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Induction of β(A4)-Amyloid in Primates by Injection of Alzheimer's Disease Brain Homogenate

Comparison with Transmission of Spongiform Encephalopathy

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Abstract

Amyloid plaques, associated with argyrophilic dystrophic neurites, and cerebral amyloid angiopathy (CAA), but no neurofibrillary tangles, were found in the brains of three middle-aged marmoset monkeys that had been injected intracerebrally (ic) 6–7 yr earlier with brain tissue from a patient with early-onset Alzheimer's disease. Such changes were not found in the brains of three age-matched control marmosets. Immunochemically the amyloid plaques and CAA stained with antibody to β(A4)-protein. The plaques and CAA displayed dichroic birefringence when stained with Congo red and viewed under polarized light. β(A4)-amyloid plaques and CAA were also found in the brain of one of two marmosets injected ic 6 yr previously with brain tissue from a patient with prion disease with concomitant β(A4)-amyloid plaques and CAA. An occasional β(A4)-amyloid plaque was found in the brains of two of four marmosets injected ic >4.5 yr previously with brain tissue from three elderly patients, two of whom had suspected (but untransmitted) CJD. No β(A4)-amyloid plaques or CAA were found in six marmosets who were older than the injected animals, in four marmosets that had not developed spongiform encephalopathy (SE) having been injected several years previously with human brain tissue from three younger patients with suspected or atypical prion disease, or in 10 younger marmosets who had undergone various neurosurgical procedures. Seventeen marmosets injected in the same way with brain tissue from patients or animals with SE developed SE 17–49 mo after injection. These results suggest that β(A4)-amyloidosis is a transmissible process comparable to the transmissibility of SE.

Index Entries: β(A4)-amyloid plaques; cerebral amyloid angiopathy; spongiform encephalopathy; primates; transmission studies.

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Introduction

The demonstration in the mid-1960s that kuru and Creutzfeldt-Jakob disease (CJD) were transmissible experimentally *(1,2)* set them apart from the other human neurodegenerative diseases and suggested that they were related to the sheep disease, scrapie, which had been shown to be transmissible in the 1930s *(3)*. In recent years, however, the picture has changed again. The evidence that genetic disposition, based in the "prion" protein (PrP) gene, rather than exposure to an infectious agent is the crucial factor in determining disease occurrence in at least a proportion of cases, and that the transmissible spongiform encephalopathies (TSEs) belong to a wider group of diseases (which can be described collectively as prion diseases), some cases of which are neither spongiform *(4)* nor readily transmissible (e.g., ref. *5* and below) has reemphasized the similarities between the TSEs and the other human neurodegenerative diseases. The increasing appreciation of the importance of the amyloidogenic nature of prion protein *(6)* in the TSEs has made these diseases particularly relevant to other neurodegenerative diseases that involve amyloid production, of which Alzheimer's disease is the most important example. The recent demonstration that production of β(A4)-amyloid, like the amyloidogenic protein PrP^{SC}, can be induced by intracerebral (ic) injection of affected brain material across species *(7)* further increases the similarities between prion disease and other diseases that involve cerebral amyloidosis, even though the full pathology of Alzheimer's disease (AD) has yet to be shown to be transmissible. This article considers the emerging similarities between the transmissible and nontransmissible dementias with particular consideration of the pathogenesis of amyloidosis.

Materials and Methods

Animals

All experiments were carried out in common marmosets *(Callithrix jacchus)* born and housed in the same colony. The colony contains about 200 marmosets of heterogeneous genetic background, care having being taken to breed from animals more distantly related than first cousins. The founder animals of this colony came from various UK sources between 10 and 18 yr ago, i.e., about two to four generations.

Since senescence could be influenced by environmental factors, we have considered longevity specifically in this colony. In one cohort of 20 animals, four were killed before the age of 10 yr (one because of obstetric difficulties, one because of persistent diarrhea, and two because of debility and osteomalacia). Of the remaining 16, eight survive as healthy breeding animals aged >11 yr, seven were killed in an elderly condition (characterized by moderate weight loss, graying fur, tooth decay, reduced fecundity, and sometimes decreased mobility) aged between 10 and 11 yr, and one was found dead at 10 yr. The oldest marmoset known to us (but resident in another colony) is reported to be 17 yr old. Thus, the experimental animals in whom the presence of β(A4)-amyloid is reported in this article, whose ages ranged from 6 to 8 yr 6 mo, cannot be regarded as elderly.

A total of 33 marmosets have received ic injections of brain material. Their brains have all been examined histologically and compared with the brains of nine uninjected marmosets and eight marmosets that had received other neurosurgical procedures.

Injection Procedure

Fresh cerebral tissue was taken at necropsy and stored at –40°C until used. Injection material was prepared as a 10% weight/vol homogenate of tissue in 0.85% sterile saline. Animals were premedicated with 0.05 mL ketamine (100 mg/mL) intramuscularly (im) and were anesthetized with 1.0 mL/kg alphaxolone-alphadalone (Saffan, Glaxovet, Glaxo, UK) im. An injection of 50 µL homogenate was made ic into each of six sites (i.e., a total of 0.3 mL). Injections were made stereotactically using a 19-swg hypodermic needle into the caudate nucleus, hippocampus, and parietal cortex in the left hemisphere and the nucleus accumbens, amygdala, and parietal cortex in the right hemisphere. All animals recovered well from the injection procedure and were behaving normally within a day or two.

Time-Course of the Experiment

All animals were housed with other animals (which, in the case of animals injected with brain material from patients with SE, had been injected with the same material). They were observed daily and were treated with antibiotics for any minor intercurrent infections. Animals were killed when at least three observers were convinced that the animals

were showing neurological signs or after a period of 4.5–6.5 yr if no behavioral abnormality was detected. In animals that developed SE, the onset of signs could be exceedingly subtle, nonspecific, and protracted, or of intermediate duration, or acute, progressing from well to moribund in a few hours *(8)*.

Histology

All animals were killed using an overdose of pentobarbitone. Brains were fixed in 10% formalin in saline. The monkeys' brains were usually cut coronally into seven blocks and embedded in paraffin wax. Sections of the brains of animals injected with brain tissue from the patient with Alzheimer's disease and all the animals that were used as controls were stained with hematoxylin-eosin, hematoxylin-van Gieson, luxol fast/cresyl violet, Congo red, thioflavin-T, Glees' silver impregnation method, and, immunohistochemically, using antibodies to β(A4)-protein (Dako, High Wycombe, UK), τ protein (Dako) and PrP (kindly supplied by J. Hope, Edinburgh). The brains of the animals that developed SE were examined only by conventional histology, at a time at which the relevant antibodies were not readily available.

Cases from Which Brain Tissue Was Collected

Case 1 was a 56-yr-old male who died 5 yr after the onset of forgetfulness and 4 yr after a clinical diagnosis of Alzheimer's disease had been made. Three months prior to death, he was incoherent and could walk only with difficulty. One month prior to death, he was bedridden and in the end stage of dementia.

There is no known family history, but his mother died prematurely (of cancer). No mutations were found in the PrP gene, and the APP gene is currently being checked for mutations. Neuropathological examination (CJB) revealed moderate cerebral atrophy with ventricular enlargement, massive numbers of senile plaques and neurofibrillary tangles (NFTs), and widespread meningeal and cortical congophilic amyloid angiopathy (CAA). The amyloid seen in plaques and in vessels stained strongly using antibodies to β(A4)-protein. There was no SE or astrocytic hyperplasia. Sections sent to the CJD Neuropathology Surveillance Laboratory, Edinburgh, did not stain with antibody to PrP-protein. A neuropathological diagnosis of Alzheimer's disease was made.

Case 2 (E.U. of ref. *9*) was a 62-yr-old female with a 5-yr history of progressive dementia and ataxia. She belonged to a pedigree in which neurodegenerative disease is linked to a PrP^{102} proline to leucine substitution *(10)*. Previous attempts to transmit SE from this case were not successful *(11)*. Neuropathological examination (LWD) revealed SE in cortex and diencephalon, neuronal loss, astrocytic hyperplasia, and extensive deposition of multicentric and unicentric plaques in cerebral and cerebellar cortex. There were also some NFTs in the hippocampus, some neuritic plaques, and extensive CAA. Sections stained immunohistochemically with antibodies to β(A4)-protein demonstrated the presence of CAA and plaques. Sections stained with antibody to PrP demonstrated the presence of plaques. The histological appearance was characteristic of Gerstmann-Sträussler-Scheinker (GSS) syndrome with concomitant β(A4)-amyloidosis.

Miyazono et al. *(12)* found colocalization of PrP protein and β(A4)-protein in the same plaques in the brains of several cases with a PrP^{102} gene mutation and a GSS phenotype. A similar finding is reported in patients with a PrP^{198} mutation *(13)*. This suggests that the β(A4)-amyloid in case 2 may have been generated by the prion disease process rather than consisting of coincidental Alzheimer's disease or age-related β(A4)-amyloidosis.

Case 3 was a 79-yr-old female who died of bronchopneumonia. She had been resident in a psychiatric hospital for 37 yr with a diagnosis of schizophrenia. She had had a frontal leucotomy shortly after admission. Neuropathological examination (CJB) showed moderate neuronal loss, astrocytic hyperplasia, and areas of SE. There were a few amyloid plaques, some of which stained with antibodies to PrP and some to β(A4)-protein, but no NFTs. A diagnosis of possible CJD superimposed on a long-standing psychiatric illness was made. PrP was not detected in frozen brain by Western blotting.

Case 4 was an 81-yr-old male who died of bronchopneumonia 3 mo after admission to a psychiatric hospital. The time of onset of his dementia was unclear, but may have been up to several years before. Neuropathological examination (CJB) revealed some cortical areas of neuronal cell loss, gliosis, and SE, but no NFTs. There were a few plaques, some of which stained with antibodies to PrP and some to β(A4)-protein. A diagnosis of possible CJD was made. PrP was not detected in frozen brain by Western blotting.

Case 5 was a 74-yr-old male who died following a myocardial infarction. His brain was used as a control in our early attempts to transmit SE to a new recipient species, i.e., the common marmoset. An elderly brain was used because we needed a case that was not likely to develop SE at a later age to compare to a familial case of GSS at a time before linkage to the PrP gene had been established. He had not been diagnosed as suffering from any neurological or psychiatric condition, although subsequent neurochemical analysis of his brain revealed cholineacetyltransferase levels that were only 32% of controls, and identical to the levels found in a group of 12 patients with a clinical and pathological diagnosis of Alzheimer's disease (A. Cross, personal communication and ref. *14*). No histological data are available for this brain.

Case 6 (case 1 of refs. *15,16*) was a 71-yr-old female who died 8 wk after the onset of ataxia and dementia. Neuropathological examination (F. R. Wells, London) revealed widespread neuronal loss, gliosis, and severe SE. There were no plaques, CAA, or NFTs. A diagnosis of CJD was made.

Case 7, a first cousin of case 2, was a 51-yr-old female with a 3-yr history of dementia and mild ataxia. She carried a PrP^{102} proline to leucine mutation *(10)*. PrP was not detected in frozen brain tissue by Western blotting. Neuropathological examination (LWD) revealed no SE or gliosis. Some amyloid deposits, which stained with antibodies to PrP, but not β(A4)-protein, were seen in the neocortex of the frontal and parietal lobes, and in the hippocampus and cerebellum *(17)*. A diagnosis of atypical prion dementia was made.

Case 8 (J. C. of ref. *9*) was a 46-yr-old female who died after a 6-yr history of ataxia and dementia. She was a distant cousin of cases 2 and 7 of this article and carried a PrP^{102} proline to leucine mutation *(10)*. Neuropathological examination (LWD) revealed marked SE and many multicentric plaques that stained with antibodies to PrP. There were no NFTs, or CAA, and immunostaining with antibodies to β(A4)-protein were negative. A diagnosis of GSS was made. PrP was detected in frozen brain by Western blotting.

Case 9 was a 45-yr-old male with a 4-yr history of progressive dementia. He had a 144-bp insertion in the PrP gene and came from a large pedigree in which neurodegenerative disease was linked to this insertion *(18,19)*. Neuropathological examination (I. Janota, London) revealed minimal SE, no plaques, no NFTs, and no CAA. Immunostaining with antibodies to PrP and β(A4)-protein were both negative. A diagnosis of atypical prion dementia was made.

Case 10 was a 46-yr-old female with a 4-yr history of progressive dementia. Neuropathological examination (R. Perry, Newcastle) revealed some spongiform change in the temporal cortex, but no NFTs. A diagnosis of possible, atypical CJD of long duration was made.

Case 11 (patient 1 of ref. *20*) was a 34-yr-old male who had received injections of human pituitary-derived growth hormone from the age of 12–15 yr. He died after an 8-mo dementing illness. Neuropathological examination (LWD) revealed extensive SE and plaque formation. These plaques stained with antibody to PrP, but not β(A4)-protein. The diagnosis was iatrogenic prion disease.

Case 12 was a Friesian cow (PG91/87, ref. *8*) with natural bovine spongiform encephalopathy (BSE). Neuropathological diagnosis was made by G. A. H. Wells, Weybridge.

Case 13 was a Greyface sheep (85/29, ref. *8*) with natural scrapie. Neuropathological diagnosis was made by H. Fraser, Edinburgh.

Case 14 was a marmoset (number 3 of ref. *15*) that developed ataxia and was killed 22 mo after ic injection with brain material from case 6. The brain showed severe SE, but no plaque formation.

Case 15 was a marmoset (number 6 of ref. *15*) that developed ataxia and was killed 32 mo after ic injection with brain material from case 8. The brain showed severe SE, but no plaque formation.

Results

β(A4)-Amyloid in the Brains of Marmosets Injected ic with Brain from a Case of Severe, Early Onset Alzheimer's Disease

In the brains of the three marmosets injected 6.5 yr previously with brain tissue from case 1 (who had severe Alzheimer's disease), there were moderate numbers of abnormal structures visible on the silver-stained sections (*see* Table 1). The abnormalities consisted of a spectrum of argyrophilic structures. The smallest consisted of the aggregation of a few argyrophilic bodies grouped around an area of relatively unstained neuropile. These argyrophilic bodies ranged in size from <1 μm to about 10 μm and were attached to axons, suggesting that they were swollen degenerating axonal terminals, i.e.,

Table 1
Monkeys Injected with Brain Tissue from Patient with Alzheimer's Disease

Case	Age at death	Monkey	Time since injection	Age at death	Monkey neuropathology		
					Plaques	CAA	β-protein
1	56	Turmeric	6 yr 5 mo	8 yr 3 mo	++	++	++
1	56	Baal	6 yr 5 mo	8 yr 3 mo	++	++	++
1	56	Abel	6 yr 8 mo	8 yr 6 mo	++	++	++
1	56	Jacob	10 mo	2 yr 8 mo	—	—	—
—	—	Irad	Uninjected	8 yr 2 mo	—	—	—
—	—	Apollo	Uninjected	8 yr 7 mo	—	—	—
—	—	Micah	Uninjected	8 yr 9 mo	—	—	—

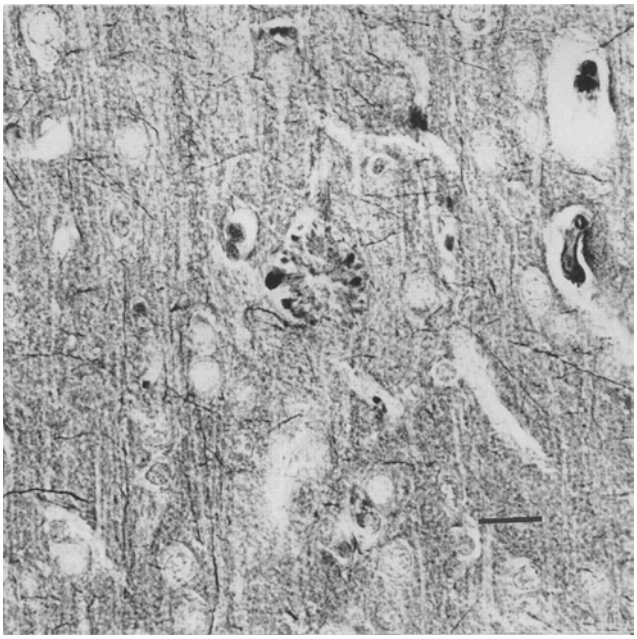

Fig. 1. Neuritic plaque of the "primitive" type seen in the cerebral cortex of monkey Turmeric, injected with brain material from case 1. Silver-stained section. Bar = 25 μm.

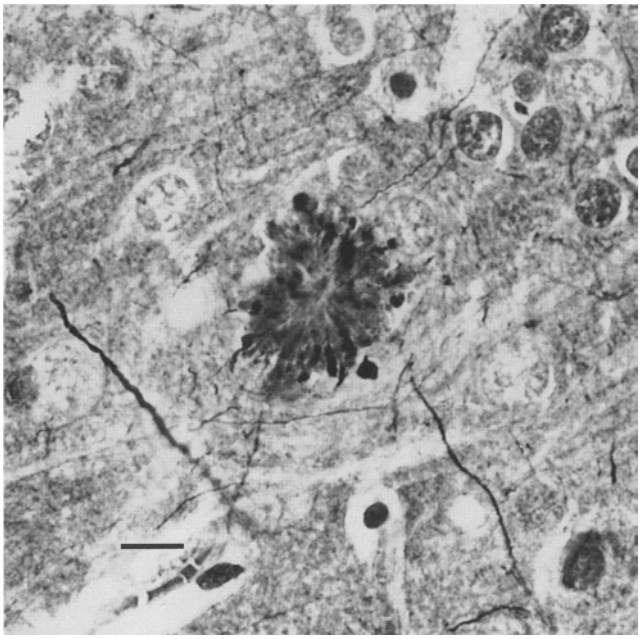

Fig. 2. "Mature" neuritic plaque seen in cerebral cortex of monkey Haydn, injected with brain material from case 2. Silver-stained section. Bar = 15 μm.

retraction balls (*see* Fig. 1). The majority of the argyrophilic bodies, however, were larger, consisting of densely stained components arranged in round clumps of about 30 μm diameter. These components were usually club-shaped with the thicker end facing outward from an acellular, relatively paler staining center (*see* Fig. 2). These abnormalities therefore had the appearance of neuritic, senile plaques of varying degrees of "maturity."

Immunohistochemistry revealed the presence of plaques that stained with antibodies to β(A4)-protein (*see* Fig. 3). These plaques usually had a wheel-like appearance consisting of an amorphous hub, a fine mesh of radiating spokes, and a faint rim. Substantial amounts of CAA were also visible throughout the meningeal vessels (*see* Fig. 4) on the cerebral cortical and, to a lesser extent, cerebellar surface and in the small intracortical vessels (*see* Fig. 5). In addition to β(A4)-protein staining within the walls of the intracortical vessels, there were also many perivascular β(A4)-protein staining aggregations, some of which had a plaque-like structure.

The CAA (*see* Fig. 6), and to a lesser extent, the plaques (*see* Fig. 7) showed dichroic birefringence

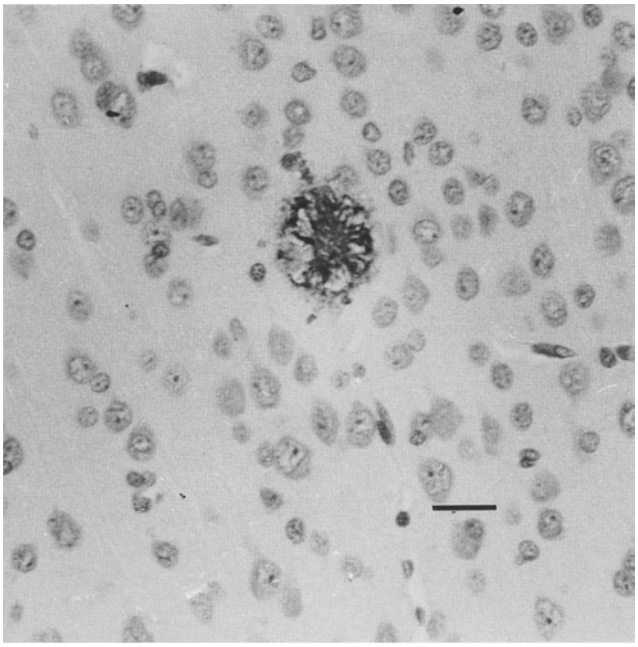

Fig. 3. β(A4)-protein positive cerebrocortical plaque seen in monkey Turmeric, injected with brain from case 1. Note the "hub, spoke, and rim" distribution of the stain. Bar = 25 μm.

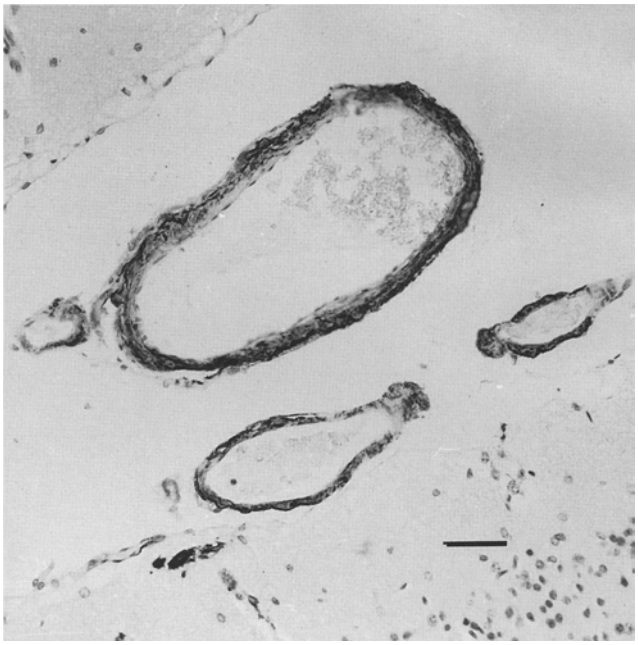

Fig. 4. CAA in meningeal blood vessel in monkey Abel, injected with brain material from case 1. Section immunostained for β(A4)-protein. Bar = 60 μm.

when stained with Congo red and viewed under polarized light. The CAA showed bright fluorescence when stained with thioflavin-T and viewed under UV light.

There was no evidence of NFTs on silver-stained sections, and no structures stained with antibodies to τ protein or to PrP protein. Hematoxylin/eosin staining did not reveal SE or astrocytosis. Electron microscopy was not undertaken.

The plaques were found throughout the cerebral cortex (*see* Fig. 8) and in the amygdala, but were absent from the basal ganglia, thalamus, cerebellum, and white matter. Plaques were not found differentially at the position of the ic injections.

One animal (Jacob) injected with brain from case 1 died of pancreatitis 10 mo after injection. No pathology was seen in the brain of this animal. This suggests that the β(A4)-amyloid seen in the animals that lived for much longer developed very slowly (*see also* animals that were killed shortly after being injected with brain from case 5 *below*).

The brains of three exactly aged-matched, uninjected, control marmosets from the same colony were examined contemporaneously. No abnormal structures were found on silver- or immunohistochemically stained sections in these animals.

β(A4)-Amyloid in the Brains of Marmosets Injected ic with Brain from Elderly Patients

The brain of one (Haydn) of two marmosets injected with brain material from case 2 (a 62-yr-old female with prion dementia and concomitant β[A4]-amyloid plaques and CAA) was also found to contain a number of β(A4)-protein plaques and some β(A4)-positive CAA when examined with silver stain and immunohistochemically (*see* Table 2). Staining with antibody to PrP was negative. There were no SE and no NFTs. The brain of the other animal (Mozart) showed no detectable pathology, although there was a degree of fixation artifact.

The brain of the marmoset injected with brain from case 3 (a 79-yr-old female with some β[A4]-amyloid plaques and some SE) was found to contain an occasional plaque that stained with antibody to β(A4)-protein, but not PrP-protein, but no CAA, no SE, and no NFTs. The brain of the marmoset injected with brain from case 4 (an 81-yr-old male with a diagnosis of possible CJD) appeared normal.

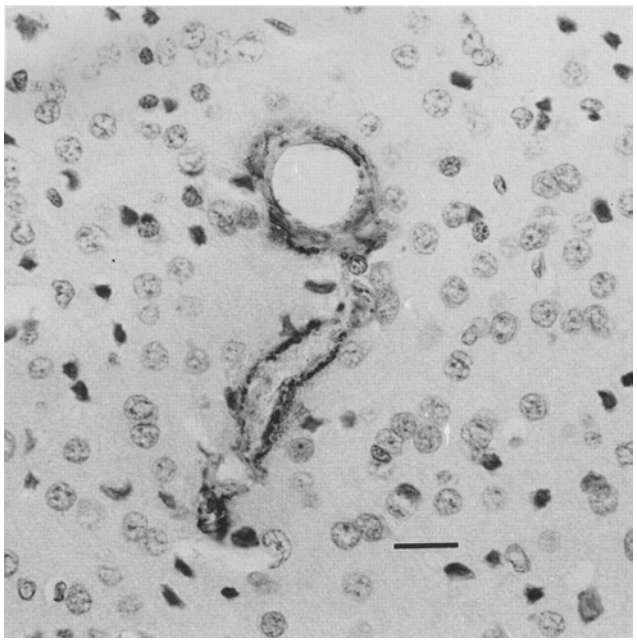

Fig. 5. CAA in intracortical blood vessel in monkey Abel, injected with brain material from case 1. Section immunostained for β(A4)-protein. Bar = 25 μm.

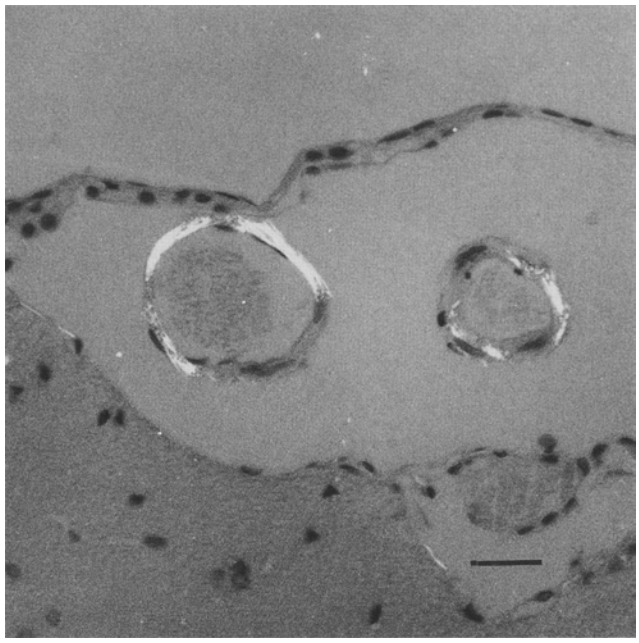

Fig. 6. CAA in meningeal blood vessel in monkey Abel, injected with brain material from case 1. Section stained with Congo red and photographed using polarized light. Bar = 25 μm.

The brain of the marmoset injected 5.5 yr previously with brain from case 5 (a 74-yr-old male who died of a myocardial infarction, but for whom there was neurochemical evidence of age-related neuropathology) was found to contain an occasional plaque and a trace of CAA, but no other pathology. The brains of three other marmosets that had been injected with brain from case 5, 3 mo, 10 mo, and 4.5 yr earlier appeared normal.

Five marmosets injected with brain from the only other elderly patient (case 6, a 71-yr-old female with rapidly progressive CJD) developed SE (*see* Comparison with Transmission of SE from Acquired, Sporadic, and Familial Prion Disease).

Lack of β(A4)-Amyloid in the Brains of Marmosets Injected ic with Brain from Younger Patients

No β(A4)-amyloid plaques or CAA was found in the brains of 10 marmosets injected with brain from patients aged under 60 yr whose brains did not contain β(A4)-amyloid (*see* Table 3). Four of these marmosets had been injected with brain from cases 7, 9, or 10 (atypical prion dementia or possible CJD) and had survived for 6 yr without developing neurological signs or SE. This indicates that the β(A4)-amyloid seen in animals described in the first two sections of Results had not developed merely as the consequence of the injection of any brain tissue, but suggests, rather, that this pathology developed as a consequence of the nature of the injected brain material, i.e., because it contained β(A4)-amyloid. Four marmosets had been injected with brain from case 8 (GSS) and two with brain from case 11 (iatrogenic prion disease). All six of these animals developed SE, but no other pathology was evident *(15,17)* (*see* Comparison with Transmission of SE from Acquired, Sporadic, and Familial Prion Disease).

Comparison with Uninjected Animals

Table 4 shows that six marmosets from the same colony who were older than those described in the first two sections of Results were not found to have β(A4)-amyloid plaques or CAA in their brains. This indicates that the animals described in these sections did not show these pathological changes because of their age. Eight marmosets were examined who had received excitotoxic lesions of either the vertical limb of the diagonal band or the nucleus basalis of Meynert as part of other experiments

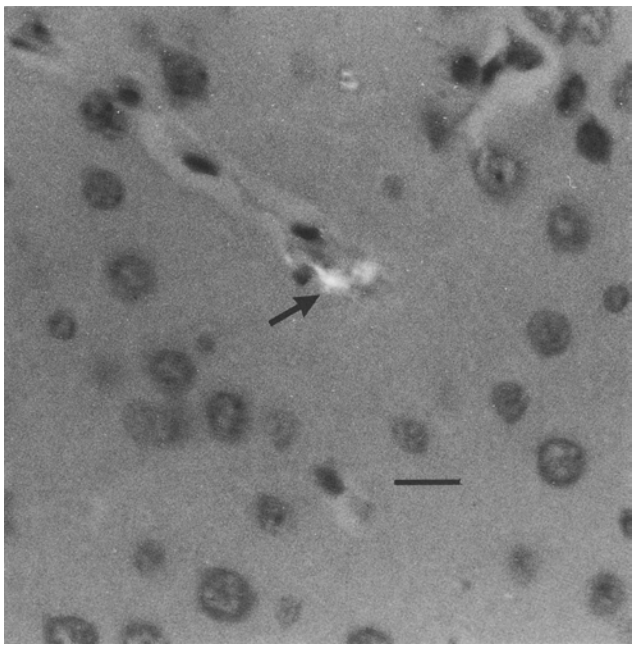

Fig. 7. Perivascular plaque in monkey Abel, injected with brain material from case 1. Section stained with Congo red and photographed using polarized light. Bar= 15 μm.

(21,22) some months earlier. Although neuronal loss and gliosis were observed at the lesion site, there were no plaques on silver-stained sections. This indicates that the pathology seen in animals described in the first two sections was unlikely to be the consequence of trauma or experimentally induced neuronal cell death.

Comparison with Transmission of SE from Acquired, Sporadic, and Familial Prion Disease

Table 5 shows the development of SE in 17 marmosets injected with brain from human and animal cases of SE. It is of interest that SE did not develop in marmosets injected with brain from six patients with suspected prion disease. Cases 3, 4, and 10 had some SE, but no PrP was found on Western blotting in cases 3 and 4. (It was not tested in case 10). Cases 2, 7, and 9 came from families in which neurodegenerative disease was linked to mutations in the PrP gene, although there was little or no SE in cases 7 and 9. PrP was not detected by Western blotting in case 7, and was not tested in cases 2 and 9. Injection of brain from case 8, who was related to cases 2 and 7, but who had severe SE and PrP on Western blotting, resulted in SE in 4/4 marmosets. In a recent retrospective analysis, Brown et al. *(23)* failed to find PrP by Western blotting in a large series of cases of suspected CJD and other neurodegenerative diseases from which transmission of SE to primates failed. There would therefore appear to be a relationship between the degree of SE, the detection of PrP by Western blotting, and the ease of transmission of SE to primates. It is difficult to argue, however, that cases that carried a mutation in the PrP gene and died of a dementing illness in later middle age did not have a prion disease even when SE was not found, PrP was not detected, and transmission studies failed. This suggests that prion disease is sometimes neither spongiform nor transmissible.

Discussion

These results suggest that the formation of β(A4)-amyloid plaques and CAA, whether occurring initially as part of the neuropathology of Alzheimer's disease, of another neurodegenerative disease, or of aging, is a process that can be transmitted by ic injection of affected brain tissue across species. It is important to ask, however, whether the plaques and angiopathy in the animals could be age-related and therefore not necessarily a consequence of the nature of the injected material. In an extensive comparative study, Dayan *(24)* found CAA as the most frequently encountered pathology in very elderly animals, but even this was a rare finding. Walker et al. *(25)*, in a study of primate brains, observed CAA, but not parenchymal plaques in squirrel monkeys 22–27 yr old, although Selkoe et al. *(26)* did find plaques and CAA in two squirrel monkeys aged 20 and 23 yr. Neuritic plaques and CAA have been found in the brains of aged macaques *(26–28)*. These plaques and more diffuse amyloid deposits immunostained positively with antibodies to β(A4)-protein *(26,29)*. Naturally occurring β(A4)-amyloid has therefore only been reported in monkeys that have exceeded about 75% of the maximum life-span of that species. The marmosets with β(A4)-amyloid reported in this article had only achieved 50% of the maximum life-span of this species.

Of equal importance is the question of whether the amyloid seen in the animals' brains could consist only of the injected human amyloid and not of *de novo* amyloid of animal origin. There are various reasons why this is unlikely to be the case, although none of these provide definitive proof.

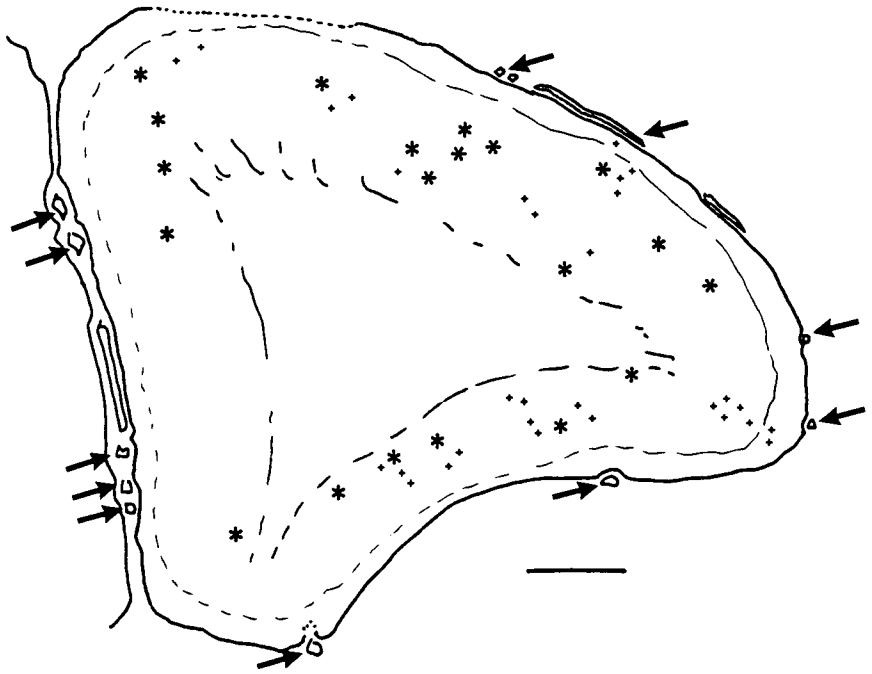

Fig. 8. Camera lucida drawing of coronal section through frontal lobe of monkey Baal, injected with brain material from case 1. Section immunostained for β(A4)-protein. Arrows indicate the main deposits of CAA. Small crosses indicate intracortical CAA and/or perivascular amyloid. Asterisks indicate position of well-formed plaques. Note: no injections were made in the frontal cortex. Bar = 1 mm.

1. Plaques were found bilaterally in frontal cortex, temporal cortex, the Sylvian fissure, cingular cortex, amygdala, and occasionally, in posterior cortical areas and hippocampus. Human brain tissue had been injected unilaterally into caudate, nucleus accumbens, hippocampus, amygdala, and two sites in the parietal cortex. The presence of plaques in areas that had not been injected and the absence of plaques in areas in which brain tissue had been deposited make it unlikely that the plaques were composed of injected material.
2. In silver-stained sections, long axonal processes could be seen terminating in argyrophilic retraction balls within the plaques. On immunostaining, the plaques were seen to consist of a central area of β(A4)-protein deposits, fine fibrillar radiating structures, and a faint outer rim. This degree of structural organization is consistent with a pathological process of plaque formation in the animal, rather than the persistence of human amyloid debris from the injected material. Similarly, the β(A4)-staining of the CAA was structurally embedded within the vessel walls, especially the large cerebral arteries. Its appearance was the same as naturally occurring CAA.
3. No β(A4)-amyloid or silver-staining plaques were seen in the brain of monkey Jacob, which was killed 10 mo after being injected with brain from case 1, suggesting that the plaques seen in the monkeys that survived longer had developed slowly over the course of some years.

β(A4)-amyloid plaques have not been previously reported in any nonhuman primates used for transmission studies of prion disease or other neurodegenerative diseases, including Alzheimer's disease *(30,31)*, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and ALS-PD of Guam *(30)*, as well as Huntington's and Pick's diseases *(31)*. Those experiments are, however, not strictly comparable. The primate brains in those experiments were not assessed using silver-staining and immunohistochemical methods. Without these methods, we would not have detected pathology in our animals. Furthermore, the animals in these earlier studies were allowed to survive until they developed a rapidly progressive motor disorder. The animals that died were subsequently found to

Table 2
Monkeys Injected with Brain Tissue from Elderly Patients

Case	Age at death	Monkey	Time since injection	Age at death	Monkey neuropathology		
					Plaques	CAA	β-protein
2	62	Haydn	4 yr 7 mo	6 yr 0 mo	++	++	++
2	62	Mozart	5 yr 0 mo	6 yr 5 mo	—	—	—
3	79	Joachim	5 yr 10 mo	6 yr 11 mo	(+)	—	(+)
4	81	Jezebel	5 yr 10 mo	6 yr 11 mo	—	—	—
5	74	Moloch	5 yr 7 mo	7 yr 2 mo	(+)	(+)	(+)
5	74	Cain	4 yr 8 mo	6 yr 6 mo	—	—	—
5	74	Isaac	10 mo	2 yr 8 mo	—	—	NT
5	74	Beatrice	3 mo	5 yr 7 mo	—	—	NT
6	71	*See* Table 5					

Table 3
Monkeys Injected with Brain Tissue from Younger Patients

Case	Age at death	Monkey	Time since injection	Age at death	Monkey neuropathology		
					Plaques	CAA	β-protein
7	51	Adah	5 yr 8 mo	7 yr 2 mo	—	—	—
7	51	Dove	5 yr 10 mo	6 yr 10 mo	—	—	—
8	46	*See* Table 5					
9	45	Reuben	5 yr 10 mo	7 yr 3 mo	—	—	—
10	46	Hamadan	5 yr 10 mo	7 yr 2 mo	—	—	—
11	34	*See* Table 5					

have SE on conventional histological examination. The remaining animals are either still alive or died of intercurrent illness, in which case the brain would sometimes be autolysed.

The common marmoset *(Callithrix jacchus)* has not been used for transmission experiments outside our laboratory. This species may be particularly suitable for transmission of β(A4)-amyloid because of an interaction between transmission time and the life-span of the animal. In our experiments, the transmission time was about a third of the maximum life-span of this species. Transmissions of β(A4)-amyloid to rodents may not be possible because they do not live long enough for the pathological process to develop, whereas the process of β(A4)-amyloidosis may progress more slowly in animals with a longer life-span making transmission times impracticably long. Marmosets may also be susceptible to β(A4)-amyloidosis for genetic reasons. There is complete amino acid homology in the 695 residue transcripts of the APP gene and almost complete homology in the 751 and 770 residue transcripts between humans and monkey (cynomolgous species), but not rodents *(29)*. This degree of homology may extend to all simians (including marmosets), rendering them susceptible to an amyloidogenic response following ic injection with brain tissue containing human β(A4)-amyloid.

It may be of interest that the brains of the marmosets inoculated with human brain material that contained large amounts of β(A4)-amyloid themselves contained more β(A4)-amyloid than that found in the brains of marmosets inoculated with human brain material containing only small amounts of β(A4)-amyloid. Furthermore, the brains of marmosets injected with brain from the patient with Alzheimer's disease contained both β(A4)-amyloid plaques and CAA, having been inoculated with human brain that also contained both plaques and CAA. These two forms of β(A4)-amyloid are both degradation products of APP, but differ slightly in the number of amino acid residues in humans *(32)*. It is not possible to determine from this experiment whether each type of β(A4)-amyloid leads to the formation of more of the same type in the marmoset, but it would seem more likely that the local environment (blood vessels or parenchyma) determines the type of amyloid deposition by a process

Table 4
Elderly Uninjected Animals

Monkey	Time since injection	Age at death	Monkey neuropathology		
			Plaques	CAA	β-protein
Boaz	—	8 yr 10 mo	—	—	—
Burdock	—	9 yr 3 mo	—	—	—
Janet	—	9 yr 6 mo	—	—	—
Craig	—	11 yr 0 mo	—	—	—
Rum	—	>11 y	—	—	—
Charlie	—	>11 y	—	—	—

Table 5
Monkeys that Developed Spongiform Encephalopathy

Case	Diagnosis	Number of monkeys	Mean time since injection, mo ± SEM	Mean age at death, ± SEM
6	Sporadic CJD	5	21 ± 1	2 yr 8 mo (±1 mo)
8	PrP102 GSS	4	30 ± 2	3 yr 11 mo (±2 mo)
11	Iatrogenic prion disease	2	21 ± 2	2 yr 10 mo (±2 mo)
12	Cow BSE	2	49 ± 1	5 yr 4 mo (±1 mo)
13	Sheep scrapie	2	41 ± 3	4 yr 7 mo (±3 mo)
14,15	Marmoset SE passaged from case 6 *(14)* and case 8 *(16)*	2	17 ± 0	3 yr 9 mo (±0 mo)

of *in situ* degradation of amyloid precursor protein. This is analogous to the situation in prion disease where the PrP found in amyloid plaques is a different breakdown product of PrP 33-35^{SC} from the preamyloid PrP found in diffuse parenchymal deposits *(33)*.

The transmission of β(A4)-amyloidosis resembles the transmission of prion disease in which the presence of the amyloidogenic protein PrP^{SC} (which is associated with SE in prion disease) induces the formation of host-derived PrP^{SC} on ic injection, with concomitant SE and, in some circumstances, PrP-positive plaques in the host. This suggests that the pathogenesis of Alzheimer's disease and the transmissible neurodegenerative diseases may be similar, despite the fact that different amyloidogenic proteins are involved in each case. It should be stressed, however, that it is the process of β(A4)-amyloidosis, not Alzheimer's disease, that has been shown to be transmissible in this experiment. The monkeys that developed β(A4)-amyloid were not obviously debilitated (although they were not assessed by formal neuropsychological testing), and their brains did not contain NFTs. As such, they did not have Alzheimer's disease, although we cannot say what would have happened if they had been permitted to live for a longer period. β(A4)-amyloid deposition is probably the earliest neuropathological change in Alzheimer's disease *(34)*. β(A4)-amyloid may induce neurodegeneration and contribute to the formation of NFTs *(35,36)*.

There are further marked similarities between transmissible and nontransmissible neurodegenerative diseases. In both types of disease, cases can be classified as familial, sporadic, or acquired, and an interaction exists between this classification and age at onset across all these diseases *(37)*. Familial forms of prion disease are associated with mutations within the PrP gene on chromosome 20 in humans (for review, *see* ref. *38*). Specific mutations within the APP gene on human chromosome 21 are associated with disease in HCHWA-Dutch type *(39,40)* and in some pedigrees with Alzheimer's disease *(41–44)*, although Alzheimer's disease in other pedigrees has been linked to other chromosomes *(45,46)*. Another mutation in the APP gene *(47)* may also result in CAA and/or neuritic plaque formation. It has been proposed that sporadic cases of prion disease (which are not associated with mutations within the prion gene) may result from the

rare stochastic conversion of normal prion protein (PrP^C) to the abnormal isoform (PrP^{SC}) *(38,48)*. Mutations in the APP gene have not been found in sporadic cases of Alzheimer's disease *(43,44)*. Thus, it seems likely that the primary structure of the normal precursor protein in both prion disease and β(A4)-amyloid diseases contributes to the probability of occurrence of a change in posttranslational protein processing, which can also occur with a lower probability as a consequence of age-related events, leading to familial or sporadic forms of disease, respectively, each with a characteristic age at onset. Acquired forms of prion disease occur as a consequence of contamination *(49)*, whereas acquired forms of β(A4)-amyloidosis, e.g., dementia pugilistica *(50)*, occur as a consequence of trauma. There is no epidemiological evidence to suggest that Alzheimer's disease is ever acquired by contamination.

Although the induction of SE in animals following ic injection of brain tissue from patients with kuru *(1)* and CJD *(2)* was originally taken as evidence for the involvement of a transmissible virus, the persistent failure to identify a virus and the subsequent greater understanding of the pathogenesis of prion diseases has led to a reevaluation of the implications of experimental transmissibility *(37,51)*. It has been established in experimental transmission of prion disease across species that following the introduction of foreign PrP^{SC}, it is the host PrP^C that is converted to the abnormal PrP^{SC} form *(52)*. Thus, it would appear that it is a pathogenetic mechanism that is being transferred from one brain to another leading to SE and, in some mouse models, to PrP-amyloid deposition. A similar mechanism may be involved in the experimental induction of β(A4)-amyloid. If it is argued that familial and sporadic cases of prion disease involve transmissible mechanisms without the inevitable conclusion that all human cases are acquired by contamination, then it can also be argued that β(A4)-amyloidosis is a transmissible process without the implication that Alzheimer's disease and other β(A4)-amyloid diseases are acquired by contamination.

The demonstration of disease-associated mutations in the β(A4)-amyloid precursor protein (APP) gene in some pedigrees with early onset familial Alzheimer's disease suggests that certain aspects of APP metabolism are primary to this disease process and that other features of Alzheimer's disease, e.g., neurochemical changes and tangle formation, may be secondary and separable from the primary pathology. In particular, neurofibrillary tangle formation is separable from β(A4)-amyloid deposition, since tangles may be absent from elderly brains that contain moderate amounts of β(A4)-amyloid, but tangles are found in some pedigrees with prion disease without β(A4)-amyloid *(53)*. However, it is unlikely that APP with a mutant amino acid sequence is necessary for β(A4)-amyloid deposition, because no mutations are found in sporadic Alzheimer's disease, some β(A4)-amyloid plaques are found in the brains of the "normal" elderly, and β(A4)-amyloid deposition is found in middle-aged Down's syndrome patients in which there is overproduction of normal APP *(34)*. This suggests that β(A4)-amyloid deposition could occur in any brain, the key feature of etiology being the probability of its occurrence, which would determine severity and age of onset of disease. Recent evidence demonstrates that soluble β(A4)-amyloid is produced by normal healthy brain tissue *(54,55)* and suggests that the pathological process in Alzheimer's disease consists of a failure of β(A4)-amyloid breakdown, i.e., that the key pathological event occurs after β(A4)-amyloid has been produced. One possibility is that insoluble β(A4)-amyloid may act as a precipitating and enhancing factor in β(A4)-amyloid deposition. Maggio et al. *(56)* have reported that β(A4)-amyloid plaques adsorb synthetic β(A4)-amyloid peptides in vitro. Partial synthetic homologs of the first 28 amino acids of β(A4)-amyloid protein can spontaneously form amyloid-like fibrils *(57,58)*, and the same may be true for the whole 4-kDa β(A4)-protein *(59)*.

The present findings suggest that close parallels can be expected in the molecular genetics of β(A4)-amyloid and prion diseases. It is already known that several dominant point mutations in the precursor genes are linked to disease in familial β(A4)-amyloid diseases and prion diseases, and that different mutations result in a different characteristic phenotype, although with considerable overlap in both cases (β[A4]-amyloid, ref *60*; prion disease, ref. *38*). The hypothesis that during disease, β(A4)-amyloid and PrP^{SC} convert the normal to the abnormal isoform of each protein implies that in both cases the protein product of two complementary alleles may be involved in the disease process and that heterozygosity may affect this interaction. That such post-translational interactions between the protein products of both alleles can occur is demonstrated by the observation that the activated form of the mutant P53 protein can convert the protein product of the wild-type P53 gene into the abnor-

mal form *(61)*. Homozygosity for the PrP^{129} polymorphism contributes to the occurrence of sporadic prion disease *(62)*, as well as to age at onset and disease progression, at least in familial disease *(63,64)* and to vulnerability to acquired prion disease *(65)*. The same effects of homozygosity for polymorphisms in the APP gene may also occur in Alzheimer's disease.

In experimental systemic amyloidosis, a constituent of amyloid-containing tissue, known as amyloid-enhancing factor (AEF), can act as a nucleating agent in accelerating its own production and the formation of amyloid deposits *(66)*, thereby shortening the time course of experimental amyloidosis from 2 wk to 4 d in rodents. It is possible that this form of experimental amyloidosis may also be transmissible *(67)*.

Thus, it can be postulated by analogy with the other forms of amyloidosis that the conversion of naturally occurring soluble β(A4)-amyloid protein into an insoluble β-pleated sheet conformation that then aggregates into amyloid plaques is a probabilistic or stochastic event that occurs at a low level in normal, but aged brains. The probability of occurrence of this event may be enhanced by mutations that determine the amino acid sequence of β(A4)-amyloid protein and/or that affect β(A4)-protein metabolism, thereby producing severe disease at an earlier age in familial cases. Human neuroblastoma (M17) cells transfected with constructs expressing one of the mutant APPs recently shown to be linked to familial Alzheimer's disease produced five times more β(A4)-containing, carboxyl terminal APP derivative than cells expressing wild-type APP and released six times more β(A4) into the culture medium *(68)*. In Down's patients, overproduction of β(A4)-amyloid protein with a normal amino acid sequence may also enhance the probability and speed of pathological change producing Alzheimer-type pathology. Various forms of trauma, including ischemia *(69)* and neuronal damage *(70)*, increase APP production and may therefore contribute to pathogenesis. A single trauma could occasionally result in Alzheimer's disease at a much later time (e.g., ref. *71*) if an increase in APP production triggered a self-perpetuating amyloidogenic mechanism. An association between Alzheimer's disease and previous head injury has been observed (*see* ref. *72*). If the presence of the insoluble form of β(A4)-amyloid increases the production of more of the insoluble form of β(A4)-amyloid by acting as a "seeding" or "nucleating" agent or template, then the increase in pathology will be exponential, resulting in a rapid increase in pathology at a late age. Furthermore, pathology would appear to spread around the brain between areas connected by cortico-cortical pathways *(73)*. The demonstration of substantial amounts of β(A4)-amyloid in the form of plaques and CAA in the brains of monkeys inoculated 6 yr previously with brain tissue containing β(A4)-amyloid (but not in age-matched controls) may be regarded as a key piece of evidence in favor of the model of amyloidosis outlined here.

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