Fantasy of a "Virus" from the Inorganic World: Pathogenesis of Cerebral Amyloidoses by Polymer Nucleating Agents and/or "Viruses"

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A. Introduction

Kuru and the transmissible virus dementias are in a group of virus-induced slow infections that we have described as subacute spongiform virus encephalopathies (SSVEs) because of the strikingly similar histopathological lesions they induce. Scrapie, mink encephalopathy, and the chronic wasting disease with spongiform encephalopathy of captive mule deer and of captive elk all appear, from their histopathology, pathogenesis, and the similarities of their infectious agents, to belong to the same group (Gajdusek and Gibbs 1975; Gajdusek et al. 1965, 1966; Masters et al. 1981 a, b; Williams and Young 1980, 1982; Williams et al. 1982). The basic neurocytological lesions in all these diseases are 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, spongiform change or status spongiosis of gray matter and extensive neuronal loss (Beck et al. 1975, 1982; Klatzo et al. 1959).

These atypical infections differ from other diseases of the human brain, that have been subsequently demonstrated to be slow virus infections, in that they do not evoke a virus-associated inflammatory response in the brain (i.e., no perivascular cuffing or invasion of the brain parenchyma with leukocytes); they usually show no pleocytosis nor marked rise in protein in the cerebrospinal fluid throughout the course of infection (Gajdusek 1985 b; Gajdusek and Zigas 1957, 1959; Traub et al. 1977). Furthermore, they show no evidence of an immune response to the causative virus and there are no recognizable virions in sections of the brain visualized by electron microscopy, whereas in other virus encephalopathies virions have been readily observed. Instead, they show ultrastructural alteration in the plasma membrane that lines the vacuoles (Beck et al. 1982), piled up neurofilament in some swollen nerve cells (Beck et al. 1975, 1982; Klatzo et al. 1959; Lampert et al. 1971) and strange arrays of regularly arrayed tubules that look like particles in cross-section in postsynaptic processes (Baringer et al. 1979, 1981; David-Ferreira et al. 1968; Field and Narang 1972; Field et al. 1969; Lamar et al. 1974; Narang 1973; 1974 a, b; Narang et al. 1972, 1980; Vernon et al. 1970; ZuRhein and Varakis 1976).

The pursuit of the transmissibility and virus etiology of kuru (Gajdusek and Zigas 1957, 1959; Gajdusek et al. 1966; Klatzo et al. 1959) and the presenile dementia of the Creutzfeldt-Jakob disease (CJD) type (Gajdusek 1977; Gajdusek and Gibbs 1975; Gibbs et al. 1968) has led to the definition of the unconventional viruses as a new group of microbes, which, because of their very atypical physical, chemical, and biological properties, has stimulated a worldwide quest to elucidate their structures and resolve the many paradoxes they present to the basic tenets of microbiology and to solve the enormous clinical and epidemiological problems these viruses pose. The

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unanticipated ramifications of the discovery of these slow infections and the peculiar properties of the unconventional viruses, which have even challenged the central dogma of modern molecular biology, have led to a series of discoveries each of which have wide implications to microbiological and neurobiological research (Braig and Diringer 1985; Diringer et al. 1983; Gajdusek 1977, 1984, 1985a, b, c; Gajdusek and Gibbs 1975; Goldgaber et al. 1987a, b; Masters et al. 1981a, b, 1985a, b; Multhaup et al. 1985; Oesch et al. 1985; Prusiner 1982, 1984; Prusiner et al. 1983, 1984; Robertson et al. 1985; Rohwer 1984a, b, c; 1985, unpublished work; Rohwer and Gajdusek 1980; Rohwer et al. 1979). These are summarized below.

B. Interference with Axonal Transport. Amyloid Formation from Precursor Protein in Alzheimer's Disease and Normal Aging and in Slow Virus Infections

The cytoskeleton of all cells contains three ultrastructurally distinct elements made of fibrous macromolecules; microtubules 24 nm in diameter, intermediate filaments 10 nm in diameter, and microfilaments about 5 nm in diameter and composed of polymerized actin.

Neurofilaments, also called neuronal intermediate filaments, are antigenically distinct from the intermediate filaments of other cells. They extend from the cell body down the whole length of the axon; they are composed of three proteins of 200, 150, and 68 kilodaltons (kDa), respectively. Our work on the etiology of kuru (Gajdusek 1977, 1984, 1985a, b, c; Gajdusek and Gibbs 1975; Gajdusek and Zigas 1957, 1959; Gajdusek et al. 1965, 1966; Klatzo et al. 1959) and on the cause of amyotrophic lateral sclerosis (ALS) and parkinsonism with dementia (PD) with the early appearance of neurofibrillary tangles (NFT; Anderson et al. 1979; Chen 1981) in the populations in high incidence foci in the Western Pacific (Gajdusek 1988a; Gajdusek and Salazar 1982; Garruto et al. 1982, 1984, 1986; Perl et al. 1982) has pointedly emphasized that this molecular complex is not a static cytoskeletal structure, but moving fibers, responsible for the slow component of axonal transport of lysosomes, enzymes, and transmitter molecules to the presynaptic terminals (Gajdusek 1984, 1985a, b, c).

Interference with axonal transport may be responsible for a stagnation or pooling of cytoskeletal elements, and subsequent degradation of the sequestered cytoskeletal molecule(s) or matrix protein(s) in which they rest to form amyloid fibrils of paired helical filaments (PHFs) in the neurofibrillary tangles (NFTs) and the neuritic plaques that characterize Alzheimer's disease (Autilio-Gambetti et al. 1983; Gambetti et al. 1983a; Bizzi et al. 1984; Dahl and Bignami 1985; Gambetti et al. 1983a, b; Hirano and Inoue 1980; Hirano et al. 1984a, b; Inoue and Hirano 1979; Klatzo et al. 1965; Rasool and Selkoe 1985; Selkoe et al. 1986; Sotelo et al. 1980b; Terry and Pena 1965). Furthermore, it now appears that amyloid deposits in the nervous system, particularly the amyloid plaques of Alzheimer's disease and those of Down's syndrome, Pick's disease, and normal aging, and the perivascular accumulations of amyloid in the CNS, and in the vascular walls extending out into the meninges, are derived from a precursor matrix protein (amyloid B-protein or A4) trapped in these cytoskeletal accumulations, while the paired helical filaments of NFTs may represent yet further intracellular degradation of the same precursor matrix protein in the stagnated cytoskeletal elements (Glenner and Wong 1984a, b; Gredert et al. 1988; Guiroy et al. 1987; Kidd et al. 1985; Ksiezak-Reding et al. 1988a, b; Masters et al. 1985a, b; Schubert et al. 1988; Wischik et al. 1988a, b). We earlier presumed that the precursor for these brain amyloid fibrils is the 200-kDa component of the protein triad from which 10-nm neurofilaments are formed or microtubule-asso-

ciated proteins tau (MAP-tau; Anderton et al. 1982; Gajdusek 1984, 1985a). However Goldgaber et al. (1987a, b) have isolated, sequenced, characterized, and localized on chromosome 21 cDNA clones coding for this precursor protein from the adult human brain cDNA library. Kang et al. (1987) have also isolated and sequenced the cDNA coding for the same protein from the fetal human brain cDNA library, and Tanzi et al. (1987) and Robakis et al. (1987) have isolated the same gene. Down's syndrome patients carry three copies of this gene and overexpress this precursor and thus express NFTs, amyloid plaque cores (APCs), and congophilic angiopathy (CA) 50 years earlier than normal subjects (Delabar et al. 1987), but we cannot reconfirm that AD patients carry three copies of the gene as we first reported (Delabar et al. 1987). However, since virtually everyone shows NFTs, APCs, or CA by 100 years of age, no abnormal gene is needed for the production of this amyloid.

The normal amyloid precursor B-protein gene is overexpressed in brains of Alzheimer's disease patients (Cohen et al. 1988; Higgins et al. 1988; Schmechle et al. 1988). It also encodes several different mRNAs that are identical except for the expression of a 168-bp long exon, or an added 57-bp long exon immediately following, at position 289 (between the G and TT) of a GTT valine codon) of the full mRNA without either of these inserts. The 168-bp long exon shares 50% homology to Kunitz-type protease inhibitors, with all six cysteine residues conserved (Goldgaber et al. 1988; Kitaguchi et al. 1988; Ponte et al. 1988; Tanzi et al. 1988).

The 4100 Dalton subunit polypeptide (42 amino acids) of vascular amyloid (Glenner and Wong 1984a, b), APCs (Masters et al. 1985b), and also the PHF from NFTs of Alzheimer's disease patients (Masters et al. 1985a) all have the same amino acid sequence with progressively more N-terminal heterogeneity (Masters et al. 1985a, b). This indicates that vascular amyloid deposits are least degraded from the parent host-B-protein, core amyloid of amyloid plaques next, and the amyloid polypeptide of PHF is most degraded from this same precursor B-protein. Although protein components of microtubules (and tubulin or MAP proteins) might well be the precursor or parent protein we seek, we now find that in all conditions where these masses of amyloid appear (perivascular or in neuritic or amyloid plaques and NFTs) there is a pooling or piling up of neurofilament in perikaryon and axonal swellings. It appears that the B-protein precursor of amyloid, the gene for which we have identified on chromosome 21 in man, is a membrane anchored or excreted matrix element caught in this mass of collapsed, pooled cytoskeletal elements (Schubert et al. 1988).

In fact, Hirano has demonstrated ultrastructurally minute masses of amyloid fibers and of regular paracrystalline arrays of particles or tubules within packed masses of piled up NF in spheroids that have formed from such swollen perikarya or axonal swellings in motor neurons of the spinal cord in ALS (Inoue and Hirano 1979; Hirano and Inoue 1980; Hirano et al. 1984a, b). Kirschner et al. (1986) have pointed out that the helical structure of the 200-kDa component of neurofilaments does not lend itself to degeneration to the B-pleated sheet structure common to all brain amyloids, and that perhaps MAP-tau is the more likely precursor. It too is accumulated in pooled masses of neurofilaments (Grundke-Iqbal et al. 1986; Kosik et al. 1986; Wood et al. 1986). This now seems not to be the case, although copolymerization or firm associations of the amyloid B-protein fibrils with a B-pleated peptide derived from MAP-tau appears to be likely (Gajdusek 1988b; Guiroy and Gajdusek 1988). However, we do not yet know the normal function of the precursor protein for amyloid polypeptide formation.

Thus, interference with axonal transport of neurofilaments may be a basic mechanism of pathogenesis that leads to

(a) pooling of the cytoskeletal elements associated proteins and matirx proteins in the perikaryon or axonal cylinders and lysis of the neuron as in ALS and other motor neuron diseases; (b) amyloid and neuritic plaque formation, from degradation in Alzheimer's disease and many other CNS degenerations of a precursor B-protein, which is anchored to the external membrane surface or excreated from the cell; and, finally, (c) neurofibrillary tangle formation with the same precursor protein further modified to form paired helical filaments probably copolymerized or otherwise associated with numerous proteins of the cytoskeletan. We know that the precursor B-protein is synthesized in neurons and probably also in microglial and oligodendroglial and some cerebrovascular endothethial cells (Bahmanyar et al. 1987; Fukatsu et al. 1984a, b; Goldgaber et al. 1987a, b; Schmechle et al. 1988). It could be released into the extracellular space by all of these cells.

C. Two Forms of Amyloid in Cerebral Plaques

The larger, more regular amyloid plaques of kuru, of Creutzfeldt-Jakob disease (CJD) and its Gerstmann-Sträussler variant, and of scrapie are composed of a scrapie-specific amyloid protein (PrP_{27-30}), a degradation product of a host-specified larger protein (PrP_{35-37}). The cDNA for this precursor protein has been sequenced (Oesch et al. 1985) and much of the oligonucleotide sequence confirmed by amino acid sequencing of parts of the isolated precursor (Multhaup et al. 1985). The gene for this precursor protein of the amyloid of the transmissible dementias is located on chromosome 20 in man, 2 in mouse.

Prusiner calls this scrapie-specified host-specified protein his "prion" protein (PrP_{27-30}; Bendheim et al. 1984; Bolton et al. 1985; Multhaup et al. 1985; Oesch et al. 1985; Prusiner 1982, 1984; Prusiner et al. 1983, 1984; Rohwer 1984c). In CJD the amyloid in plaque cores carries the same immunologic specificity as those of kuru and scrapie (Bendheim et al. 1984; Bockman et al. 1985; Braig and Diringer 1985; Brown et al. 1986a; Manuelidis 1985; Manuelidis et al. 1985) and amino acid sequences as does the purified 27- to 30-kDa protein of scrapie-associated fibrils (SAF; or "prion protein" PrP_{27-30}). The microheterogeneity of the CJD plaque polypeptide is the result of cleavage from different regions of the same host precursor protein (PrP_{35-37}; Multhaup et al. 1988).

The amyloid of Alzheimer's and Down's syndrome is composed of a self-aggregating 4.1-kDa amyloid polypeptide subunit of 42 amino acids (B-protein or A4 protein; Glenner and Wong 1984a; Masters et al. 1985a, b; Wong et al. 1985). The cDNA clones coding for this amyloid subunit have been isolated and characterized by Goldgaber and Lerman and their coworkers (1987a, b) and by Kang et al. (1987), Robakis et al. (1987), and Tanzi et al. (1987). This is a precursor protein for a different amyloid from that of kuru-CJD-scrapie. It is specified by a gene on chromosome 21 in man, 16 in mouse. On the other hand the amyloid of CJD is made by polymerization of a heavily glycosylated 27- to 30-kDa glycoprotein closely related to the scrapie-specific protein from scrapie-associated fibrils (SAFs; Bendheim et al. 1985; Bolton et al. 1985; Multhaup et al. 1985).

Thus, there are two forms of brain amyloid: that of the transmissible dementias and that of Alzheimer's disease, aged Down's syndrome, Guamanian ALS/PD, and normal aging (Merz et al. 1986). The respective precursors are specified by different genes located in man in chromosome 20 and 21, respectively (in mouse on chromosome 2 and 16, respectively). To determine whether these amyloids are formed from a neuronal, microglial, or serum-borne precursor has been the problem. It now appears that the amyloid in NFTs is formed from neuronal synthesized precursor; extracellular amyloid of plaques and congophilic angiopathy may be of microglial and vas-

cular endothelial origin (Fukatsu 1984a, b; Higgins et al. 1988).

The mechanism of processing that produces the regularly oriented birefringent configuration of B-pleated sheets of amyloid proteins is not known. The known sequences of the amyloid in perivascular deposits (Glenner and Wong 1984a, b; Wong et al. 1985), plaque cores (Masters et al. 1985b), and PHFs of neurofibrillary tangles (Masters et al. 1985a; Guiroy et al. 1987), which are all alike, do not correspond with the amino acid sequence of the SAF protein (PrP; Multhaup et al. 1985; Oesch et al. 1985; Prusiner et al. 1984). Furthermore, neither precursor shows any homology with the sequences for the major components of the cytoskeleton: the three protein component neurofilaments, α- or β-tubulin or MAP II or MAP-tau or actin (Geisler et al. 1985; Lewis and Cowan 1985). Thus, we are dealing with two different precursor proteins and two different amyloid polypeptides, or small proteins, derived from them in the transmissible and the nontransmissible dementias. The two host-specified precursor proteins are not yet identified with a known function or structure in normal cells.

In the course of scrapie infection the tertiary and quarterinary structure of the precursor protein of 35–37 kDa is altered to render it insoluble and protease resistant. Once cleaved to the 27–30 kDa fragment, it is easily polymerized into fibrils. Both forms are apparently infectious.

D. Scrapie-Associated Fibrils

In preparations of scrapie-affected brain suspensions in a density gradient, Merz and Somerville have demonstrated amyloid-like two-stranded fibers – each fiber composed of two protofibrils – that increase in quantity with virus titer (Merz – 1981; 1984a, b). We have found these structures in brains of CJD patients and in brains of primates with experimental CJD and kuru but not in normal control brains or brains of patients with other neurodegenerative diseases (Brown et al. 1985; Gajdusek 1985c; Merz et al. 1984a, b). It has been postulated that these structures may represent the scrapie or CJD or kuru infectious agent (Gajdusek 1985b; Merz et al. 1981, 1984a, b; Prusiner 1984; Prusiner et al. 1983). Such structures bring to mind the filamentous plant viruses and filamentous phage fd which are of about the same diameters. However, no nucleic acid has been demonstrated in purified preparations of SAF proteins (PrPs). SAFs are yet the more intriguing since they are the central core of "cigar-like" tubulofilamentous structures in scrapie and CJD brains (Narang et al. 1987), but obscured by an outer coat of proteinaceus material and an inner coat of single-stranded host-derived DNA (Narang et al. 1988).

These scrapie-associated fibrils (SAFs) which may be the infectious agents are distinguishable ultrastructurally from the paired helical filaments (PHFs) of neurofibrillary tangles and the fibrils of brain amyloid (Merz et al. 1981; 1984a, b). However, their similarity is misleading since these do not share antigenicity with the PHFs of NFTs or with the amyloid fibrils in amyloid plaques of aging, Alzheimer's disease, and Down's syndrome. Thus, some antisera, both polyclonal and monoclonal, to the PHFs of Alzheimer's disease NFTs cross-react with the purified subunit protein of amyloid from plaque cores of senile plaques (Autilio-Gambetti et al. 1983; Gambetti et al. 1983a, b; Rasool and Selkoe 1985; Selkoe et al. 1986). However, most antisera to amyloid plaque cores do not react with NFTs. Neither of these antisera react, however, with SAFs of scrapie (Kingsbury et al. 1985; Manuelidis 1985).

Antibodies to the 27- to 30-kDa subunit protein of SAFs (or Prusiner's "prion proteins", PrP_{27-30}) cross-react strongly on Western blots with the subunit protein of SAFs from CJD- (Bendheim et al. 1985; Brown et al. 1985) and kuru- (Brown et al. 1986a) affected brains. However, such SAF-specific sera

do not react with neurofilaments or with PHfs or plaque core amyloid from Alzheimer's disease (Bendheim et al. 1985; C. J. Gibbs and D. C. Gajdusek unpublished data).

E. Viruses Provoking No Immune Response and Evidencing No Nonhost Antigen

The CJD-kuru-scrapie-like slow viruses first invade the reticuloendothelial cells and particularly low-density lymphocytes in the spleen. Yet, they provoke no antibody response which can be demonstrated using as antigen live virus preparation of highly infectious titers (Gajdusek 1985a, b; Gajdusek and Gibbs 1975; Kasper et al. 1981; McFarlin et al. 1971). With the inability to demonstrate any antiviral antibody response or any immune response directed against nonhost viral components or capable of neutralizing the virus activity, these unconventional viruses are unique in their immunologic behavior. Natural and experimental infections with these viruses elicit no antibody response in the host nor does immunosuppression with whole-body radiation, cortisone, antileukocytic serum, or cytotoxic drugs alter the incubation period, progress, or pattern of disease, or duration of illness to death. Finally, in vivo and in vitro study of both B-cell and T-cell function revealed no abnormality early or late in the course of illness and no in vitro sensitization of the cells taken from diseased animals to high-titer preparations of these viruses (Gajdusek 1977, 1985b, c; Gajdusek and Gibbs 1975). Since high-titer infective material in both crude suspension and highly purified also fails to elicit an immunologic response against nonhost components, even when used with adjuvants, this becomes the first group of microbes in which such immunologic inertness has been demonstrated, which has evoked the speculation that the replication of these viruses does not involve production of a virus-specified nonhost antigen (Gajdusek 1977; Prusiner 1982). Instead, their protein component must be specified by host genes and thus be recognized as self.

The amyloid 27- to 30-kDa protein obtained from highly purified preparations of SAFs (prion protein, PrP_{27-30}) has now been shown to be infectious (Ceroni et al. 1989; Piccardo et al. 1989; Safar et al. 1989a, b, c) and is a subunit of the SAFs which are a fibrillary aggregation of such subunits. It aggregates into dimer, tetramer, octomer, and hexadecamer polymers, as does the different subunit polypeptide (4.1 kDa) of amyloid of Alzheimer's disease and aging brain (Braig and Diringer 1985; Masters et al. 1985b; Multhaup et al. 1985). Antibody to this same scrapie amyloid protein has been made in rabbits and such polyclonal antibody reacts well with SAFs by an enzyme-linked immunosorbent assay (ELISA; Brown et al. 1985), Western blotting technique (Brown et al. 1985, 1986a; Manuelidis et al. 1985), and gold-bead decoration immunoelectron-microscopy (Manuelidis et al. 1985). Such antibodies to the scrapie SAFs cross-react well with the SAFs of kuru and of CJD and the Gerstmann-Sträussler form of CJD (Bendheim et al. 1985; Brown et al. 1985; Manuelidis et al. 1985) and already provide a quick means of diagnosis of these diseases (Brown et al. 1986a). These antisera to SAFs cross-react with the amyloid plaques of kuru, Creutzfeldt-Jakob disease, and scrapie, but they do not cross-react with the amyloid plaques of Alzheimer's disease or the aging brain (Brown et al. 1985; C. J. Gibbs, D. C. Gajdusek, unpublished data; Kitramoto et al. 1986).

F. Enormous Resistance to Physical and Chemical Inactivation

The demonstration of the resistance of the unconventional viruses to high concentrations of formaldehyde or glutaraldehyde, psoralens, and most other antiviral and antiseptic substances (Brown et al. 1982a, 1986b), and to ultraviolet

(UV) and ionizing radiation, ultrasonication, and heat, and the further demonstration of iatrogenic transmission through implanted surgical electrodes, contaminated surgical instruments, and corneal transplantation, injections of human growth hormone derived from pituitary glands obtained from cadavers (Brown et al. 1985), and dura mater obtained from cadavers and "sterilized" by ionizing radiation, and possibly through dentistry, has led to the necessity of changing autopsy room and operating theater techniques throughout the world as well as the precautions used in handling older and demented patients. Many of the gentle organic disinfectants, including detergents and the quarterinary ammonium salts, often used for disinfection and even hydrogen peroxide, formaldehyde, ether, chloroform, iodine, phenol and acetone, are inadequate for sterilization of the unconventional viruses, as is the use of the ethylene oxide sterilizer. More recently, it has been shown that formaldehyde-fixed brain tissue is much more resistant to inactivation by autoclaving than is unfixed fresh scrapie infected brain (Taylor and McConnell 1988). This demands revision of previously acceptable procedures for decontamination and disinfection (Brown et al. 1982a, b, 1984, 1986b).

These unconventional viruses are also resistant, even when partially purified, to all nucleases, to β-propiolactone, ethylenediaminetetraacetic acid (EDTA), and sodium deoxycholate. They are moderately sensitive to most membrane-disrupting agents in high concentration such as phenol (60%), chloroform, ether, urea (6 *M*), periodate (0.01 *M*), 2-chloroethanol, alcoholic iodine, acetone, chloroform-butanol, hypochlorite, and alkalai, to chaotropic ions such as thiocyanate and guanadinium and trichloroacetate, and to proteinase K and trypsin when partially purified (Prusiner 1982), but these only inactivated 99% to 99.9% of the infectious particles leaving behind highly resistant infectivity (Rohwer 1984b). Sodium hydroxide (1.0 *N*) and hypochlorite (5%), however, quickly inactivate over 10^5 ID_{50} of the virus (Brown et al. 1984). They have a UV inactivation action spectrum with a six fold increased sensitivity at 237 nm over that at 254 nm or 280 nm, and 50-fold increased sensitivity at 220 nm (Gibbs et al. 1977; Haig et al. 1969; Latarjet 1979; Latarjet et al. 1970). Moreover, they show remarkable resistance to ionizing radiation that would indicate a target size, if such a naive calculation is applicable to a highly aggregated "semisolid" array of associated proteins, of under 100000 kDa (Gibbs et al. 1977; Latarjet 1979; Latarjet et al. 1970; Rohwer and Gajdusek 1980).

However, many investigators have seen regular arrays of particles that appear to be tubular structures seen in cross-section, in postsynaptic terminals of neurons in experimental animals infected with CJD, kuru, and scrapie (Baringer et al. 1979; 1981; David-Ferreira et al. 1968; Field and Narang 1972; Field et al. 1969; Lamar et al. 1974; Narang 1973; 1974a, b; Narang et al. 1972, 1980, 1987, 1988; Vernon et al. 1970; ZuRhein and Varakis 1976). Structures more typical of virions are not recognized on electron microscopic study of infected cells in vivo or in vitro, nor are they recognized in highly infectious preparations of virus concentrated by density-gradient banding in the zonal rotor.

These atypical properties have led to the speculation that the infectious agents lack a nucleic acid, and that they may be a self-replicating protein (perhaps by derepressing or causing misreading of cellular DNA bearing information for their own synthesis), even a self-replicating membrane fragment which serves as a template for laying down abnormal plasma membrane, including itself (Bendheim et al. 1985; Bolton et al. 1982, 1984, 1985; Gajdusek 1984, 1985a, b, c; Oesch et al. 1985; Prusiner 1982, 1984; Prusiner et al. 1983, 1984). I have often suggested that they are catalyzing and organizing the specific degradation of a host-speci-

fied precursor protein, autocatalytically producing themselves in the process (Gajdusek 1977, 1984, 1985a, b, c). More recently I have suggested that the fibril amyloid enhancing factors offer a good model for scrapie replication (Gajdusek 1988b; Guiroy and Gajdusek 1988).

Analogies with defective of "contaminated" seed crystals of simple nucleating molecules specifying the crystallization of their own distinct crystal structure come to mind as to mineral nucleation of protein crystallization (McPherson and Shlichta 1988). The presence of mineral deposits in neurons in the form of hydroxyapatites often containing aluminum (Bizzi et al. 1984; Nikaido et al. 1972; Perl and Brody 1980), silicon (Austin 1978; Austin et al. 1973; Garruto et al. 1986; Iler 1985; Nikaido et al. 1972), and other atoms as the antecedents to NFT formation with the aymloid protein of PHFs has been shown. Such deposits are exceptionally intense in the high incidence foci of ALS, parkinsonism-dementia, and associated early appearance of NFTs in the Western Pacific (Gajdusek and Salazar 1982; Garruto et al. 1982, 1984, 1986; Perl et al. 1982). More recently, Masters et al. (1985a) and Candy et al. (1986) have found silicon and aluminum deposits in the center of amyloid plaque cores in Alzheimer's disease. The aluminum silicate, perhaps in the form of montmorillonites, are in the center of amyloid plaque cores. Candy et al. have thus suggested because of this location, that they are the initiating elements of the amyloid deposition (Candy et al. 1986). Thus, we wonder whether a nucleus of a cation-binding mineral lattice may initiate the change to amyloid configuration of the normal host scrapie precursor protein (Iler 1985; Rees and Cragg 1983; Weiss 1981).

G. Mendelian Single Gene Autosomal Dominant Inheritance Determines Expression in Familial CJD

CJD became the first human infectious disease in which a single gene was demonstrated to control susceptibility and occurrence of the disease. The CJD virus is isolated from the brain of such familial cases. The autosomal dominant behavior of the disease in such families, including the appearance of the disease in 50% of siblings who survive to the age at which the disease usually appears, has evoked the possibility of virus etiology in other familial dementias. The presence of CJD patients in the families of well-known familial Alzheimer's disease, and the familial occurrence of the spinocerebellar ataxic form of Creutzfeldt-Jakob disease, the Gerstmann-Sträussler syndrome, which is also transmissible, have led to renewed interest in familial dementias of all types (Masters et al. 1981a, b; Traub et al. 1977). Thus, we are trying to determine the chromosomal location of the gene determining familial focus of CJD, in order to discover the effect this gene has on the processing of the precursor protein (PrP_{35-37}) of scrapie amyloid (PrP_{27-30}).

H. Autoantibody to 10-nm Neurofilament in SSVE Patients

The demonstration by Sotelo et al. of a very specific autoantibody directed against 10-nm neurofilaments and no other component of the CNS in over 60% of the patients with kuru and CJD as a phenomenon appearing late in the disease, was the first demonstration of an immune phenomenon in the SSVEs and an exciting new avenue of approach for the study of the transmissible dementias (Aoki et al. 1982; Bahmanyar et al. 1983, 1984; Sotelo et al. 1980a, b). This autoantibody behaves like many other autoantibodies such as the rheumatoid factor and the anti-DNA antibody in lupus and the antithyroglobulin antibody in Hashimoto's thyroiditis in that it is often present in normal subjects, and more often present in subjects closely related to the patients. Although found in more than one-half of patients with transmissible virus dementia, it was not detected in

40% of patients with classical CJD. It does develop in other gray matter diseases, including Alzheimer's and Parkinson's diseases, but at far lower incidence than in CJD (Bahmanyar et al. 1983; Sotelo et al. 1980a). Furthermore, it was not detected in patients with other immune diseases such as disseminated lupus erythematosis and chronic rheumatoid arthritis (Bahmanyar et al. 1983). We have demonstrated that on Western blots separating the three proteins comprising the 10-nm neurofilament triad of 200 kDa, 150 kDa, and 68 kDa, most positive sera have antibodies directed against the 200-kDa protein with some cross-reaction with the 150-kDa protein, some sera react better with the 150-kDa protein, and rare sera only with the 68-kDa protein, thought to be an internal component of the neurofilament (Bahmanyar et al. 1984; Toh et al. 1985a, b). Sheep with scrapie, however, often react best with a 62-kDa neurofilament-associated protein (Toh et al. 1985b). Some authors found a higher incidence than we have of these specific antibodies in normal subjects (Stefansson et al. 1985). Nonetheless, the same problem is posed. Why are there antibodies to the neurofilament proteins and not to other CNS antigens?

I. Unconventional Viruses: Subviral Pathogens, Perhaps Devoid of a Nucleic Acid or a Non-host Protein

The scrapie virus has been partially purified by density-gradient sedimentation in the presence of specific detergents. Scrapie virus has been over 1000-fold purified relative to other quantifiable proteins in the original brain suspension (Bolton et al. 1982, 1984; Diringer et al. 1983; Manuelidis and Manuelidis 1983; Multhaup et al. 1985; Prusiner et al. 1984; Rohwer and Gajdusek 1980; Rohwer et al. 1979). In such preparations the virus is susceptible to high concentrations of proteinase K and trypsin digestion, but it is not inactivated by any nuclease (Prusiner 1982). Sedimented, washed, and resuspended virus has been banded into peaks of high infectivity with the use of cesium chloride, sucrose, and metrizamide density gradients in the ultracentrifuge. Attempts to demonstrate a nonhost nucleic acid in scrapie-virus preparations using DNA homology and transfection and nuclease inactivation have been unsuccessful (Borras and Gibbs 1986; Borras et al. 1982, 1986; Hunter et al. 1976). No significant quantities of nucleic acid are present in purified preparations of 27- to 30-kDa SAF associated-protein (PrP_{27-30}), and such preparations were first found to be noninfectious (Diringer et al. 1983; Manuelidis 1985; Multhaup et al. 1985; Oesch et al. 1985), but have been shown to be highly infectious (Ceroni et al. 1989; Piccardo et al. 1989; Safar et al. 1989a, b, c).

The atypical action spectrum for inactivation of scrapie virus by UV should not be taken as proof that no genetic information exists in the scrapie virus as nucleic acid molecules, since Latarjet has demonstrated similar resistance to UV and a similar UV action spectrum for microsomes (Gibbs et al. 1977; Haig et al. 1969; Latarjet 1979; Latarjet et al. 1970). Ultraviolet resistance 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 kDa). However, we may read this UV-resistance at face value as the first clear evidence that we were dealing with an infectious polypeptide.

Moreover, the unconventional viruses possess numerous properties in which they resemble classical viruses (Gajdusek 1977, 1985b; Rohwer 1984a, b; 1985, unpublished work; Rohwer and Gajdusek 1980), and some of these properties suggest far more complex genetic interaction between virus and host than one might expect for genomes with a molecular mass of only 10^5 kDa. Rohwer has shown that the scrapie virus replicates in hamster brain at a constant rate, with no eclipse phase, and with a doubling time

of 5.2 days (Rohwer 1985, unpublished work). Examination of the kinetics of its inactivation and the demonstrated association or aggregation of scrapie virus particles into polymers or clusters that can be disrupted by ultrasonication have cast doubt on the calculation of its small size from ionizing radiation inactivation data and inferences about its structure from resistance to chemical inactivating agents. Thus, aggregates make necessary "multiple hits" for inactivation, whereas free virus is killed by a single event (Rohwer 1985, unpublished work).

In plant virology we have been forced to modify our concepts of a virus to include subviral pathogens such as the newly described viroids causing 11 natural plant diseases – potato spindle tuber disease, chrysanthemum stunt disease, citrus exocortis disease, Cadang-Cadang disease of coconut palms, cherry chloratic mottle, cucumber pale fruit disease, hop stunt disease, avacado sunblotch disease, tomato bunchy top disease, tomato "planta macho" disease, and burdock stunt disease – and the virusoids of four natural plant diseases (velvet tobacco mottle virus, solanum nodiflorum mottle virus, lucerne transient streak virus, subterranean clover mottle virus) to which we may turn for analogy (Diener and Hadidi 1977; Sänger 1982). All of the viroids are small circular RNAs containing no structural protein or membrane and they have all been fully sequenced and their fine structures determined. They have only partial base pairing as the circle collapses on itself. They contain only 246 to 574 ribonucleotides and replicate by a "rolling circle" copying of their RNA sequences in many sequential rotations to produce an oligomeric copy, which is then cut into monomers or sometimes dimers. No protein is synthesized from their genetic information, and only the replication machinery of the cell is used. These subviral pathogens have caused us to give much thought to possible similarities to the unconventional viruses. However, we and others have shown that the unconventional viruses differ markedly from the plant viroids on many counts (Diener and Hadidi 1977; Gajdusek 1985 b, c; Prusiner 1982; Sänger 1982); in fact, many of their properties are diametrically opposite to those of the viroids. Thus, the intellectually stimulating analogies of the unconventional viruses to viroids and virusoids prove to be spurious, yet these subviral pathogens of plants have served to alert us to the possibility of extreme departure from conventional virus structures.

Recent work on amyloid enhancing factors, particularly fibril amyloid enhancing factor (Niewold et al. 1987) strongly suggests that an autocatalytic nucleating process directing fibril growth according to its own specified fibril structure appears to give us the most challenging model for scrapie replication (Gajdusek 1988 b; Guiroy and Gajdusek 1988). The newer work of Safar and his coworkers (Ceroni et al. 1989; Piccardo et al. 1989; Safar et al. 1989 a, b, c) clearly shows that the normal host-specified scrapie precursor protein (PrP_{35-37}) is converted to an infectious form by configurational change in secondary and tertiary structure of the noninfectious precursor. It will require the work of crystallographers to define molecularly how this de novo configurational change to an infectious polypeptide is autoinduced and autopatterned.

J. Concluding Hypothesis – Fantasy of a "Virus" from the Inorganic World

We are at an exciting moment in the study of the unconventional viruses. Either the SAF-associated protein (PrP_{27-30}) and its infectious progenitor, the infectious form of the scrapie precursor protein (PrP_{35-37}) are the infectious agent directing its own synthesis by nucleation and autopatterned crystallization or by augmentation (and alternative splicing) of its host gene, or this protein is simply an elegant molecular biological "high-tech" demonstration of what we

have known for a long while, namely, that amyloid is found in the CNS in all of these diseases and is a distant byproduct of the cell damage caused by the virus. In that case we are still in search for the atypical virus.

If the alteration of a host precursor protein to the self-polymerizing, insoluble, protease-resistant amyloid-like infectious scrapie-specific 35- to 37-kDa protein from a host protein by autoinduced configurational change in secondary structures or by posttranslational processing, a glycosylation (Bolton et al. 1985; Manuelidis et al. 1985; Multhaup et al. 1985), phosphorylation (Sternberger et al. 1985), peptide bond hydrolysis, cleavage, with proteolytic truncation at both termini, cross-linkage, altered splicing and repacking (Connors 1985; Masters et al. 1985a) is the basic growth process of scrapie replication, then the hydroxyapatites-aluminum silicate inorganic nidi in NFTs and in the center of amyloid plaque cores in Alzheimer's disease may signal that *this mineral-protein complex is the nucleating agent that has proved so elusive*. We must allow for the possibility that such a mineral-amyloid complex might in the proper milieu of the interior of a cell replicate slowly and regularly as it degrades a 35- to 37-kDa host precursor matrix protein (Oesch et al. 1985) to the amyloid we see in SAFs (PrP) and the amyloid plaques of these infections. In Alzheimer's and Pick's diseases and Down's syndrome a 4.1-kDa polylpeptide or its polymers complexes as an amyloid protein to a calcium-aluminum silicate apparently can self-replicate and self-aggregate as it autocatalytically degrades a precursor protein, presumably the amyloid B-protein precursor now identified, to the mineral-amyloid aggregates or paracystalline arrays we see in neurofibrillary tangles (Garruto et al. 1984; Perl et al. 1982;) and the amyloid plaque cores (Austin 1978; Candy et al. 1986; Masters et al. 1985a; Nikaido et al. 1972). Only in the nondividing neuron does this slow degenerative process eventually kill the cell. Thus, our atypical slow "virus" may simply be similar to a crystal template directing its own crystallization or "crystal lattice" from a source of presynthesized host protein precursors and an inorganic cation receptor nucleant. This remains a still-tenable hypothesis. If so, we wonder whether inorganic polymer chemistry and crystallography may provide better insights than the normal paradigms of modern molecular biology (Connors 1985; Iler 1985; Weiss 1981).

The calcium, aluminum, and silicon deposits have only been found in the center of cores of amyloid plaques and in neurofibrillary tangles. Thus, they remain candidates for the initiation or nucleation phase of amyloidogenesis in these degenerative amyloidoses of brain. In the slow virus infections the microfibril or oligomer of the scrapie-associated protein (PrP_{27-30}) may be its own nucleating agent and crystallization template. In these infections no mineral deposits have been found in the center of cores of the amyloid plaques (unpublished data).

I would prefer to call the infectious agent of scrapie a *virus*, even if it proves to be as romantically exotic as a polypeptide directing an auto-catalytically patterned degradation of a stagnated, pooled host-specified matrix protein to a glycosylated amyloid. The potent abstract concept of a virus as a self-specifying transmissible entity requiring the machinery of the host for its replication does not specify any specific structure. Mathematicians playing with computers have not hesitated to use the term "virus" for the "virus infections" of computer memories they have produced (Dewdney 1985a, b). Dewdney (1984), with his Core Wars program, initiated computer virology. The facts that software viruses contain no nucleic acid nor are nucleic acids in any way involved in the pathology that these viral diseases produce has not prevented computer scientists from appropriately calling them viruses (Denning 1988).

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