Experimental Subacute Spongiform Virus Encephalopathies in Primates and Other Laboratory Animals

Abstract. *The host range of subacute spongiform virus encephalopathies is described. The asymptomatic incubation period and the duration of the illnesses in various species of animal hosts is discussed along with information on additional species of Old World and New World monkeys and the domestic cat, which have been shown to be susceptible to subacute spongiform virus encephalopathies.*

The discovery that human kuru can be transmitted to the chimpanzee after 18 to 24 months of incubation (*1*) led to an extensive use of this species to elucidate the pathogenesis of this disease and the characterization of the virus (*2*). This led to the discovery that the Creutzfeldt-Jakob (C-J) type of presenile dementia, a pathologically and symptomatically similar neurological disease, is also transmissible to the chimpanzee after an asymptomatic incubation period of 10 to 14 months (*3*). Other Old World and New World subhuman primates appeared to be resistant to both viruses during the first 2 years after inoculation. However, on longer observation several species of monkeys have developed both kuru and C-J disease after extraordinarily long, silent, asymptomatic incubation periods (*4*). We have now shown that many species of primates, including both Old World and New World monkeys, are susceptible to both diseases. Moreover, the two slow virus induced subacute spongiform encephalopathies of domestic animals, scrapie of sheep and goats (*5*), and transmissible mink encephalopathy (*6*) produce spongiform encephalopathy in Old World and New World monkeys. We now report the extended range of host susceptibility for these viruses (Table 1). The successful transmissions of C-J disease to cats and to Old World monkeys have not been reported, nor has a summary of our unsuccessful attempts to transmit kuru and C-J disease to smaller animals.

We wish to transmit the slow virus infections to a host less expensive, in less danger of becoming extinct, and more easily housed in the laboratory than the chimpanzee and the spider monkey. Since no indicators of the viruses are available other than infectivity, clinical disease, and neuropathological lesions in an experimental animal, and since no immunological response has been demonstrated, and no cytopathic effect occurs in vitro in infected cell cultures (*7*), a host with shorter incubation period is needed. The experimentally infected primates provide a very precise animal model of human disease; the etiology and patho-

Table 1. Host range of subacute spongiform virus encephalopathies. All data on kuru and Creutzfeldt-Jakob disease are from our laboratory. Marmoset experiments were conducted in collaboration with Peterson and co-workers (*9*). All data on scrapie were obtained in our laboratory except those for sheep, goats, rats, and hamsters (*10*); mink (*11*); and voles (*12*). All data on mink encephalopathy are from Marsh *et al.* (*6*) except for our own data on chimpanzees, squirrel monkeys, and transmissible mink encephalopathy in sheep (*13*); putative mouse transmission (*14*) has not been confirmed. Parentheses indicate the total number of months that animals have been on test without developing clinical disease; NT, not tested.

Host	Kuru		Creutzfeldt-Jakob disease		Scrapie		Transmissible mink encephalopathy	
	Incubation period (months)	Duration of disease (months)	Incubation period (months)	Duration of disease (months)	Incubation period (months)	Duration of disease (months)	Incubation period (months)	Duration of disease (months)
Man	60–> 240	4–12	4–5	4–48				
Chimpanzee	10–59	1–15	11–26	4–6	(107)		(39)	
New World monkeys								
Spider	10–51	1–13	21–37	1–6	(28)		NT	
Squirrel	9–44	1–4	13–25	1–4	14	2	8–11	1
Capuchin	10–45	1–6	29–39	5–12	(20)		NT	
Woolly	33	4	21	4	NT		NT	
Marmoset	31–38	< ½	43	< ½	NT		NT	
Old World monkeys								
Rhesus	101	2	(53)		(107)		11–33	< 1–8
Bushbaby	(65)		16	2	NT		NT	
Stump-tailed	(60)		(60)		NT		16	1
Cynomolgus	(119)		(53)		65	2	NT	
Mangabey	(75)		41	2½	NT		NT	
African green	(93)		33–47	< 1–12	(107)		NT	
Sheep	(87)		(60)		5–12	1–6*	48	< 1
Goat	(74)		(24)		8–17	3–5†	20–36	3–5
Calf	NT		NT		NT		(13)	
Mink	(33)		(39)		12–19	1–2	4–33	1–2
Ferret (albino)	NT		NT		NT		14–15	1
Ferret (black)	(55)		(39)		(51)		(39)	
Cat (domestic)	(30)		30	2	NT		(40)	
Raccoon	NT		NT		NT		6	
Skunk (striped)	NT		NT		NT		6	
Mice	(48)		(48)		4–17	< 1–2	(24)	
Rats	(51)		(46)		7–10	< 1	NT	
Hamster (golden)	(36)		(36)		9–12	< 1	18	< 1
Hamster (Chinese)	(36)		(36)		4–5	3	NT	
Gerbil	(24)		(24)		4–5	< 1	NT	
Vole	NT		NT		2–4	1–2 days	NT	
Guinea pig	(72)		(72)		(24)		(1)	
Rabbit	(36)		(36)		(36)		(3)	

* For natural sheep scrapie, the incubation period is 30 to 60 months; the duration of the disease is 1 to 6 months (*15*). † For natural goat scrapie the incubation period is 6 to 48 months; the duration of the disease is less than 1 to 2 months (*16*).

genesis are the same as in the diseases in man.

We have been unable to transmit either of the two human diseases to nonprimate hosts. We would like to infect smaller laboratory animals, but we were unsuccessful in our attempts to infect more than 20 strains of inbred and randomly bred mice and mice immunosuppressed before and after inoculation with cortisone, antilymphocyte serum, cyclophosphomide, and x-irradiation. Neonatally thymectomized and splenectomized mice have also failed to develop disease. We have not been able to confirm an earlier report that kuru was transmitted to mice that were x-irradiated after inoculation (*8*).

Other small laboratory animals, such as rats, Syrian hamsters, guinea pigs and rabbits, hens, ducks, and dogs have not been susceptible to either kuru or C-J disease. However, we have now one domestic cat, inoculated 30 months previously with brain tissue from a C-J patient, that developed the disease with accompanying pathognomic lesions of neuronal vacuolation, glial proliferation, and spongiosis in the cerebral cortical gray matter. Thus, the host ranges of the viruses of kuru and C-J disease have been extended, and there is reason to hope that the agents may eventually be adapted to mice or other small and inexpensive laboratory animals and that the disease will then have a shorter incubation period.

Note added in proof: Since this report was written we have confirmed the transmission of kuru and C-J disease to additional nonhuman primates. Histopathological lesions of kuru have been observed in the absence of clinical kuru in a gibbon ape and a mangabey which died of intercurrent illnesses 9 months and 2 months, respectively, after inoculation. Clinically recognizable C-J disease has been diagnosed and confirmed histologically in rhesus monkeys (65 to 66 months of incubation), a stump-tailed monkey (61 months of incubation), and a cynomolgus monkey (61 months of incubation); and early histopathological lesions of C-J disease have been observed in a mangabey and a pig-tailed monkey, both of which died of intercurrent illnesses 2 months after inoculation. Attempts to serially propagate kuru and C-J disease in the aforementioned species of animals have already been initiated.

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Induction of an Enzyme in Genetically Deficient Rats after Grafting of Normal Liver

Abstract. *Tissue from normal rat livers was grafted onto the livers of rats that were genetically deficient in bilirubin uridine diphosphate glucuronyltransferase activity. Twelve weeks after the grafting operation, the liver of the recipient rats had bilirubin uridine diphosphate glucuronyltransferase activity.*

The induction or transfer of enzyme activity would be an attractive mode of therapy for some of the enzyme deficiency diseases. Rugstad and his co-workers (*1*) have shown that bilirubin uridine diphosphate (UDP) glucuronyltransferase (E.C. 24.1.17) activity could be transferred into enzyme deficient homozygous Gunn rats. This was accomplished by subcutaneous transplantation of a clonal strain of rat hepatoma cells (*2*). However, transplantation of a viable neoplastic tissue holds little promise of a direct clinical application. In these studies, we grafted small amounts of normal Wistar rat liver into the liver of its enzyme deficient mutant strain, the homozygous Gunn rat, and measured the effects of the grafts upon bilirubin UDP glucuronyltransferase activity and serum bilirubin concentrations in the Gunn rats.

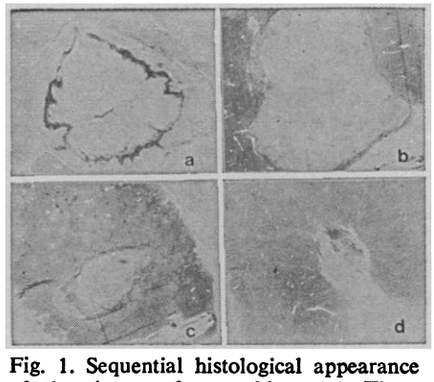

Fig. 1. Sequential histological appearance of the tissues after grafting. (a) Three weeks after the graft procedure. The pale graft is surrounded by fibrotic tissues and some inflammatory cells. (b) Six weeks after the graft procedure. The graft seems to be near the periphery. Fibrotic tissues cannot be seen. (c) Nine weeks after grafting. The size of the tissues has diminished. (d) Twelve weeks after grafting. No visible graft tissue can be identified. The location of the graft can be identified by the cleft.

Homozygous Gunn rats were identified by their yellow color shortly after birth. Their ears were punched for subsequent identification. They were weaned at 21 days of age, a blood sample was taken from the orbital sinus cavity, and serum bilirubin concentration was determined by absorbance at 450 nm (Cary model 15 recording spectrophotometer). Elevated bilirubin concentrations in homozygous animals indicated that these rats were deficient in UDP glucuronyltransferase activity. At 6 to 8 weeks, these rats were checked again for serum bilirubin concentration and were used as recipients in the grafting experiments. Donor and recipient rats were matched for age and sex, then simultaneously anesthesized by an intraperitoneal injection of 1 ml of an aqueous solution of tribromoethanol (2 percent). Approximately 5 percent of the liver of each Gunn rat was removed with a uterine punch biopsy forceps. We replaced the punch biopsies of each Gunn rat liver with identical sections from the liver of a normal Wistar rat donor.