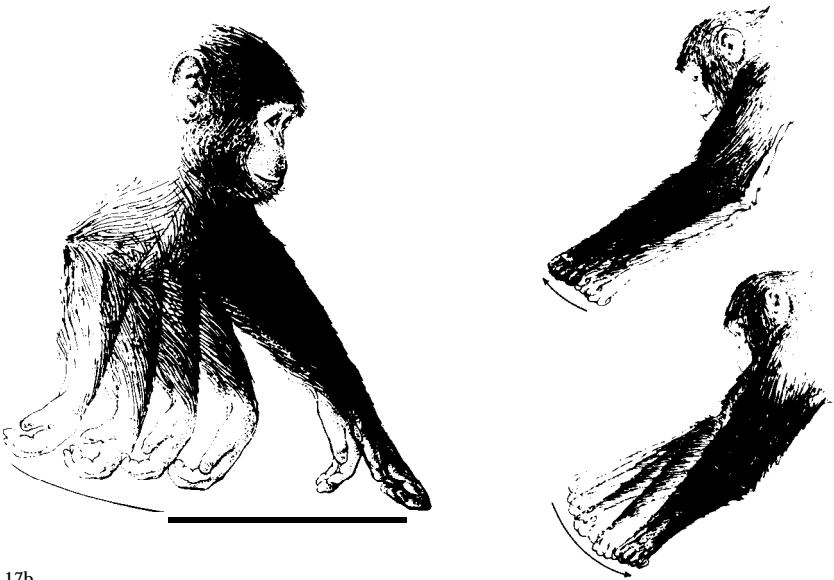
Figure 16a. Chimpanzee with a vacant facial expression and a drooping lip, a very early sign of kuru preceding any "hard" neurological signs. Most animals show this sign for weeks or even months before further symptoms of kuru are detectable other than subtle changes in personality.

16b. Three successive views of the face of a chimpanzee with early kuru drawn from cinema frames. Drooping lower lip is an early sign of kuru.

16c. Face of a normal chimpanzee drawn in three successive views from successive cinema views.



17a



17b

Figure 17a. Chimpanzee with early experimentally-induced kuru eating from floor without use of prehension. This "vacuum cleaner" form of feeding was a frequent sign in early disease in the chimpanzee when tremor and ataxia were already apparent (From: Asher, D. M. et al., In: *Nonhuman Primates and Human Diseases*, W. Montagna and W. P. McNulty, Jr., eds., Vol. 4, 1973, pp. 43-90).

17b. Range of movement in forelimbs in walking: left, normal chimpanzee; right, chimpanzee in stage 2 of experimental kuru. Quantitative assessment was made by studying individual frames of Research Cinema film (24).



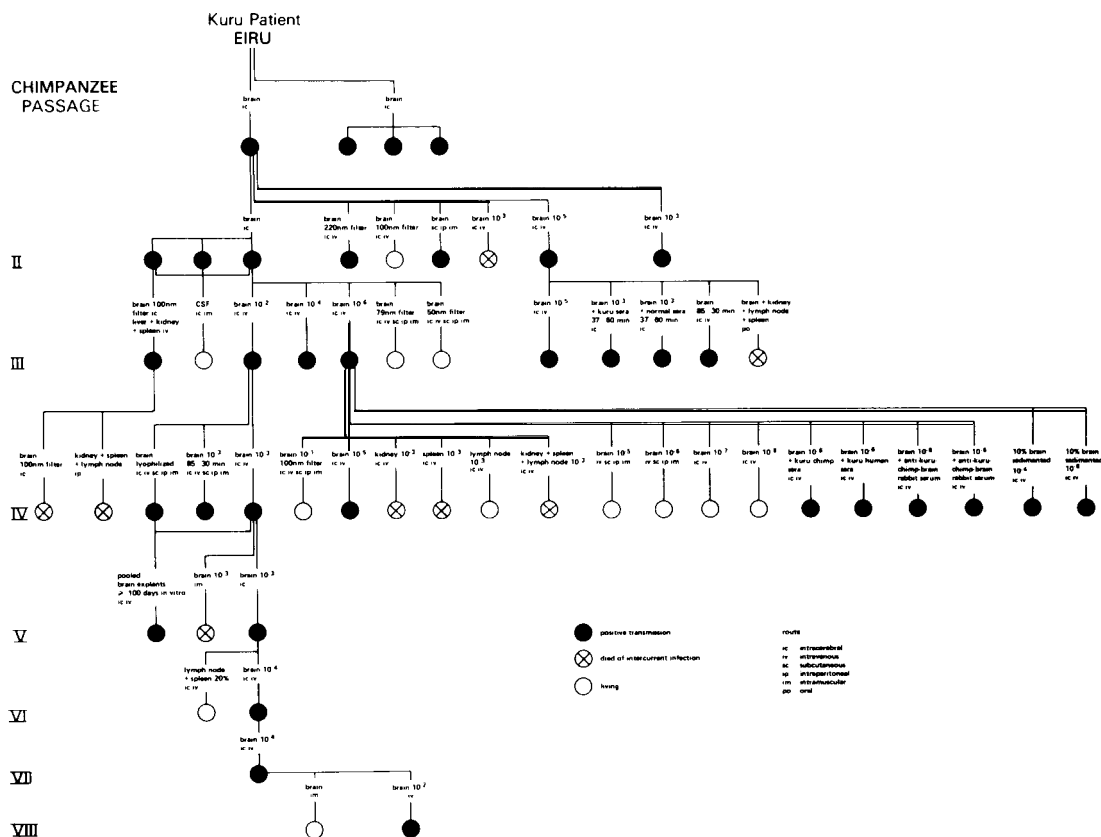


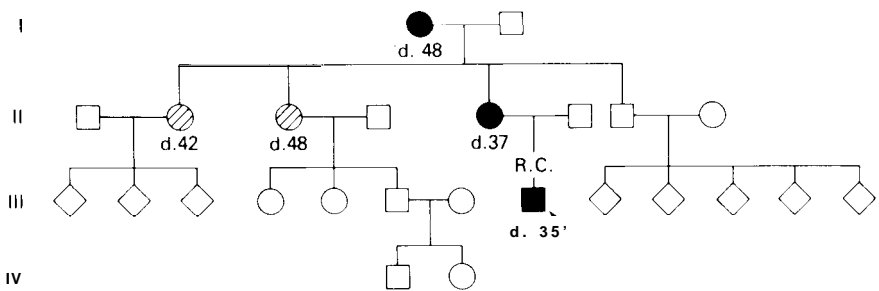
Figure 18. Kuru transmission experiments in chimpanzees, illustrating the early extensive use of this rare and diminishing species and significant curtailment of chimpanzee inoculations after the 4th chimpanzee passage. It was at this time that we discovered that New World monkeys could be used in lieu of the chimpanzee, although they required considerably longer incubation periods. The experiments indicate failure of the agent to pass a 100 nm or smaller filter. They also show the failure of a conventional virus neutralization test, using only 10 infectious doses of kuru virus to neutralize the virus using sera from patients with kuru or from chimpanzees with experimental kuru or antisera made by immunizing rabbits with kuru chimpanzee brain. In these experiments, kidney, spleen and lymph node have not yielded virus, and although chimpanzee brain has had a titer above  $10^6$  by intracerebral inoculation, at  $10^3$  dilutions such brain suspensions inoculated by peripheral routes have not produced disease. In the 3rd passage (on the left), liver, spleen and kidney given intracerebrally, presumably caused disease since 100 nm filtrates of infectious brain have regularly failed to produce the disease; the affected 3rd passage animal had received both inocula.

TRANSMISSIBLE VIRUS DEMENTIAS (CREUTZFELDT-JAKOB DISEASE)

Creutzfeldt-Jacob disease (CJD) is a rare, usually sporadic, presenile dementia found worldwide; it has a familial pattern of inheritance, usually suggestive of autosomal dominant determinations in about 10 % of the cases (Fig. 19). The typical clinical picture includes myoclonus, paroxysmal bursts of high voltage slow waves on EEG, and evidence of widespread cerebral dysfunction. The disease is regularly transmissible to chimpanzees (3, 33), New and Old World monkeys (Tables 9 and 10) and the domestic cat (Tables 11 and 12) (23, 32), with pathology in the animal indistinguishable at the cellular level from that in the natural disease or in experimental kuru (Fig. 20) (3, 43). We have recently confirmed in our laboratory reports of transmission of CJD from human brain to guinea pigs (48, 48a). In spite of a recent convincing report of transmission of CJD from human brain to mice (5, 5a) we have not yet succeeded in transmitting CJD or kuru to mice.

As we have attempted to define the range of illness caused by the CJD virus, a wide range of clinical syndromes involving dementia in middle and late life have been shown to be such slow virus infections associated with neuronal vacuolation or status spongiosus of gray matter and a reactive astrogliosis.

CJD GENEALOGY CHART

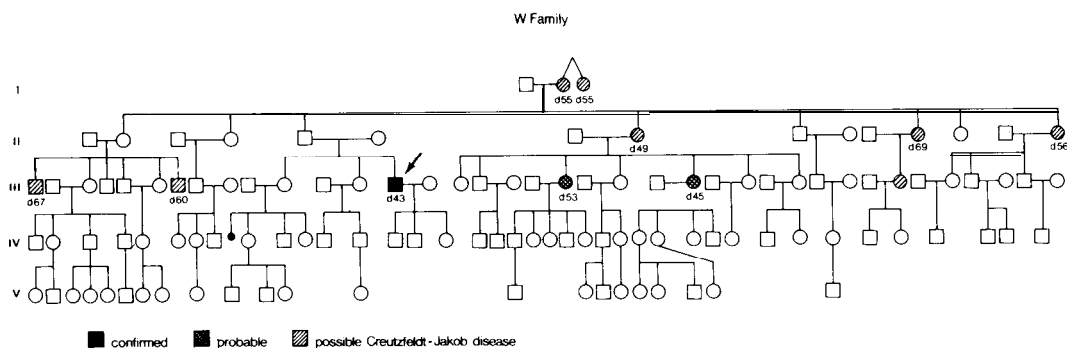


Creutzfeldt-Jakob disease was a rare, almost unknown disease; now cases of transmissible virus dementia are regularly found wherever they are looked for. Better ascertainment of cases, the study of familial aggregations and an unusually high prevalence in Libyan Jews, and the possibility of occupational hazard and transmission through corneal transplant provide promising epidemiological leads to the understanding of the natural history of the disease, and hence to its prevention.

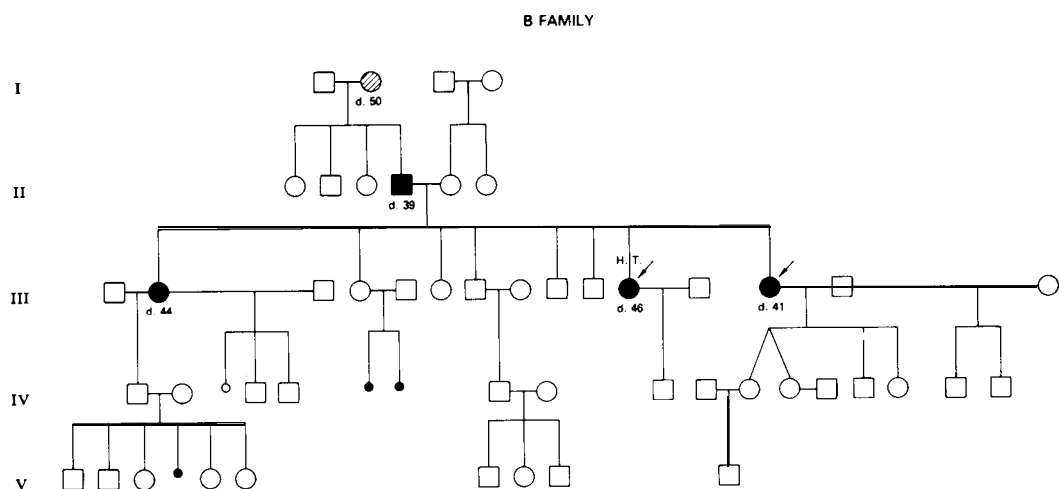
- Creutzfeldt-Jakob disease confirmed pathologically
- ▨ Probable Creutzfeldt-Jakob disease
- / Transmitted to chimpanzee from brain tissue inoculated intracerebrally

Figure 19. Subacute spongiform virus encephalopathy has been transmitted to chimpanzees or New World monkeys from 8 patients with transmissible virus dementias of a familial type. Ten percent of CJD patients have a history of similar disease in kinsmen.

19a. Genealogical chart shows a family with 5 cases of CJD over three generations, suggesting autosomal dominant inheritance. From patient R. C., the disease has been transmitted to a chimpanzee.



19b. This family has 11 members suffering from CJD-like disease in three generations. From the brain tissue of patient J. W., obtained at autopsy, the disease has been transmitted to a squirrel monkey.



19c. This family has 5 cases of CJD over three generations, again suggesting autosomal dominant inheritance. From the brain tissue of patient H. T., obtained at autopsy, the disease has been transmitted to two squirrel monkeys.

These even include cases that have been correctly diagnosed as brain tumors (glioblastoma, meningioma), brain abscess, Alzheimer's disease, progressive supranuclear palsy, senile dementia, or stroke, or Kohlmeier-Degos disease (27), at some time in their clinical course (51, 62). Hence, the urgent practical problem is to delineate the whole spectrum of subacute and chronic neurological illnesses that are caused by or associated with this established slow virus infection. Because some 14% of the cases show amyloid plaques akin to those found in kuru, and many show changes similar to those of Alzheimer's disease, in addition to the status spongiosus and astrogliosis of CJD, and because other cases also involve another neurological disease as well as CJD (50, 51, 62), we have started to refer to the transmissible disorder as transmissible virus dementia (TVD).

Since our first transmission of Creutzfeldt-Jakob disease, we have obtained brain biopsy or early postmortem brain tissue on over 200 cases of pathologically confirmed CJD. The clinical, laboratory, and virus investigations of these cases have been summarized in a recent report (62) that extends and updates our earlier report of 35 cases (56). We have been aware of occasional clustering of cases in small population centers, admittedly lacking in natural boundaries, and the unexplained absence of any cases over periods of many years in some large population centers where, at an earlier date, cases were more frequent.

Table 9. Species of laboratory primate susceptible to the subacute spongiform virus encephalopathies

In man	
Kuru	<i>Apes</i> : chimpanzee, gibbon <i>New World monkeys</i> : capuchin, marmoset, spider, squirrel, woolly <i>Old World monkeys</i> : African green, bonnet, cynomolgus macaque, mangabey, rhesus, pig-tailed macaque
Creutzfeldt-Jakob disease	<i>Apes</i> : chimpanzee <i>New World monkeys</i> : capuchin, marmoset, spider, squirrel, woolly <i>Old World monkeys</i> : African green, bushbaby, cynomolgus macaque, mangabey, patas, pig-tailed macaque, rhesus, stump-tailed macaque
In animals	
Scrapie	<i>New World monkeys</i> : capuchin, spider, squirrel <i>Old World monkeys</i> : cynomolgus macaque, rhesus
Transmissible mink encephalopathy	<i>New World monkeys</i> : squirrel <i>Old World monkeys</i> : rhesus, stump-tailed macaque

Table 10. Species of laboratory primates susceptible to subacute spongiform encephalopathies

	Incubation periods (in months)			
	Kuru	CJD	Scrapie	TME
Apes				
Chimpanzee ( <i>Pan troglodytes</i> )	10—82	11—71	(111)	(72 +)
Gibbon ( <i>Hylobates lar</i> )	+(10)	NT	NT	NT
New World monkeys				
Capuchin ( <i>Cebus albifrons</i> )	10—15	29—34	NT	NT
Capuchin ( <i>Cebus apella</i> )	11—61	11—47.5	32—35.5	NT
Spider ( <i>Ateles geoffroyi</i> )	10—85.5	4—50	38	NT
Squirrel ( <i>Saimiri sciureus</i> )	8—50	5—41	8—63	8—13
Marmoset ( <i>Saguinus sp.</i> )	1.5—36	18—54	NT	NT
Woolly ( <i>Lagothrix lagothricha</i> )	33	21	NT	NT
Old World monkeys				
African green ( <i>Cercopithecus aethiops</i> )	18	33—49.5	(109)	NT
Baboon ( <i>Papio anubis</i> )	(114)	47.5	NT	NT
Bonnet ( <i>Macaca radiata</i> )	19—27	(43)	NT	NT
Bushbaby ( <i>Galago senegalensis</i> )	(104)	16	NT	NT
Cynomolgus macaque ( <i>Macaca fascicularis</i> )	16	52.5—60	27—72	NT
Patas ( <i>Erythrocebus patas patas</i> )	(120)	47—60.5	NT	NT
Pig-tailed macaque ( <i>Macaca nemestrina</i> )	70	+(2)	NT	NT
Rhesus ( <i>Macaca mulatta</i> )	15—103	43—73	30—37	17—33
Sooty mangabey ( <i>Cercocebus atys</i> )	+(2)	+(2)—43	NT	NT
Stump-tailed macaque ( <i>Macaca arctoides</i> )	(120)	60	NT	13
Talapoin ( <i>Cercopithecus talapoin</i> )	(1 +)	64.5	NT	NT

Numbers in parentheses are the number of months elapsed since inoculation, during which the animal remained asymptomatic.

Table 11. Nonprimate hosts for experimental subacute spongiform encephalopathies

In man:	
Kuru	Ferret, mink
Creutzfeldt-Jakob disease	Cat, ferret, guinea pig, ?mouse (5, 5a), hamster (48b)
In animals:	
Scrapie	Gerbil, goat, hamster, mink, mouse, rat, sheep, vole
Transmissible mink encephalopathy	Ferret, goat, hamster, mink, opossum, raccoon, sheep, skunk

Table 12. Creutzfeldt-Jakob disease in cats

Inoculum	Incubation period (months)	Duration (months)
Primary passage		
Human brain	30	2
Serial passage		
Cat brain (passage 1)	19—24	4—5 1/2
Cat brain (passage 2)	18—24	

This geographic and temporal clustering does not apply, however, to a majority of cases and is unexplained by the 10% of the cases that are familial. Matthews has recently made a similar observation in two clusters in England (50). There are two reports of conjugal disease in which husband and wife died of CJD within a few years of each other (30, 50).

The prevalence of CJD has varied markedly in time and place throughout the United States and Europe, but we have noted a trend toward making the diagnosis more frequently in many neurological clinics in recent years, since attention has been drawn to the syndrome by its transmission to primates (3, 33). For many large population centers of the United States, Europe, Australia, and Asia, we have found a prevalence approaching one per million with an annual incidence and a mortality of about the same magnitude, as the average duration of the disease is 8 to 12 months. Matthews (50) found an annual incidence of 1.3 per million in one of his clusters, which was over 10 times the overall annual incidence for the past decade for England and Wales (0.09 per million). Kahana et al. (40) reported the annual incidence of CJD ranging from 0.4 to 1.9 per million in various ethnic groups in Israel. They noted, however, a 30-fold higher incidence of CJD in Jews of Libyan origin above the incidence in Jews of European origin. From recent discussions with our Scandinavian colleagues it is apparent that an annual incidence of at least one per million applies to Sweden and Finland in recent years.

Probable man-to-man transmission of CJD has been reported in a recipient of a corneal graft, which was taken from a donor who was diagnosed retrospectively to have had pathologically confirmed CJD (12). The disease occurred 18 months after the transplant, an incubation period just the average for chimpanzees inoculated with human CJD brain tissue (32, 62). From suspension of brain of the corneal graft recipient we succeeded in transmitting CJD to a chimpanzee although the brain had been at room temperature in 10% formol-saline for seven months (26a). More recently we learned that two of our confirmed cases of TVD were professional blood donors until shortly before the onset of their symptoms. To date, there have been no transmissions of CJD from blood of either human patients or animals affected with the experimentally transmitted disease. However, we have only transfused two chimpanzees each with more than 300 ml of human whole blood from a different CJD patient

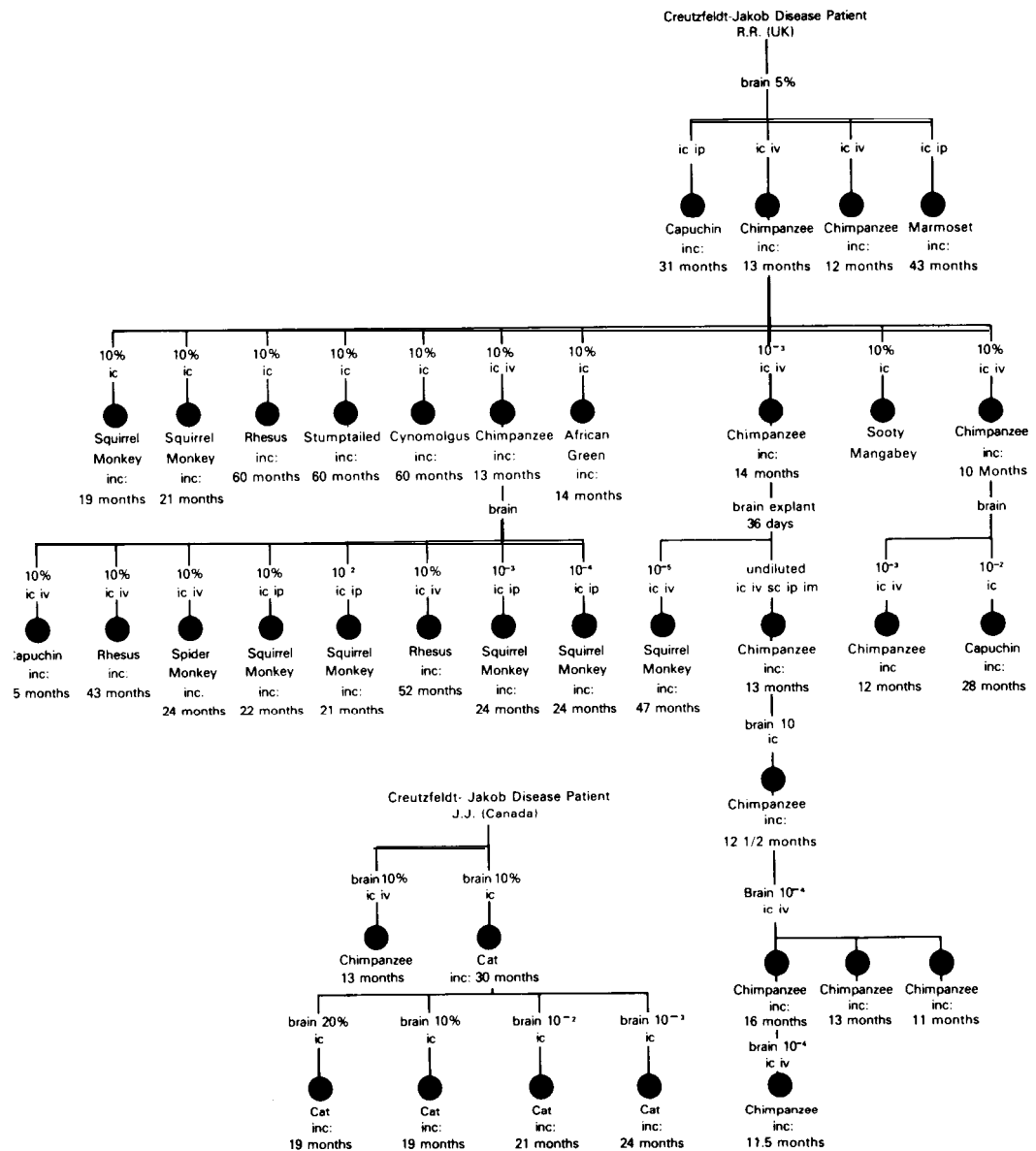


Figure 20. Six serial passages of CJD in chimpanzees, starting with brain tissue from a biopsy of a patient (R. R.) with CJD in the United Kingdom (U. K.). Also shown is transmission of the disease directly from man to the capuchin monkey and marmoset, and from chimpanzee brain to three species of New World monkeys (squirrel, capuchin, spider monkeys), and to six Old World species (rhesus, stumptailed, cynomolgus, African green, pigtailed, and sooty mangabey). Incubation periods in the *New World* monkeys ranged from 19 to 47 months, and in the *Old World* monkeys from 43 to 60 months. The pigtailed macaque and the sooty mangabey showed positive CJD pathology when sacrificed without

within the past several months. Finally, the recognition of TVD in a neurosurgeon (27), and more recently in two physicians, has raised the question of possible occupational infection, particularly in those exposed to infected human brain tissue during surgery, or at postmortem examination (61, 63).

The unexpectedly high incidence of previous craniotomy in CJD patients noted first by Nevin et al. (51) and more recently by Matthews (50) and by ourselves (62), raises the possibility of brain surgery either affording a mode of entry for the agent or of precipitating the disease in patients already carrying a latent infection. The former unwelcome possibility now seems to be a reality with the probable transmission of CJD to two young patients with epilepsy from the use of implanted silver electrodes sterilized with 70% ethanol and formaldehyde vapor after contamination from their use on a patient who had CJD. The patients had undergone such electrode implantation for stereotactic electroencephalographic localization of the epileptic focus at the time of correctional neurosurgery (3a).

Two patients with transmissible virus dementias were not diagnosed clinically or neuropathologically as having CJD, but rather as having Alzheimer's disease (62). In both cases the disease was familial: in one (Fig. 21) there were six close family members with the disease in two generations; in the other both the patient's father and sister had died of presenile dementia. The diseases as transmitted to primates were clinically and pathologically typical subacute spongiform virus encephalopathies, and did not have pathological features of Alzheimer's disease in man. More than 30 additional specimens of brain tissue from non-familial Alzheimer's disease have been inoculated into TVD-susceptible primates without producing disease. Therefore, although we

clinical disease. A third passage to the chimpanzee was accomplished using frozen and thawed explanted tissue culture of brain cells that had been growing *in vitro* for 36 days. Using  $10^3$ ,  $10^4$ , and  $10^4$  dilutions of brain, respectively, the 4th, 5th, and 6th chimpanzee passages were accomplished. This indicates that the chimpanzee brain contains >50,000 infectious doses per gram, and that such infectivity is maintained in brain cells cultivated *in vitro* at 37° C for at least one month. The lower left shows transmission of CJD from a second human patient (J. T.) to a cat with a 30 month incubation and serial passage in the cat with 19 to 24 month incubation.



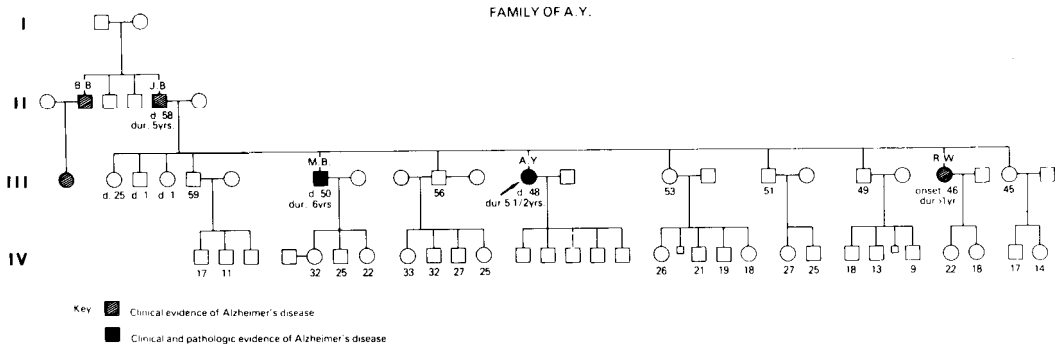
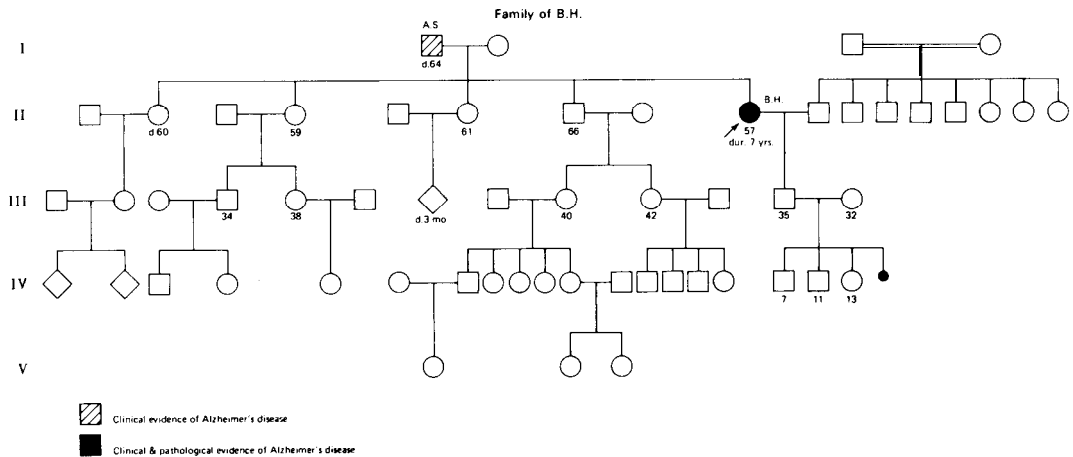


Figure 21a. Y family. Brain tissue obtained from patient A. Y. at biopsy induced subacute spongiform encephalopathy in a squirrel monkey 24 months after intracerebral inoculation. The patient, a 48-year old woman who died after a 68 month course of progressive dementia, quite similar in clinical aspects to the progressive dementia from which her father and brother had died at 54 and 56 years of age, respectively, was diagnosed clinically and neuropathologically as suffering from Alzheimer's disease. Her sister is at present incapacitated by a similar progressive dementia of 4 years' duration. Although the transmitted disease in the squirrel monkey was characterized by severe status spongiosis, none was seen in the patient. although amyloid plaques and neurofibrillary tangles were frequent.



21b. H family. Brain tissue obtained from patient B. H. at surgical biopsy induced subacute spongiform encephalopathy in a squirrel monkey and a capuchin monkey 29 1/2 months and 43 months, respectively, after intracerebral inoculations. The patient, a 57 year old woman, has had slowly progressive dementia and deterioration for the past 7 years. Neuro-pathological findings revealed abundant neurofibrillary tangles and senile plaques and no evidence of status spongiosis. The patient's father, A. S., had died at age 64 following several years of progressive dementia, behavioral change and memory loss. B. H. is presently alive and institutionalized.

cannot claim to have transmitted the classical sporadic Alzheimer's disease to primates, we are confronted with the anomaly that the familial form of Alzheimer's disease has, in these two instances, transmitted as though it were CJD.

The above findings have added impetus to our already extensive studies of Huntington's chorea, Alzheimer's and Pick's diseases, parkinsonism-dementia, senile dementia, and even "dementia praecox", the organic brain disease associated with late uncontrolled schizophrenia.

## SCRAPIE

Scrapie is a natural disease of sheep, and occasionally of goats, that has widespread distribution in Europe, America, and Asia. Affected animals show progressive ataxia, wasting, and frequently severe pruritis. The clinical picture and histopathological findings of scrapie closely resemble those of kuru; this permitted Hadlow (35) to suggest that both diseases might have similar etiologies. As early as 1936, Cuillé and Chelle (5b) had transmitted scrapie to the sheep, and its filterable nature and other virus-like properties had been demonstrated two to three decades ago (26). Because scrapie is the only one of the subacute spongiform virus encephalopathies that has been serially transmitted in mice, much more virological information is available about this agent than about the viruses that cause the human diseases.

Although scrapie has been studied longer and more intensely than the other diseases, the mechanism of its spread in nature remains uncertain. It may spread from naturally infected sheep to uninfected sheep and goats, although such lateral transmission has not been observed from experimentally infected sheep or goats. Both sheep and goats, as well as mice, have been experimentally infected by the oral route. It appears to pass from ewes to lambs, even without suckling; the contact of the lamb with the infected ewe at birth appears to be sufficient, because the placenta itself is infectious (39). Transplacental versus oral, nasal, optic, or cutaneous infection in the perinatal period, are unresolved possibilities. Older sheep are infected only after long contact with diseased animals; however, susceptible sheep have developed the disease in pastures previously occupied by scrapied sheep.

Both field studies and experimental work have suggested genetic control of disease occurrence in sheep. In mice, there is evidence of genetic control of length of incubation period and of the anatomic distribution of lesions, which is also dependent on the strain of scrapie agent used. Scrapie has been transmitted in our laboratory to five species of monkeys (Tables 9 and 10) (23, 31, 32), and such transmission has occurred using infected brain from naturally infected sheep and from experimentally infected goats and mice (Figures 22a, b, c). The disease produced is clinically and pathologically indistinguishable from experimental CJD in these species.

# TRANSMISSION OF U. S. STRAIN (C-506) OF SHEEP SCRAPIE TO MICE AND NON-HUMAN PRIMATES ON PRIMARY AND SERIAL PASSAGE

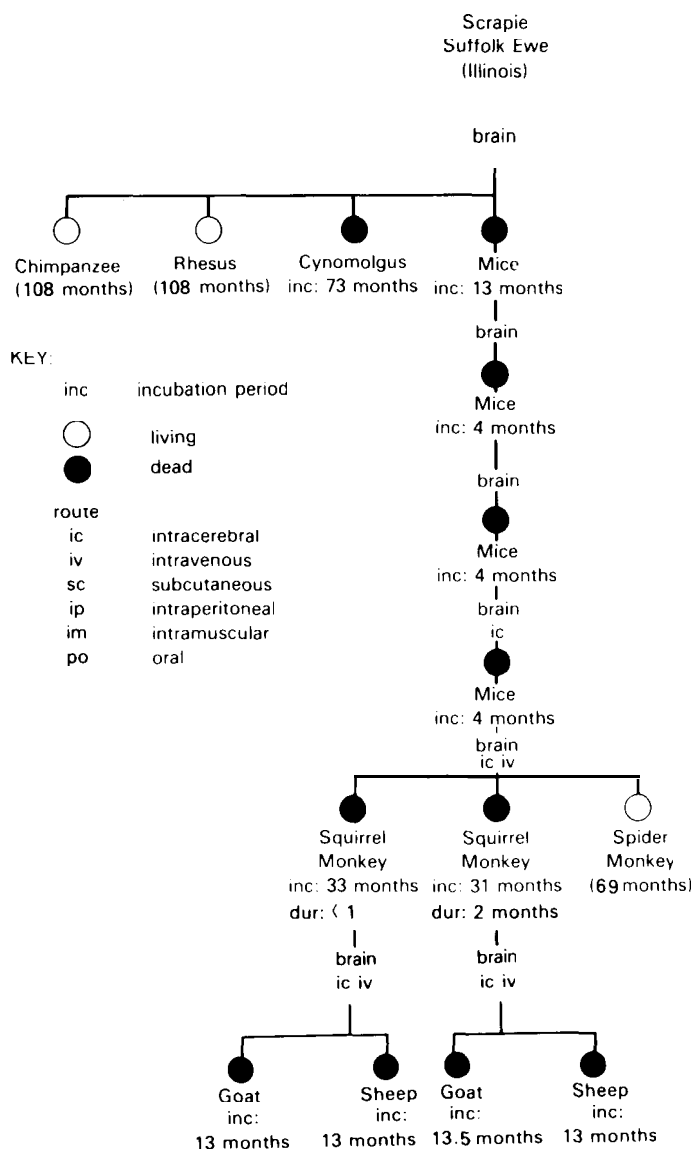
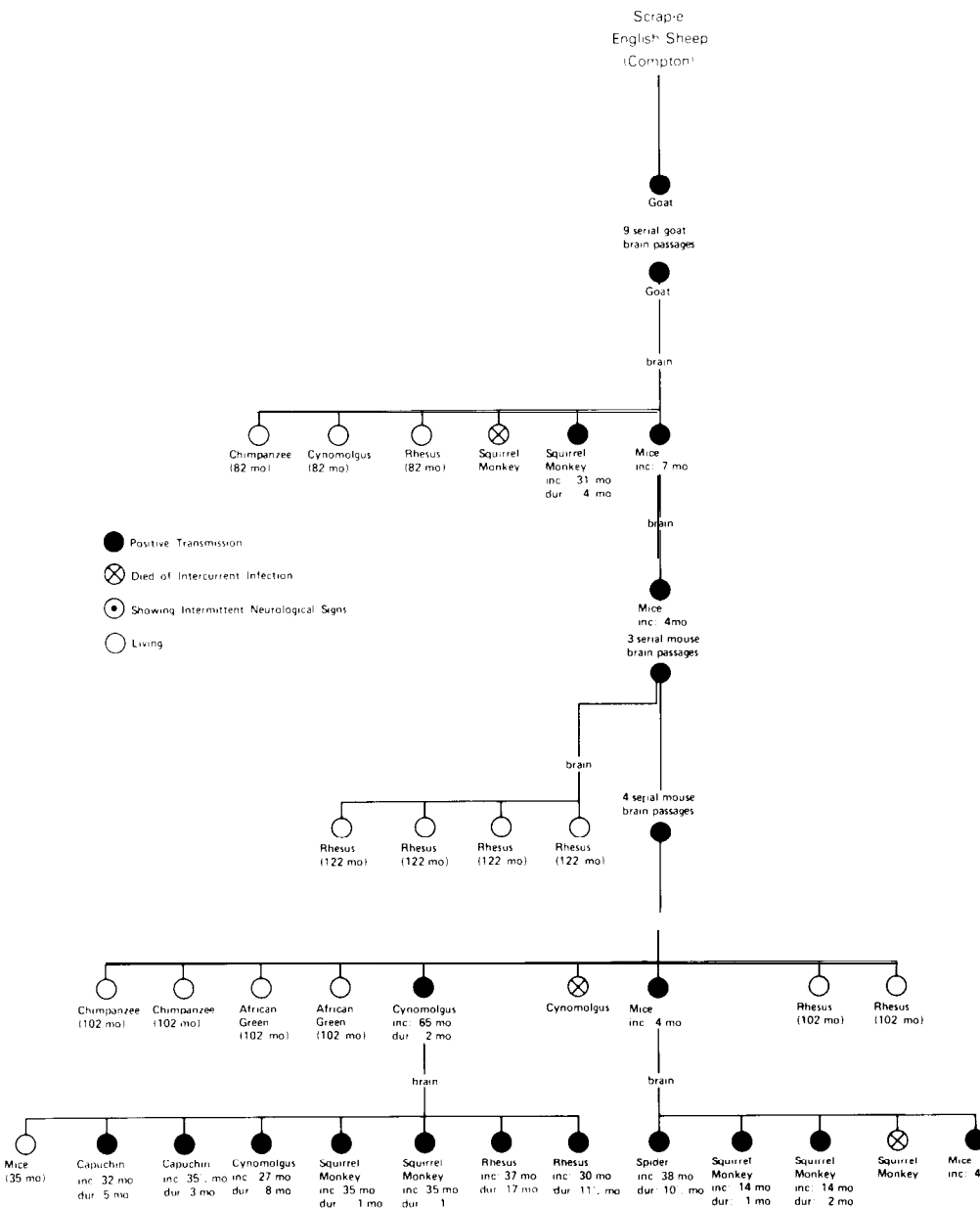


Figure 22. Scrapie has been transmitted to three species of New World monkeys and two species of Old World monkeys (Tables 9, 10).

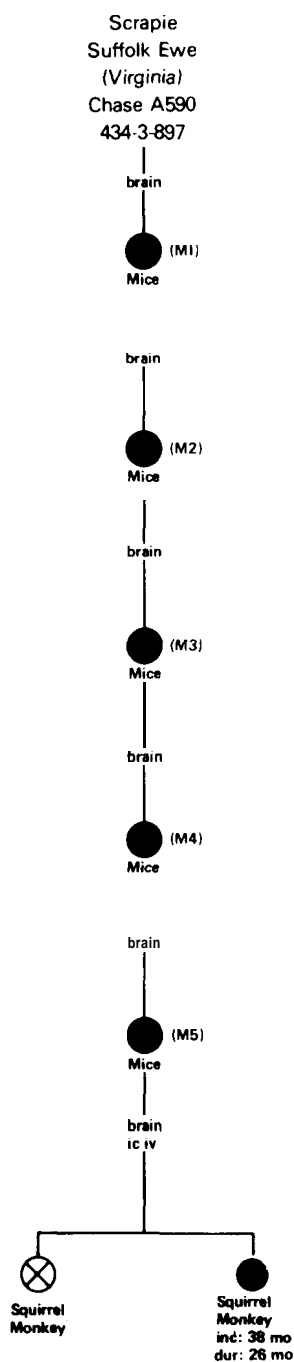
22a. Transmission of scrapie from the brain of a scrapie-infected Suffolk ewe (C506) in Illinois to a cynomolgus monkey, and from the 4th mouse passage of this strain of scrapie virus to two squirrel monkeys. Incubation period in the cynomolgus was 73 months and in the squirrel monkeys 31 and 33 months. A chimpanzee and a rhesus monkey inoculated 109 months ago with this sheep brain remain well, as does a spider monkey inoculated 70 months ago with brain from the 4th passage of the C506 strain of scrapie in mice.

TRANSMISSION OF GOAT-ADAPTED AND MOUSE-ADAPTED SCRAPIE  
(COMPTON STRAIN) TO NON-HUMAN PRIMATES



22b. Primary transmission of goat-adapted scrapie (Compton, England strain) to the squirrel monkey and to mice and the transmission of mouse-adapted scrapie to two species of Old World and three species of New World monkeys. Numbers in parentheses are the number of months elapsed since inoculation, during which the animal remained asymptomatic.

TRANSMISSION OF MOUSE-ADAPTED SHEEP SCRAPIE  
(U.S. STRAIN 434-3-897) TO A SQUIRREL MONKEY

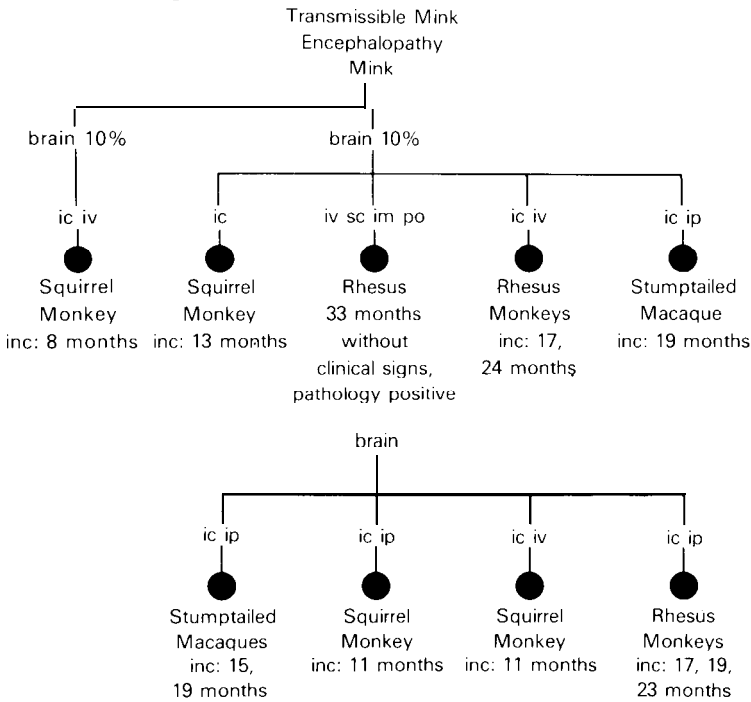


22c. Transmission of mouse-adapted sheep scrapie (U. S. strain 434-3-897) to a squirrel monkey 38 months following intracerebral inoculation with a suspension of scrapie-infected mouse brain containing  $10^{4.3}$  infectious units of virus per ml. This animal showed signs of ataxia, tremors and incoordination, and the disease was confirmed histologically. See (b) for an explanation of symbols.

TRANSMISSIBLE MINK ENCEPHALOPATHY

Transmissible mink encephalopathy (TME) is very similar to scrapie both in clinical picture and in pathological lesions. On the ranches on which it developed, the carcasses of scrapie-infected sheep had been fed to the mink; presumably the disease is scrapie. The disease is indistinguishable from that induced in mink by inoculation of sheep or mouse scrapie. Like scrapie, TME has been transmitted by the oral route, but transplacental or perinatal transmission from the mother has not been demonstrated. Physicochemical study of the virus has thus far revealed no differences between TME and the scrapie virus (42, 49).

The disease has been transmitted to the squirrel, rhesus, and stump-tailed monkey (Tables 9 and 10; Fig. 13), and to many nonprimate hosts, including the sheep, goat, and ferret, but has not been shown to transmit to mice (Table 11). In monkeys the illness is indistinguishable from experimental CJD in these species.



Information from R.F.Marsh, R.J.Eckroade, R.P.Hanson,  
C.J.Gibbs, and D.C.Gajdusek

Figure 23. Transmissible mink encephalopathy (TME), a rare disease of American ranch mink, is possibly a form of scrapie. The clinical picture and histopathological lesions attendant in the brain, resemble that of scrapie, and scrapie sheep carcasses were fed to mink on ranches on which TME appeared. The disease is transmissible to sheep, goats, certain rodents and New and Old World monkeys. Illustrative data on the primary transmissions of transmissible mink encephalopathy to one species of New World monkey and two species of Old World monkeys, and serial passage of the virus in squirrel, rhesus and stumptailed monkeys are presented in this Figure. Incubation periods are shown in months that elapsed between inoculation and onset of clinical disease. (Figure includes information from our laboratory and from R. F. Marsh, R. J. Eckroade, and R. P. Hanson.)

## ORIGIN AND SPREAD OF KURU

Unanswered crucial questions posed by all of these agents are related to their biological origin and mode of survival in nature. The diseases they evoke are not artificial diseases, produced by researchers tampering with cellular macromolecular structures, as some would have it. They are naturally occurring diseases, for none of which do we know the mode of dissemination or maintenance which is adequate to explain their long-term persistence. For kuru we have a full explanation of the unique epidemiological findings and their change over the past two decades: the contamination of close kinsmen within a mourning family group by the opening of the skull of dead victims in a rite of cannibalism, during which all girls, women, babes-in-arms, and toddlers of the kuru victim's family were thoroughly contaminated with the virus (15, 17, 21). The disease is gradually disappearing with the cessation of cannibalism and has already disappeared in children, with progressively increasing age of the youngest victims (Figs. 7-9, 24, 26). **However, this does not provide us with a satisfactory explanation for the origin of kuru. Was it the unlikely event of a sporadic case of worldwide CJD, which in the unusual cultural setting of New Guinea produced a unique epidemic? We now have the report of a spontaneous case of CJD in a 26 year old native Chimbu New Guinean from the Central Highlands, whose clinical diagnosis was proved by**



Figure 24. A Fore mother mourning over the body of her dead daughter, who has just died of kuru. The deep decubitus ulcer below her right hip indicates her chronic debility, which is in contrast to her good nutritional state. Men, and already initiated boys, rarely participated in the mourning rite around the corpse, and even more rarely in the dissection and preparation of the kuru victim's flesh for its ritual endocannibalistic consumption.



Figure 25. All cooking, including that of human flesh from diseased kinsmen, was done in pits with steam made by pouring water over the hot stones, or cooked in bamboo cylinders in the hot ashes. Children participated in both the butchery and the handling of cooked meat, rubbing their soiled hands in their armpits or hair, and elsewhere on their bodies. They rarely or never washed. Infection with the kuru virus was most probably through the cuts and abrasions of the skin, or from nose-picking, rye rubbing, or mucosal injury.

light- and electronmicroscopic examination of a brain biopsy specimen (24, 37a). Serial passage of brain in main in successive cannibalistic rituals might have resulted in a change in the clinical picture of the disease, with modification of the virulence of the original agent.

If such spontaneous CJD is not related to the origin of kuru, another possibility might be that the serial brain passage that occurred in this ritual inoculation of brain from successive victims in multiple sequential passages into their kinsmen yielded a new neurotropic strain of virus from some well-known virus. Finally, in view of what occurs in the defective replication of measles virus in patients with SSPE, we must wonder if a ubiquitous or, at least, a well-known virus may not be modified into a defective, incomplete, or highly integrated or repressed agent *in vivo* in the course of its long masked state in the individual host. Such a new breed of virus may no longer be easily recognizable either antigenically or structurally, because of failure of full synthesis of viral subunits or of their assembly into a known virion. Therefore, we may ask if kuru does not contain some of the subunits of a known agent, modified by its unusual passage history (15, 16, 22).





26a



26b



26c

Figure 26a. An Awa boy just before first stage initiation, while still living in the women's house with his sisters and small pigs. At this age, boys were already well trained in the use of bows and arrows in hunting.

26b. Youthful Awa toxophilite, already a warrior.

26c. Young Awa warriors in their boy's house.



27a



27b



27c



27d

Figure 27. Boys of prepubertal age were removed from the women's houses to enter the wa'e, men's house, after elaborate first-stage initiation ceremonies. Thereafter, and for the rest of their lives, they would live, eat, and sleep separately from the women. Married men did not share the houses of their wives, and sexual activity was restricted to daylight in the secluded privacy of the gardens. Three Fore boys are shown in the first stage of initiation in 4 sequences (a-d) during their ceremonial adornment, after having been held in seclusion for several days and having their nasal septa pierced.

27a. Bark strips have been braided into their hair.

27b. Bands of shells of high value to the Fore are fastened to their foreheads.

27c. Their bodies are rubbed with pig grease.

27d. They are given new bark sporans and bows and arrows.

# CONJECTURAL NATURAL HISTORY OF THE SUBACUTE SPONGIFORM VIRUS ENCEPHALOPATHIES: HYPOTHETICAL ORIGIN OF CREUTZFELDT-JAKOB DISEASE, KURU, AND TRANSMISSIBLE MINK ENCEPHALOPATHY FROM NATURAL SHEEP SCRAPIE

Scrapie has now been found to cause a disease clinically and neuropathologically indistinguishable from experimental Creutzfeldt-Jakob disease in three species of New World and two species of Old World monkeys (Tables 9 and 10). This disease occurs after either intracerebral or peripheral routes of inoculation. Natural sheep scrapie, as well as experimental goat and mouse scrapie strains of virus have caused disease in the monkeys. The Compton strain of scrapie virus, as a result of such passage through primates, develops an altered host range, for it no longer produces disease in inoculated mice, sheep and goats. A similar situation has been noted to prevail when scrapie is produced in ferrets or mink; the mink or ferret brain virus is no longer pathogenic for mice. This is also true for the virus of natural mink encephalopathy, which, presumably, had its origin in the feeding of scrapie sheep carcasses to mink on commercial mink farms.

Creutzfeldt-Jakob disease or kuru viruses may produce, after over two years of asymptomatic incubation, an acute central nervous disease with death in a few days in the squirrel monkey; even sudden death without previously noted clinical disease has been seen. The same strains of kuru or CJD viruses produce chronic clinical disease in the spider monkey, closely mimicking the human disease, after incubation periods of two years or more. The time sequence of disease progression also mimics that in man, ranging from several months to over a year until death. A single strain of kuru or CJD virus may cause severe status spongiosus lesions in many brain areas, particularly the cerebral cortex in chimpanzees and spider monkeys with minimal or no involvement of the brainstem or spinal cord, whereas in the squirrel monkey this same virus strain may cause extensive brainstem and cord lesions.

From the above findings, it is clear that neither incubation periods nor host range, nor the distribution or severity of neuropathological lesions, can be interpreted as having any significance toward unraveling the possible relationships of the four viruses causing the subacute spongiform virus encephalopathies.

As mentioned earlier, we have found that the prevalence of CJD in the United States and abroad appears to be about one per million whenever extensive neurological survey for cases is instituted. In a study in Israel, an overall prevalence in Jews of Libyan origin is 30 times as high as in Jews of European origin (40). The custom of eating the eyeballs and brains of sheep in the Jewish households of North African and Middle Eastern origin, as opposed to Jewish households of European origin, has understandably given rise to the conjecture that scrapie-infected sheep tissue might be the source of such CJD infection (37).

Figure 28 presents a conjectural schematic natural history of the subacute spongiform virus encephalopathies in which the hypothetical origin of CJD,

CONJECTURAL NATURAL HISTORY OF THE SUBACUTE SPONGIFORM VIRUS ENCEPHALOPATHIES  
HYPOTHETICAL ORIGIN OF CREUTZFELDT-JAKOB DISEASE (CJD), KURU, AND TRANSMISSIBLE MINK  
ENCEPHALOPATHY (TME) FROM NATURAL SCRAPIE OF SHEEP

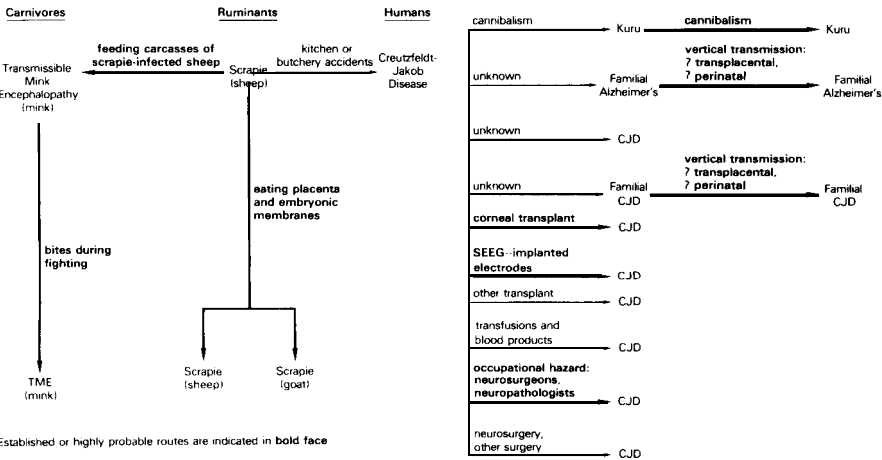


Figure 28.

kuru, and TME from natural scrapie in sheep is proposed with possible routes of transmission indicated. However, such games of armchair speculation provide schemata that cannot yet be tested. They may, nevertheless, have heuristic value. In the absence as yet of proven antigenicity or identified infectious nucleic acid in the agents, neither serological specificity nor nucleic acid homology can be used to answer the compelling question of the relationship between the viruses of kuru, transmissible virus dementia, scrapie, and transmissible mink encephalopathy.

The possibility that the viruses of all four of the subacute spongiform virus encephalopathies are not just closely related agents, but different strains of a single virus which have been modified in different hosts, is easily entertained. The passage of sheep scrapie into other sheep and into goats, at least by the route of feeding of material contaminated with placenta and embryonic membrane (53), and into mink from feeding carcasses of scrapied sheep, are established paths of scrapie transmission. In view of the experimental transmission of scrapie to monkeys, there is serious cause for wonder whether kitchen and butchery accidents involving the contamination of skin and eyes may not be a possible source of CJD in man (36a, 37). We believe that contamination during the cannibalistic ritual was the sole source of transmission of kuru from man to man, and have conjectured above that a spontaneous case of CJD may have given rise to the chain of kuru transmissions (17). The documented case of CJD from corneal transplant (12) suggests that other tissue transplantation may also be a source of infection. It is known that the virus is present in peripheral tissue, as well as in the brain. The case of CJD in a neurosurgeon who had frequently performed autopsies (27), poses a possibility of occupational hazard to the neurosurgeon and neuropathologist (61-63). Finally, the rather frequent report of neurosurgery or other surgery preceding the appearance of CJD, as noted by us (62) and by other workers (50, 51), may indicate that such surgery has been a source of infection, rather than a virus activating

incident. This seems to be a real hazard in view of the recent episode of transmission of CJD to two patients from the use of CJD-contaminated electrodes in stereotactic EEG during surgery for epilepsy (3a). The use of formaldehyde for their sterilization was, in view of the resistance of the unconventional viruses to it (26), a very unfortunate choice. The mode of transmission, which at first sight would appear to be vertical in the cases of familial CJD or familial Alzheimer's disease, remains unknown (4, 13, 50, 62). Whether infection is transovarian or occurs in utero or during parturition, or from a milk factor or some other neonatal infection, also remains unknown, although from kuru epidemiological study (i.e., failure to see kuru in children born to kuru-affected mothers since the cessation of cannibalism), we have no evidence for such transmission (17).

## PROSPECT

The elucidation of the etiology and epidemiology of a rare, exotic disease restricted to a small population isolate-kuru in New Guinea-has brought us to worldwide considerations that have importance for all of medicine and microbiology. For neurology, specifically, we have considerable new insights into the whole range of presenile dementias and, in particular, to the large problems of Alzheimer's disease and the senile dementias. The implications of vertical transmission of slow virus infections, and of host genetic control of disease expression for all genetic diseases, and the relationship of these slow virus infectious processes to those which may lead to neoplastic transformation, are obvious.

However, the major problems among the degenerative diseases: multiple sclerosis, amyotrophic lateral sclerosis, and parkinsonism remain unsolved, although there are tantalizing laboratory and epidemiological data pointing to the possible role of virus-like agents in these diseases. Perhaps the masked and defective slow infections with conventional viruses such as are seen in PML and SSPE, may provide the best leads for studying these diseases.

The foci of high incidence of amyotrophic lateral sclerosis with associated high incidence of parkinsonism-dementia complex among the Chamorro people on Guam and the Japanese of the Kii Peninsula remain continuing challenges. Our discovery (14) and reevaluation (20) of the very small but very intense focus of such motor neuron disease with associated high incidence of parkinsonism, parkinsonism-dementia, and other peculiar bradykinetic and myoclonic dementia syndromes among the Auyu and Jaqai people in a remote population of West New Guinea, suggests strongly that some common etiological factor may underly the occurrence of all these very different syndromes, as they occur strangely in this one small population and are not found in the much larger surrounding populations.

The models of lysogenicity and of subviral genetically active macromolecular structures from the study of bacterial viruses and bacterial genetics supply ample imaginative framework for an expression of our ideas of possible mechanisms of infectious pathogenesis in man. The unconventional viruses tax

even our imagination in relation to molecular biology gained from these studies in bacteria.

For a now-disappearing disease in a small primitive population to have brought us this far is ample reason for pursuing intensively the challenges offered by the still inexplicable high incidence and peculiar profusion of different neurological syndromes, pathologically distinct yet apparently somehow related to each other, which have been discovered in the several small population enclaves (14, 20, 21).

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