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Evidence for the experimental transmission of cerebral β-amyloidosis to primates

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Summary. The brains of three marmosets (*Callithrix jacchus*) injected intracerebrally 6–7 years earlier with brain tissue from a patient with early onset Alzheimer's disease were found to contain moderate numbers of amyloid plaques with associated argyrophilic dystrophic neurites and cerebral amyloid angiopathy but no neurofibrillary tangles. The plaques and vascular amyloid stained positively with antibodies to β(A4)-protein. The brains of three age-matched control marmosets from the same colony did not show these neuropathological features. The brain of one of two marmosets injected with brain tissue from a patient with prion disease with concomitant β-amyloid plaques and cerebral amyloid angiopathy also showed β-amyloid plaques and angiopathy but no spongiform encephalopathy. An occasional plaque was found in the brains of two of four marmosets injected with brain tissue from three elderly patients with age-related pathology, two of whom had an additional diagnosis of possible prion disease. Neither plaques nor cerebral amyloid angiopathy were found in six other marmosets who were older than the injected animals, in 12 further marmosets who were slightly younger but who had been injected several years previously with brain tissue which did not contain β-amyloid, or in 10 younger marmosets who had been subjected to various neurosurgical procedures. These results suggest that cerebral β-amyloidosis may be induced by the introduction of exogenous amyloid β-protein.

Keywords: β(A4)-amyloid, senile plaques, primates, Alzheimer's disease

Cerebral amyloidosis is characterized by the deposition of fibrillar protein of varying composition in brain (see Duchen 1992). Abnormal isoforms of at least four different proteins have been identified in cerebral amyloidosis. β-Amyloid is a 4-kDa protein subfragment of amyloid precursor protein (APP), the gene for which is located on chromosome 21 in man (Kang *et al.* 1987; Goldgaber *et al.* 1987). β-Amyloid is found in blood vessels in age-related cerebral amyloid angiopathy (CAA) (Vinters & Gilbert 1983; Esiri & Wilcock 1986) and hereditary cerebral haemorrhage with amyloid (HCHWA)-Dutch type (Van Duinen *et al.* 1987) and in parenchymal plaques in Alzheimer's disease, including those seen in adult Down's syndrome (Rumble *et al.* 1989), and in some atypical dementias (Mann *et al.* 1992). In HCHWA-

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Icelandic type (Gudmundsson *et al.* 1972) the abnormal protein deposit is related to cystatin C (Cohen *et al.* 1983; Ghiso *et al.* 1986) while the type of amyloid found in the pedigree described by Worster-Drought *et al.* (1940) and Plant *et al.* (1990), oculoleptomeningeal amyloidosis (Goren *et al.* 1980) and some other atypical dementias, have yet to be characterized chemically. The other known protein which can form cerebral amyloid plaques is prion protein (PrP) (Bockman *et al.* 1985; DeArmond *et al.* 1985) which is found in the transmissible spongiform encephalopathies. These latter diseases, including Creutzfeldt–Jakob disease (CJD), kuru, and Gerstmann–Sträussler–Scheinker syndrome (GSS) in humans, are known to belong to a wider group of human and animal encephalopathies referred to as prion diseases. Some cases of prion disease are neither spongiform (e.g. Collinge *et al.* 1990) nor apparently transmissible (e.g. Brown *et al.* 1991; Goldfarb *et al.* 1992) and therefore particularly resemble the other neurodegenerative diseases. Recent advances in our understanding of the protein chemistry of PrP and APP have rekindled interest in the parallels between prion disease and other neurodegenerative conditions which also involve cerebral amyloidosis. It is possible that in all these diseases amyloid deposits consist of post-translationally modified abnormal isoforms of normal precursor proteins.

A key feature of the pathogenesis of prion disease is the apparent ability of PrP^{sc} (the abnormal isoform of prion protein, which accumulates during the course of the disease) to act as a template or catalyst for the conversion of PrP^{c} (the normal cellular isoform of prion protein) to PrP^{sc} (see Prusiner 1991). A positive feedback mechanism such as this could account for the rapid development of pathology after a prolonged and apparently silent incubation period within an individual, as well as the phenomenon of transmissibility. A similar autocatalytic process could be involved in the mechanism of β-amyloid deposition in brain.

In this context, in addition to setting up a series of transmissions from patients with CJD and GSS (Baker *et al.* 1985; 1990) we also injected animals intracerebrally (i.c.) with brain tissue from patients with β-amyloid plaques and angiopathy.

Materials and methods

Animals

All inoculations were made into common marmosets (*Callithrix jacchus*), born and housed in the Clinical Research Centre marmoset colony. The founder animals of this colony came from several UK sources mainly between 1975 and 1985. The animals were therefore genetically heterogeneous.

All animals were fed an *ad libitum* diet of Mazuri New World monkey pellets, egg sandwiches, fruit and milk with vitamin supplements especially D3. Animals were observed daily on a routine basis. They were housed with non-experimental uninoculated cage-mates, with other animals injected with brain material from the same case as themselves or, occasionally, singly. With the exception of animals which developed spongiform encephalopathy (SE, see below), all animals were behaviourally normal when killed. They had occasional intercurrent respiratory and intestinal infections comparable to that seen in the rest of the marmoset colony. These infections were treated with oral antibiotics.

Injection procedure

Fresh cerebral tissue was taken at necropsy from patients and from a cow with bovine spongiform encephalopathy (BSE) and a sheep with scrapie. Tissue was stored at −40°C until used. Inoculum was prepared as a 10% weight/volume homogenate of tissue in 0.85% sterile saline. Each animal was premedicated with 0.05 ml ketamine (100 mg/ml) intramuscularly (i.m.) and was anaesthetized with 1.0 ml/kg alphaxolone-alphadalone (Saffan; Glaxovet, UK) i.m. A 50-μl injection of homogenate was made intracerebrally into each of six sites in each animal (a total of 0.3 ml). Injections were made stereotactically using a 19 s.w.g. 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 surgery and were behaving normally within 2–3 days.

Histology

Animals were killed with an overdose of pentobarbitone. Brains were fixed in 10% formalin in saline. For monkeys in experiments 1 and 2 the cerebral hemispheres were cut coronally into seven blocks, the brain-stem and cerebellum cut transversely and the blocks embedded in paraffin wax. Sections were stained with haematoxylin–eosin, haematoxylin–van Gieson, luxol fast blue-cresyl violet, Congo red, and Glees' silver impregnation for axons modified by Marsland *et al.* (1954). Sections were also stained by the immunoperoxidase method using antibodies to amyloid β-protein and τ-protein (Dako) and PrP (kindly supplied by Dr J. Hope, Edinburgh), after pretreatment with formic acid (Kitamoto *et al.* 1987). Positive and negative controls were performed concurrently with immunostains.

Table 1. Experiment 1

Homogenate from	Monkey	Age at death (years, months)	Time since injection (years, months)	Histology in monkeys				
				Plaques	CAA	NFT	SE	Immuno
Case 1 Alz. Dis.	M1	8, 3	6, 5	++	++	−	−	β-protein
β-plaques β-amyloid	M2	8, 3	6, 5	++	++	−	−	β-protein
NFT	M3	8, 6	6, 8	++	++	−	−	β-protein
Uninjected	M4	8, 2	−	−	−	−	−	−
	M5	8, 7	−	−	−	−	−	−
	M6	8, 9	−	−	−	−	−	−

Plaques, presence of neuritic plaques on silver stains; CAA, presence of cerebral amyloid angiopathy; NFT, neurofibrillary tangles; SE, spongiform encephalopathy; β-protein, indicates that plaques and CAA immunostain with antibodies to β-protein.
++, Moderate; (+), trace; −, negative.

Experiments

Expt 1. Comparison of the brains of marmosets injected with brain material from a case of Alzheimer's disease (Case 1) with the brains of age-matched control marmosets

Three marmosets (M1–M3), injected intracerebrally 6–7 years previously with brain material from a patient with early onset Alzheimer's disease (case 1), were killed for histology aged 8–9 years. Their brains were compared with those of three healthy age-matched control monkeys with no previous surgery (see Table 1).

Case 1 was a 56-year-old male who died 4 years after a clinical diagnosis of Alzheimer's disease had been made. He had presented with a 1-year history of forgetfulness, losing the thread of conversation and failing to recognize faces and names. He had lost 2 stone (13 kg) in weight. Air encephalography taken at the time of presentation showed evidence of cortical atrophy. EEG showed minimal excess of random theta components on a background of well preserved rhythmic activity. EEG 18 months later revealed definite deterioration with an excess of theta activity anteriorly. Three months prior to death his speech was incoherent and he was able to walk only with difficulty. One month prior to death he was bedridden in the end-stage of dementia. Neither parent was thought to be demented but the mother had died in her fifties (of cancer).

Neuropathological examination revealed moderate cerebral atrophy with ventricular enlargement. There were massive numbers of senile plaques (Figure 1A) and neurofibrillary tangles (NFT) (Figure 1B) and widespread meningeal and cortical congophilic angiopathy. The amyloid of the plaques and vessels immunostained strongly using antibody to β-protein (Figure 1C and D). The histological appearances were characteristic of severe Alzheimer's disease. There were no spongiform changes or astrocytic gliosis and the amyloid was not in the form of multicentric or kuru-type plaques which are characteristic of GSS or CJD. Sections sent to the CJD Neuropathology Surveillance Laboratory, Edinburgh, did not stain with antibody to PrP-protein.

Expt 2. Examination of brains of marmosets injected with brain material from suspected cases of prion dementia (Cases 2–5).

Eight marmosets (M7–M14) injected intracerebrally ~5 years previously with brain material from suspected cases of CJD (cases 2–5) or from an elderly control (case 6) were behaviourally normal when killed aged 6–7 years (see Table 2).

Case 2 (EU of Adam *et al.* 1982) was a 62-year-old lady with a 5-year history of progressive dementia and ataxia. Neuropathological examination revealed spongiform encephalopathy in cortex and deep grey nuclei, neuronal loss, astrocytic hyperplasia, and extensive amyloid deposition in the form of multicentric or unicentric plaques in cerebral and cerebellar cortex. These immunostained strongly using antibody to PrP. In addition, there were also neuritic plaques, particularly in the hippocampus, widespread amyloid angiopathy and some neurofibrillary tangles. Hippocampal plaques and vascular amyloid reacted positively with antibodies to β-amyloid. The appearances were characteristic of GSS with concomitant β-amyloidosis. This patient belonged to a pedigree in which neurodegenerative disease is associated with a PrP^{102} proline to leucine substitution (Hsiao *et al.* 1989).

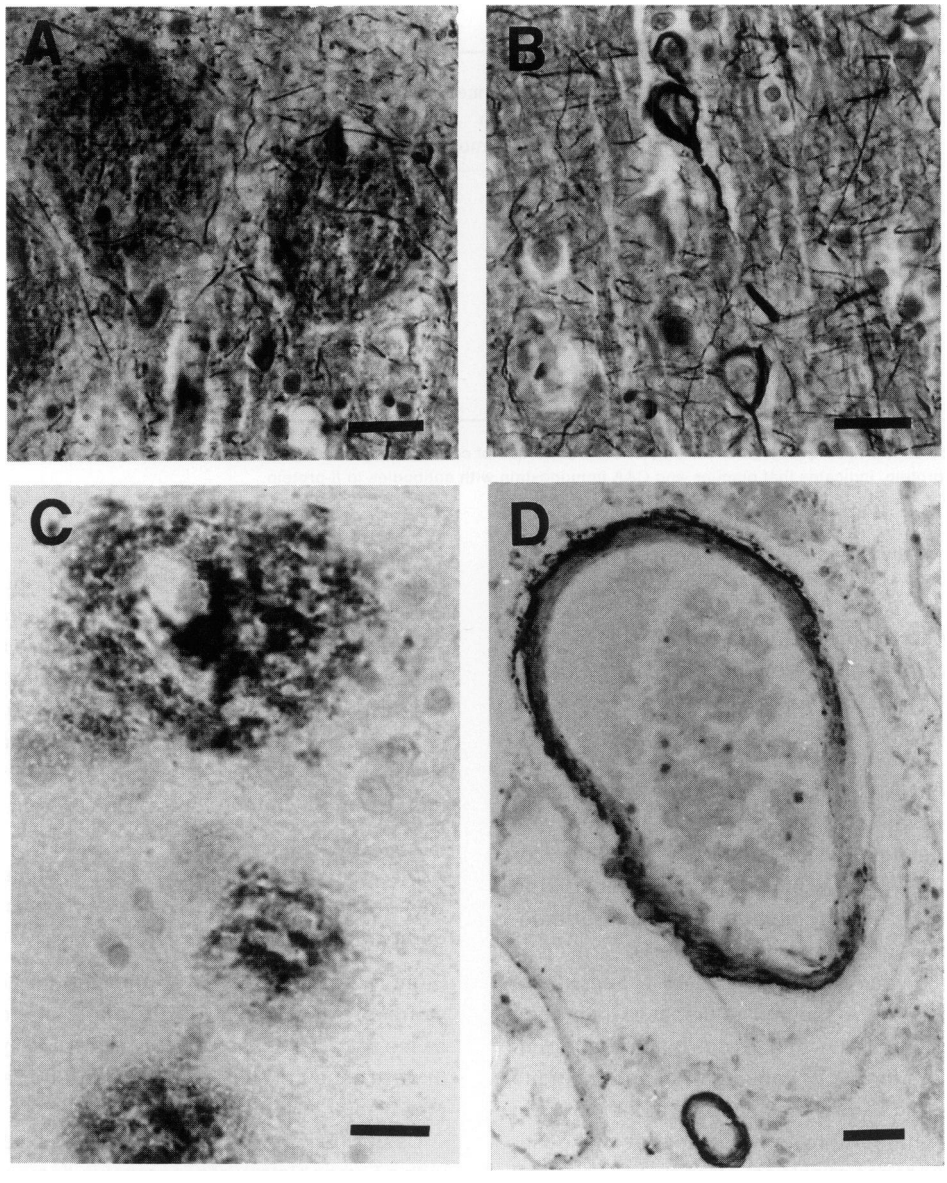

Figure 1. Sections of the occipital lobe of case 1. Silver impregnation demonstrates A, numerous neuritic senile plaques in the superficial layers of the cortex and B, many neurones containing densely argyrophilic neurofibrillary tangles in the deeper layers. Staining to demonstrate amyloid β-protein after pre-treatment with formic acid shows C, amyloid containing plaques and D, the presence of amyloid in meningeal arteriolar vessel walls. A and B: silver impregnation by modified Glees' method. C and D: immunoperoxidase staining. Bar 25 μm (A–C), 50 μm (D).

Case 3 (1st cousin of case 2) was a 51-year-old lady with a 3-year history of dementia and mild ataxia. Neuropathological examination revealed no spongiform change or gliosis. Some amyloid deposits were seen in the deep cortical areas of the frontal and parietal lobes, hippocampus and cerebellum. These deposits stained positively with antibody to PrP but were negative for β-amyloid. The neuropathological findings did not conform to the usual appearances of either GSS or CJD. A diagnosis of atypical prion dementia is appropriate.

Case 4 was a 79-year-old female who died of bronchopneumonia having been resident in a psychiatric hospital for the previous 37 years. Shortly after admission she was described as having rigidity and an 'encephalitis lethargica facies'. She had a frontal leucotomy one year after admission. She was generally

Table 2. Experiment 2

Homogenate from	Monkey	Age at death (years, months)	Time since injection (years, months)	Histology in monkeys				
				Plaques	CAA	NFT	SE	Immuno
Case 2								
β-plaques	M7	6, 0	4, 7	++	++	–	–	β-protein
β-CAA								
few NFT	M8	6, 5	5, 0	–	–	–	–	–
PrP plaques								
SE								
Case 3								
PrP plaques	M9	7, 2	5, 8	–	–	–	–	–
no CAA								
no β-plaques	M10	6, 10	5, 10	–	–	–	–	–
no NFT								
no SE								
PrP102								
Case 4								
few PrP plaques	M11	6, 11	5, 10	(+)	–	–	–	(β-protein)
few β-plaques								
no CAA								
no NFT								
SE								
Case 5								
few PrP plaques	M12	6, 11	5, 10	–	–	–	–	–
few β-plaques								
no CAA								
no NFT								
SE								
Case 6								
Elderly control	M13	7, 2	5, 7	(+)	(+)	–	–	(β-protein)
see text	M14	6, 6	4, 8	–	–	–	–	–

See Table 1 for key.

thought to have been suffering from schizophrenia. Neuropathological examination revealed moderate neuronal loss, astrocytosis and spongiform encephalopathy in the frontal and temporal cortex, corpus striatum and thalamus. A few senile plaques were seen but there were no neurofibrillary tangles. Some plaques were PrP positive but others were β-amyloid positive. The neuropathological diagnosis was CJD with moderate vascular atheroma. It is supposed that this terminal illness was superimposed on a long-standing psychiatric illness.

Case 5 was an 81-year-old man who died of bronchopneumonia 3 months after admission to a psychiatric hospital. He was demented at the time of admission and had been cared for by his wife for many years. The time of onset of dementia was unclear. Neuropathological examination revealed slight cortical atrophy and ventricular enlargement. In the temporal lobes and to a lesser extent the frontal lobes there was neuronal cell loss, gliosis and SE. There were some senile plaques but no neurofibrillary tangles and no vascular disease. Some plaques were PrP positive while others were β-amyloid positive. The neuropathological diagnosis was CJD.

Case 6. The brain from this man had been intended to be used as a control for the cases of suspected prion disease (cases 2–5). He was a 74-year-old man who died following a myocardial infarction. He had not been diagnosed as suffering from any neurological or psychiatric condition. However, subsequent neurochemical analysis of his brain revealed cholinacetyltransferase levels in the amygdala which were only 32% of control values (A. Cross, personal communication). This depletion was identical to the mean cholinacetyltransferase

depletion in a group of 12 patients with a clinical and pathological diagnosis of Alzheimer's disease (Ferrier *et al.* 1983). No histological data are available for this brain.

Supplementary observations

The brains of a further 26 marmosets used in other experiments were available for neuropathological examination and their histological appearances are relevant in the context of expts 1 and 2. All brains were stained with haematoxylin and eosin, and with silver stains; some were stained with cresyl violet and some with antibody to β-amyloid and antibody to PrP protein. These animals are listed in Table 3.

M15 was injected with brain tissue from a 45-year-old male with a 144 bp insertion in the PrP gene (case VI-21 of Poulter *et al.* 1992; Collinge *et al.* 1992). In the brain of this patient there was minimal SE, no plaques, no neurofibrillary tangles and no CAA, and immunostaining with antibodies to PrP and β-amyloid was negative. This animal was healthy when killed for histological examination. The brain was stained with haematoxylin and eosin, silver stains and antibodies to β-amyloid and PrP protein (Dr I. Janota, Institute of Psychiatry). M16 was injected with brain tissue from a 46-year-old female with mild SE but no plaques, neurofibrillary tangles or CAA. This animal was healthy when killed for histological examination using haematoxylin and eosin and silver stains (Dr R. Perry, Newcastle General Hospital).

M17–M20 were injected with brain tissue from a 46-year-old female with a clinical and neuropathological diagnosis of GSS (J.C. of Adam *et al.* 1982). She was a distant cousin of cases 2 and 3 and also carried the PrP^{102} proline to leucine mutation. Her brain had marked SE and PrP positive plaques but no neurofibrillary tangles and no CAA. Immunostaining with antibodies to β-amyloid was negative.

M21 and M22 were injected with brain tissue from a Friesian cow (PG 91/87) with histologically confirmed natural bovine spongiform encephalopathy and M23 and M24 were injected with brain tissue from a Greyface ewe (85/29) with histologically confirmed natural scrapie. These eight animals (M17–M24) were killed when they developed neurological signs and their brains have all been found to show marked SE (Baker *et al.* 1990, 1993).

A limited number of sections were available from the brains of 16 other marmosets from the same colony. M25–M30 were stained with haematoxylin and eosin, silver stains, and antibodies to β-amyloid. M31–M40 were stained with haematoxylin and eosin, cresyl violet and silver stains. M25 and M26 were healthy unoperated controls killed as part of another experiment. M27 and M28 were elderly animals both of which had been injected intracerebrally 7 years previously with human CSF as part of another experiment (Baker *et al.* 1989). M29 was an 11-year-old animal. An excitotoxic lesion was made in the nucleus basalis of Meynert ~2 weeks before it was killed. M30 was 11 years old and had had bilateral cannulae implanted several months before it was killed for the i.c. infusion of drugs. M31 became acutely ill 10 months after i.c. injection with brain tissue from case 1 and was killed. Post-mortem examination revealed acute pancreatitis. M32 was killed 10 months after injection with brain tissue from case 6. M33–M40 were eight marmosets aged 2–3 years in which excitotoxic lesions had been made in the cholinergic projection from the basal forebrain to the neocortex or hippocampus (Ridley *et al.* 1986b, 1988) ~9 months previously and from which a number of silver and cresyl violet stained sections through the lesion site were available.

Results

Expt 1. Comparison of the brains of marmosets injected with brain from a case of Alzheimer's disease (Case 1) with the brains of age-matched control marmosets (Table 1 and Fig. 2)

In the brains of the three marmosets (M1–M3) injected with brain tissue from a patient with Alzheimer's disease abnormalities were observed in the silver-impregnated sections and in those immunoreacted with amyloid β-protein antibody. Lesions were found only in grey matter including all regions of cerebral cortex and amygdaloid nucleus. They were not found in deep grey nuclei, brainstem or cerebellum. Silver impregnation showed several types of abnormality and it seems likely that they form a continuum, some lesions being earlier forms and some later, although this is, of course, conjectural. The smallest, perhaps earliest, abnormality consisted of the aggregation of a few argyrophilic bodies grouped around an area of relatively unstained neuropil (Figure 2A and B). These bodies were of variable shape, some being elongated, others rounded, varying in size from less than 1 to about 10 μm, and some being attached to axons suggesting that they were either swollen degenerating axonal terminals or 'retraction balls' (Figure 2C and D).

Many of the aggregates of argyrophilic bodies were more organized than those described above. These formed the majority of the plaque-like lesions. They consisted of densely stained bodies arranged in rounded, probably spherical, clumps of about 30 μm in diameter. These bodies tended to be club or carrot shaped radiating from a central relatively acellular paler

Table 3. Supplementary cases

Homogenate from	Monkey	Age at death (years, months)	Time since injection (years, months)	Plaques	Pathology
Attempted transmission of SE					
PrP insert	M15	7, 3	5, 10	–	–
Mild SE	M16	6, 2	5, 10	–	–
Successful transmission of SE					
GSS (PrP102)	M17	3, 10	2, 1	–	SE
	M18	3, 7	2, 5	–	SE
	M19	3, 10	2, 8	–	SE
	M20	4, 5	2, 8	–	SE
BSE (cow)	M21	5, 3	4, 0	–	SE
	M22	5, 4	4, 1	–	SE
Scrapie (sheep)	M23	4, 10	3, 8	–	SE
	M24	4, 4	3, 2	–	SE
Elderly controls					
Uninjected	M25	8, 10	–	–	–
	M26	9, 3	–	–	–
	M27	9, 6	–	–	–
	M28	11, 0	–	–	–
	M29	>11 years	–	–	–
	M30	>11 years	–	–	–
Other animals					
Case 1	M31	2, 8	10 months	–	–
Case 6	M32	2, 8	10 months	–	–
	M33–M40	2–3 years	–	–	–

staining area to end in the thicker densely stained outer ends packed together forming the periphery of the plaque (Figure 2E and F and Figure 4F). Occasionally an axon connected to the plaque lay in the surrounding neuropil.

In Congo red preparations, plaques and vessels showed dichroic birefringence in polarized light, characteristic of amyloid (Figure 4A–C).

Plaques were also demonstrated (Figure 3A and B and Figure 4D) in the anti-β-protein sections where dense solid staining was present at the centre with fibrillar processes radiating out from it, the periphery of the plaque being composed of a delicate network of lightly stained threads. We do not know whether each β-protein stained plaque was surrounded by argyrophilic bodies. No staining was observed in the τ-antibody preparations.

Occasional plaques lay immediately contiguous with vessel walls. Here the agryophilic bodies seemed loosely and haphazardly arranged rather than being a well organized rounded plaque. Perivascular plaques were also demonstrated with β-protein antibody.

Amyloid angiopathy was also found in the β-protein immunostained sections (Figure 3C and D and Figure 4E). Meningeal vessels over the entire cerebral hemispheres and also intracortical vessel walls were stained. Arterioles as well as capillaries were involved. Cerebellar and brain-stem meningeal vessels were very slightly affected, only an occasional arteriole being partially infiltrated with amyloid. No immunostained vessels were present in the white matter.

The distribution of plaques seemed to show no special predilection for any particular region such as temporal or hippocampal cortex. Nor was the distribution of plaques related to the sites of injection.

In no animal was there evidence of the formation of neurofibrillary tangles in the silver preparations. No electron microscopy was done however. There was no trace of spongiform encephalopathy or astrocytic hyperplasia in grey matter nor was there any inflammatory reaction. Histological studies were confined to the brain so it is not known whether there was evidence of systemic amyloid deposition.

The plaques in M1–M3 could not be described as very numerous but were readily found scattered throughout the cortex (Figure 5). No plaques or angiopathy were found in the silver-stained or immunostained sections of the brains of the three age-matched control marmosets (M4–M6).

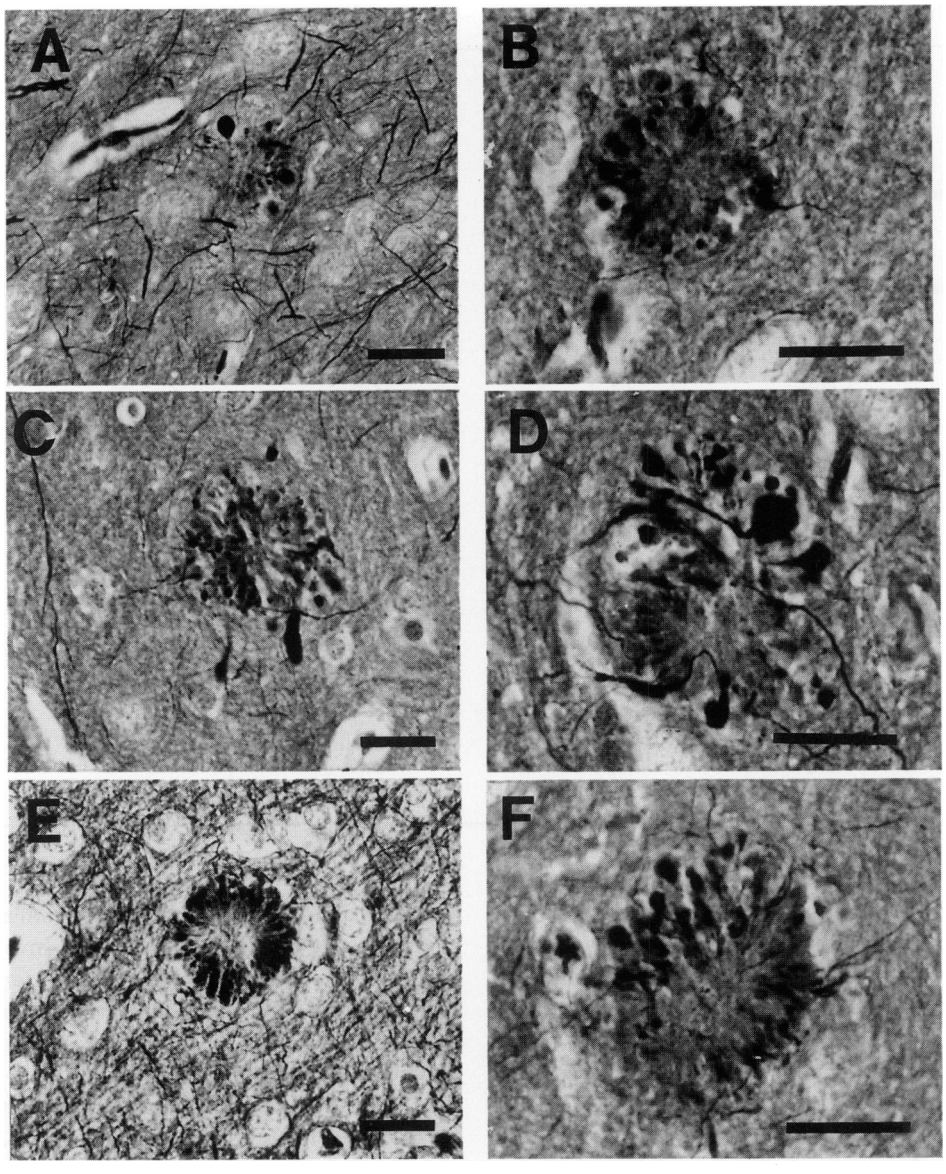

Figure 2. Sections of the cerebral cortex of marmosets M1–M3. Silver impregnation shows clusters of dystrophic neurites aggregated around a relatively unstained central core. Modified Glees' method. Bar 25 μm.

Expt 2. Examination of brains of marmosets injected with brain material from suspected cases of prion dementia (Cases 2–5) (see Table 2).

The brain of monkey M7 which had been injected with brain material from case 2 (prion dementia with concomitant β-amyloid plaques and β-CAA) contained β-amyloid plaques and β-CAA equivalent to that seen in M1–M3 of expt 1. Staining with antibody to PrP was negative and there was no SE and no neurofibrillary tangles. The brain of monkey M8, injected with the same material as M7, showed no β-amyloid deposition (and no SE). Monkeys M9 and M10, injected with material from case 3 (prion dementia without SE, β-amyloid plaques or CAA) did not have β-amyloid in their brains. M9 and M10 were slightly older and had lived slightly longer after i.c. injection than M7.

An occasional β-amyloid plaque, but no CAA, no neurofibrillary tangles and no SE, was seen in the brain of M11 (but not M12) injected with brain tissue from case 4. The brain of case 4 had shown some β-amyloid plaques. An occasional plaque was seen in the brain of M13 and there was some CAA. These features were β-amyloid positive. No plaques or CAA were seen in M14.

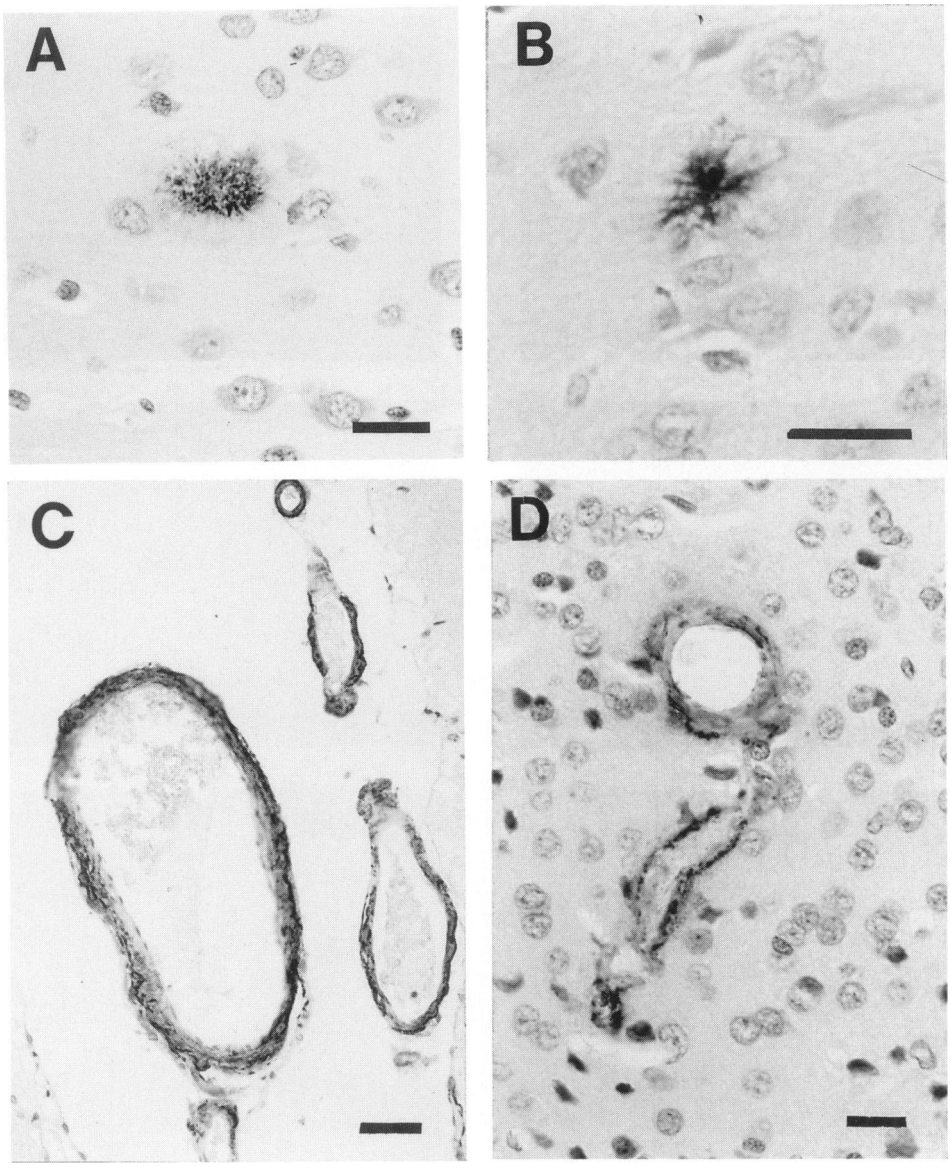

Figure 3. Sections of the cerebral cortex of marmosets M1–M3, stained by the immunoperoxidase method for amyloid β-protein. Small collections of fibrillar material radiating from a central densely stained core are shown in A and B. Amyloid infiltration is also seen in C, meningeal vessels and D, cortical vessels. Bar 25 μm (A, B and D), 50 μm (C).

M13 and M14 had been injected with brain tissue from case 6 who was 74 years old. Although histological data on his brain were not available, neurochemical measures suggested that he suffered from neurodegenerative changes.

Supplementary observations

In none of the 26 monkeys listed in Table 3 were plaques, neurofibrillary tangles or CAA seen.

Discussion

An important question is whether the β-plaques and CAA observed in animals M1–M3, M7 and, to a lesser extent, in M11 and M13 could be the result of ageing? Tables 1–3 show that the six animals with β-plaques and CAA were clearly not older than the animals without plaques. There were nine animals which did not have β-amyloid which were as old as or older than the oldest of these six animals. Nor can these six animals be considered elderly. The animals with β-plaques were aged 6–8·5 years whereas the natural lifespan of the marmoset in captivity is well in excess of 12 years. The oldest marmoset reported to us was a male aged > 16 years (H. Rothe, Göttingen University, personal communication).

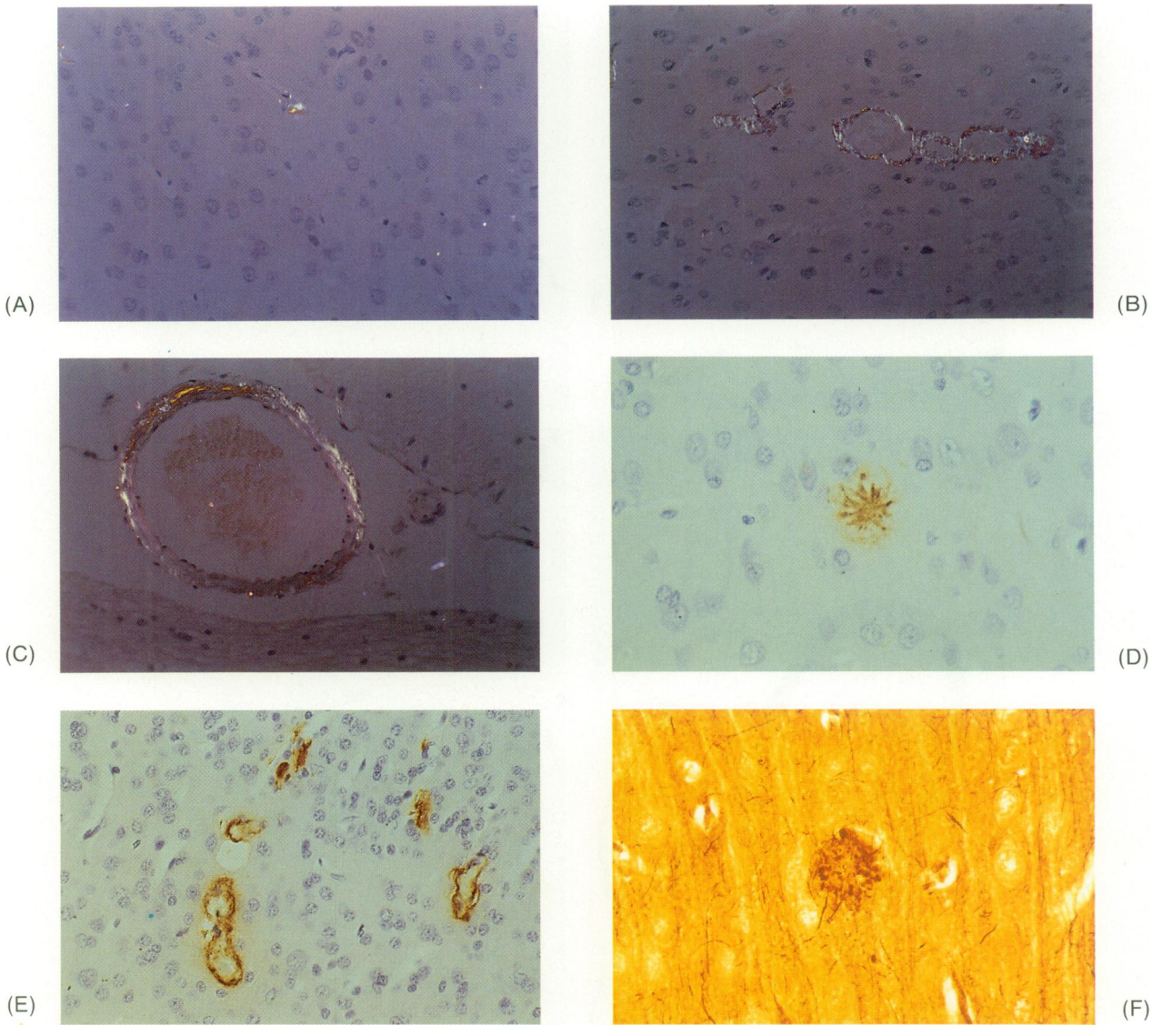

Figure 4. Sections of cortex of marmosets M1–M3. A, plaque; B, intracortical vessels; C, meningeal vessel, stained with Congo red and photographed under polarized light; D, plaque; E, cortical vessels immunostained for amyloid β-protein; F, plaque stained by Glees's silver method. A, B and C × 160; D and F × 320; E × 200.

In our colony of ~200 marmosets, animals typically develop a geriatric syndrome, comprising moderate weight loss, greying fur, tooth loss, reduced fecundity and sometimes decreased mobility, between the ages of 10 and 12 years. β-Amyloid has not previously been reported in *Callithrix jacchus*. Honavar and Lantos (1987) looked at the brains of aged common marmosets but reported mainly on ultrastructural pathology within neurones. They did not identify parenchymal or vascular amyloid.

CAA and amyloid plaques have been found in squirrel monkeys aged 20–27 years (Selkoe *et al*. 1987; Walker *et al*. 1990) and macaques aged 23–31 years (e.g. Struble *et al*. 1985; Selkoe *et al*. 1987). These plaques, together with diffuse amyloid deposits, immunostained with antibodies to β-protein. Thus, although β-amyloidosis seems to be a more common finding in primates than in other species (in which it has, nonetheless, sometimes been found, e.g. Dayan (1971)), its natural occurrence seems to be confined to animals which have reached at least 75% of the species-maximum age, whereas in this experiment β-amyloidosis was found in animals only 38–53% of the marmoset maximum age.

If the β-amyloidosis seen in these animals is not age-

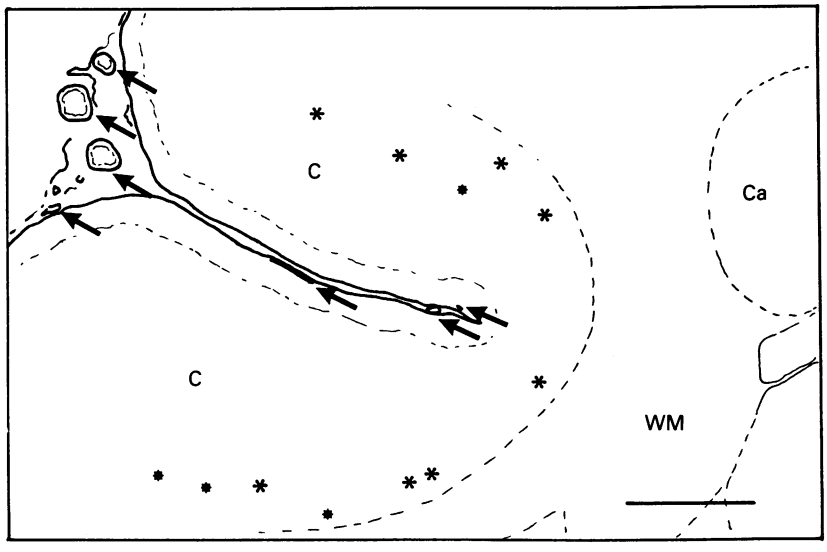

Figure 5. Camera lucida drawing of β-protein-stained coronal section of brain of marmoset M3 traversing the sylvian fissure. Arrows indicate major deposits of CAA. Large asterisks indicate position of well formed plaques. Small asterisks indicate small deposits of perivascular amyloid. C, Neocortex; Ca, caudate nucleus; WM, white matter. Bar 1 mm.

related then it must be argued that it has been induced experimentally. Tables 1–3 show that 12 animals (M9, M10, M15–24) injected with brain tissue known not to contain β-amyloid had no β-amyloid in their brains. On the other hand, 4/5 animals (M1–3, M7) injected with brain known to contain substantial amounts of β-amyloid were found to have β-amyloid plaques and CAA. A very small number of plaques or a trace of CAA was found in 2/4 animals injected with brain tissue which contained either a very small number of plaques or for which there was neurochemical evidence of age-related pathology. Two animals (M31, M32) have been discounted from consideration here because they survived only 10 months after injection. No β-amyloid was found in their brains.

These results suggest that β-amyloidosis does not occur as the non-specific result of injecting *any* brain tissue. Rather the results suggest that β-amyloid in the animals' brains is related to the presence of β-amyloid in the injected tissue. There is also some indication that the injection of brain containing large amounts of β-amyloid results in greater amounts of β-amyloid in the animal's brain.

If β-amyloidosis had developed as the result of the trauma of i.c. injection, it would be expected that its occurrence would be more evenly distributed across the 21 animals examined. If β-amyloidosis had developed as the consequence of other neurodegenerative changes, it might have been expected to appear particularly in animals M17–M24 in which the neurodegenerative changes of severe spongiform encephalopathy had occurred, or in animals M33–M40 in which neurodegeneration had been induced by the injection of excitatory neurotoxins.

β-Amyloid plaques have not previously been reported in any non-human primates used for transmission studies of prion disease or other neurodegenerative diseases including Alzheimer's disease (Gibbs & Gajdusek 1972; Masters *et al.* 1979; Brown *et al.* 1993). There are, however, methodological differences between those studies and ours. The above authors do not state the number of primate brains which have been examined with silver stains and immunohistochemistry after long survival times but it may be very small because their animals are allowed to survive indefinitely.

Our proposition is that β-amyloid plaques and CAA developed in the marmosets' brains as a consequence of having been injected with tissue containing β-amyloid. This may occur by a mechanism which is similar to that by which host PrP^{c} is converted to PrP^{sc} following i.c. injection of exogenous PrP^{sc} in experimental transmission of prion disease. In this respect the diagnosis in the patients whose brain tissue contained β-amyloid is irrelevant but it is of interest that one patient had a diagnosis of severe, early onset AD and another had a diagnosis of GSS with concomitant β-amyloidosis and a PrP^{102} mutation. In the case of the patient with AD it should be stressed that AD, *per se*, has not been transmitted. M1–M3 were not obviously debilitated (although they were not assessed by neuropsychological

testing) and their brains did not contain neurofibrillary tangles (NFTs). β-Amyloid deposition is probably the earliest neuropathological change in AD (Rumble *et al.* 1989). β-Amyloid may also induce neurodegeneration and contribute to the formation of NFTs (Kowall *et al.* 1991; Hardy & Allsop 1991).

The epidemiology of the transmissible spongiform encephalopathies and the transmission of SE from cases of genetically determined prion disease indicates that transmission from a case of SE does not prove that the case was itself acquired by contamination with infected material (Ridley *et al.* 1986a). The same logic applies to the transmission of β-amyloidosis. There is no epidemiological evidence that AD is acquired by contamination with affected tissue and the results of this experiment do not suggest that it could be except, theoretically, in certain very improbable circumstances.

It could be asked whether the β-amyloid in the marmosets could be an unusual expression of the transmission of prion disease. The neuropathology in M17–M24, together with the neuropathological findings in 20 other marmosets used for transmission studies (Baker *et al.* 1990; 1993), demonstrate that transmission from cases of GSS, CJD and iatrogenic SE (induced by injection of contaminated growth hormone) in humans and cases of BSE, scrapie and second passage of GSS and CJD in marmosets usually results in the development of SE. The failure to transmit SE in animals M7–M12, M15 and M16 may reflect the fact that they were atypical or suspected cases of prion disease for which transmission is frequently not achieved. Attempts to transmit SE from case 2 to other primate species were also unsuccessful (Masters *et al.* 1981). Case 3 achieved a diagnosis of GSS on the basis of a PrP^{102} mutation and the presence of multicentric deposits of amyloid which were immunostained with PrP antibody. The brain showed no spongiform encephalopathy (Watanabe & Duchen 1993). The remaining cases of suspected prion disease showed a sufficiently unusual clinical or neuropathological picture to have rendered the question of transmissibility of SE a useful adjunct to diagnosis. Transmission of SE from atypical cases is often not successful (Goldfarb *et al.* 1992) and its failure in these cases is not exceptional.

Despite some alarmist claims, the occurrence of prion disease with none of the hallmarks of prion disease and all of the hallmarks of another neurodegenerative disease is 'imagined' (Brown *et al.* 1993). There are no differential clinical or neuropathological features to suggest that case 1 had a prion disease rather than AD and the lack of a characteristic EEG, myoclonus, SE or PrP-staining plaques argue against a diagnosis of prion disease. Although β-amyloid plaques have been found in prion disease, the majority of these occur in elderly patients (Roberts *et al.* 1988). Occasional concomitant β-amyloidosis has been found in prion disease (as in case 2; Watanabe & Duchen 1993) but in these cases the degree of β-amyloidosis is not as extensive as that seen in case 1 which was extremely severe. Neurofibrillary tangles are an unusual feature in prion disease and, when they do occur, tend to be localized to the hippocampus. However, in the Indiana kindred, which does have a large number of NFT and plaques and a PrP^{198} mutation (Dlouhy *et al.* 1992), the plaques stain with anti-PrP as well as anti-β-amyloid antibodies (Bugiani *et al.* 1993). Thus the pathology of case 1 was typical of AD and we conclude that the amyloidosis of that and the other brains with β-amyloid has been transmitted to marmosets by intracerebral injection.

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