



HOKKAIDO UNIVERSITY

Title	Pathological studies on senile plaques, cerebrovascular amyloidosis and astrocytic gliosis in the central nervous system(CNS)of aged dogs : an animal model sharing features with CNS aging of human beings
Author(s)	Shimada, Akinori
Degree Grantor	北海道大学
Degree Name	博士(獣医学)
Dissertation Number	乙第4120号
Issue Date	1992-06-30
DOI	https://doi.org/10.11501/3089303
Doc URL	https://hdl.handle.net/2115/49824
Type	doctoral thesis
File Information	000000251736.pdf



PATHOLOGICAL STUDIES ON SENILE PLAQUES, CEREBROVASCULAR
AMYLOIDOSIS AND ASTROCYTIC GLEOSIS IN THE CENTRAL NERVOUS SYSTEM
(CNS) OF AGED DOGS: AN ANIMAL MODEL SHARING FEATURES WITH CNS
AGING OF HUMAN BEINGS

AKINORI SUMIYAMA

①

CONTENTS

PREFACE
PATHOLOGICAL STUDIES ON SENILE PLAQUES, CEREBROVASCULAR
AMYLOIDOSIS AND ASTROCYTIC GLIOSIS IN THE CENTRAL NERVOUS SYSTEM
(CNS) OF AGED DOGS: AN ANIMAL MODEL SHARING FEATURES WITH CNS
AGING OF HUMAN BEINGS

1. INTRODUCTION	1
2. MATERIALS AND METHODS	15
3. RESULTS	16
4. DISCUSSION	21
5. SUMMARY	25
6. REFERENCES	26
7. APPENDIX	27
8. INDEX	28
9. SUMMARY	29
10. REFERENCES	30
11. APPENDIX	31
12. INDEX	32
13. SUMMARY	33
14. REFERENCES	34
15. APPENDIX	35
16. INDEX	36
17. SUMMARY	37
18. REFERENCES	38
19. APPENDIX	39
20. INDEX	40
21. SUMMARY	41
22. REFERENCES	42
23. APPENDIX	43
24. INDEX	44
25. SUMMARY	45
26. REFERENCES	46
27. APPENDIX	47
28. INDEX	48
29. SUMMARY	49
30. REFERENCES	50
31. APPENDIX	51
32. INDEX	52
33. SUMMARY	53
34. REFERENCES	54
35. APPENDIX	55
36. INDEX	56
37. SUMMARY	57
38. REFERENCES	58
39. APPENDIX	59
40. INDEX	60
41. SUMMARY	61
42. REFERENCES	62
43. APPENDIX	63
44. INDEX	64
45. SUMMARY	65
46. REFERENCES	66
47. APPENDIX	67
48. INDEX	68
49. SUMMARY	69
50. REFERENCES	70
51. APPENDIX	71
52. INDEX	72
53. SUMMARY	73
54. REFERENCES	74
55. APPENDIX	75
56. INDEX	76
57. SUMMARY	77
58. REFERENCES	78
59. APPENDIX	79
60. INDEX	80
61. SUMMARY	81
62. REFERENCES	82
63. APPENDIX	83
64. INDEX	84
65. SUMMARY	85
66. REFERENCES	86
67. APPENDIX	87
68. INDEX	88
69. SUMMARY	89
70. REFERENCES	90
71. APPENDIX	91
72. INDEX	92
73. SUMMARY	93
74. REFERENCES	94
75. APPENDIX	95
76. INDEX	96
77. SUMMARY	97
78. REFERENCES	98
79. APPENDIX	99
80. INDEX	100
81. SUMMARY	101
82. REFERENCES	102
83. APPENDIX	103
84. INDEX	104
85. SUMMARY	105
86. REFERENCES	106
87. APPENDIX	107
88. INDEX	108
89. SUMMARY	109
90. REFERENCES	110
91. APPENDIX	111
92. INDEX	112
93. SUMMARY	113
94. REFERENCES	114
95. APPENDIX	115
96. INDEX	116
97. SUMMARY	117
98. REFERENCES	118
99. APPENDIX	119
100. INDEX	120
101. SUMMARY	121
102. REFERENCES	122
103. APPENDIX	123
104. INDEX	124
105. SUMMARY	125
106. REFERENCES	126
107. APPENDIX	127
108. INDEX	128
109. SUMMARY	129
110. REFERENCES	130
111. APPENDIX	131
112. INDEX	132
113. SUMMARY	133
114. REFERENCES	134
115. APPENDIX	135
116. INDEX	136
117. SUMMARY	137
118. REFERENCES	138
119. APPENDIX	139
120. INDEX	140
121. SUMMARY	141
122. REFERENCES	142
123. APPENDIX	143
124. INDEX	144
125. SUMMARY	145
126. REFERENCES	146
127. APPENDIX	147
128. INDEX	148
129. SUMMARY	149
130. REFERENCES	150
131. APPENDIX	151
132. INDEX	152
133. SUMMARY	153
134. REFERENCES	154
135. APPENDIX	155
136. INDEX	156
137. SUMMARY	157
138. REFERENCES	158
139. APPENDIX	159
140. INDEX	160
141. SUMMARY	161
142. REFERENCES	162
143. APPENDIX	163
144. INDEX	164
145. SUMMARY	165
146. REFERENCES	166
147. APPENDIX	167
148. INDEX	168
149. SUMMARY	169
150. REFERENCES	170
151. APPENDIX	171
152. INDEX	172
153. SUMMARY	173
154. REFERENCES	174
155. APPENDIX	175
156. INDEX	176
157. SUMMARY	177
158. REFERENCES	178
159. APPENDIX	179
160. INDEX	180
161. SUMMARY	181
162. REFERENCES	182
163. APPENDIX	183
164. INDEX	184
165. SUMMARY	185
166. REFERENCES	186
167. APPENDIX	187
168. INDEX	188
169. SUMMARY	189
170. REFERENCES	190
171. APPENDIX	191
172. INDEX	192
173. SUMMARY	193
174. REFERENCES	194
175. APPENDIX	195
176. INDEX	196
177. SUMMARY	197
178. REFERENCES	198
179. APPENDIX	199
180. INDEX	200
181. SUMMARY	201
182. REFERENCES	202
183. APPENDIX	203
184. INDEX	204
185. SUMMARY	205
186. REFERENCES	206
187. APPENDIX	207
188. INDEX	208
189. SUMMARY	209
190. REFERENCES	210
191. APPENDIX	211
192. INDEX	212
193. SUMMARY	213
194. REFERENCES	214
195. APPENDIX	215
196. INDEX	216
197. SUMMARY	217
198. REFERENCES	218
199. APPENDIX	219
200. INDEX	220
201. SUMMARY	221
202. REFERENCES	222
203. APPENDIX	223
204. INDEX	224
205. SUMMARY	225
206. REFERENCES	226
207. APPENDIX	227
208. INDEX	228
209. SUMMARY	229
210. REFERENCES	230
211. APPENDIX	231
212. INDEX	232
213. SUMMARY	233
214. REFERENCES	234
215. APPENDIX	235
216. INDEX	236
217. SUMMARY	237
218. REFERENCES	238
219. APPENDIX	239
220. INDEX	240
221. SUMMARY	241
222. REFERENCES	242
223. APPENDIX	243
224. INDEX	244
225. SUMMARY	245
226. REFERENCES	246
227. APPENDIX	247
228. INDEX	248
229. SUMMARY	249
230. REFERENCES	250
231. APPENDIX	251
232. INDEX	252
233. SUMMARY	253
234. REFERENCES	254
235. APPENDIX	255
236. INDEX	256
237. SUMMARY	257
238. REFERENCES	258
239. APPENDIX	259
240. INDEX	260
241. SUMMARY	261
242. REFERENCES	262
243. APPENDIX	263
244. INDEX	264
245. SUMMARY	265
246. REFERENCES	266
247. APPENDIX	267
248. INDEX	268
249. SUMMARY	269
250. REFERENCES	270
251. APPENDIX	271
252. INDEX	272
253. SUMMARY	273
254. REFERENCES	274
255. APPENDIX	275
256. INDEX	276
257. SUMMARY	277
258. REFERENCES	278
259. APPENDIX	279
260. INDEX	280
261. SUMMARY	281
262. REFERENCES	282
263. APPENDIX	283
264. INDEX	284
265. SUMMARY	285
266. REFERENCES	286
267. APPENDIX	287
268. INDEX	288
269. SUMMARY	289
270. REFERENCES	290
271. APPENDIX	291
272. INDEX	292
273. SUMMARY	293
274. REFERENCES	294
275. APPENDIX	295
276. INDEX	296
277. SUMMARY	297
278. REFERENCES	298
279. APPENDIX	299
280. INDEX	300
281. SUMMARY	301
282. REFERENCES	302
283. APPENDIX	303
284. INDEX	304
285. SUMMARY	305
286. REFERENCES	306
287. APPENDIX	307
288. INDEX	308
289. SUMMARY	309
290. REFERENCES	310
291. APPENDIX	311
292. INDEX	312
293. SUMMARY	313
294. REFERENCES	314
295. APPENDIX	315
296. INDEX	316
297. SUMMARY	317
298. REFERENCES	318
299. APPENDIX	319
300. INDEX	320
301. SUMMARY	321
302. REFERENCES	322
303. APPENDIX	323
304. INDEX	324
305. SUMMARY	325
306. REFERENCES	326
307. APPENDIX	327
308. INDEX	328
309. SUMMARY	329
310. REFERENCES	330
311. APPENDIX	331
312. INDEX	332
313. SUMMARY	333
314. REFERENCES	334
315. APPENDIX	335
316. INDEX	336
317. SUMMARY	337
318. REFERENCES	338
319. APPENDIX	339
320. INDEX	340
321. SUMMARY	341
322. REFERENCES	342
323. APPENDIX	343
324. INDEX	344
325. SUMMARY	345
326. REFERENCES	346
327. APPENDIX	347
328. INDEX	348
329. SUMMARY	349
330. REFERENCES	350
331. APPENDIX	351
332. INDEX	352
333. SUMMARY	353
334. REFERENCES	354
335. APPENDIX	355
336. INDEX	356
337. SUMMARY	357
338. REFERENCES	358
339. APPENDIX	359
340. INDEX	360
341. SUMMARY	361
342. REFERENCES	362
343. APPENDIX	363
344. INDEX	364
345. SUMMARY	365
346. REFERENCES	366
347. APPENDIX	367
348. INDEX	368
349. SUMMARY	369
350. REFERENCES	370
351. APPENDIX	371
352. INDEX	372
353. SUMMARY	373
354. REFERENCES	374
355. APPENDIX	375
356. INDEX	376
357. SUMMARY	377
358. REFERENCES	378
359. APPENDIX	379
360. INDEX	380
361. SUMMARY	381
362. REFERENCES	382
363. APPENDIX	383
364. INDEX	384
365. SUMMARY	385
366. REFERENCES	386
367. APPENDIX	387
368. INDEX	388
369. SUMMARY	389
370. REFERENCES	390
371. APPENDIX	391
372. INDEX	392
373. SUMMARY	393
374. REFERENCES	394
375. APPENDIX	395
376. INDEX	396
377. SUMMARY	397
378. REFERENCES	398
379. APPENDIX	399
380. INDEX	400
381. SUMMARY	401
382. REFERENCES	402
383. APPENDIX	403
384. INDEX	404
385. SUMMARY	405
386. REFERENCES	406
387. APPENDIX	407
388. INDEX	408
389. SUMMARY	409
390. REFERENCES	410
391. APPENDIX	411
392. INDEX	412
393. SUMMARY	413
394. REFERENCES	414
395. APPENDIX	415
396. INDEX	416
397. SUMMARY	417
398. REFERENCES	418
399. APPENDIX	419
400. INDEX	420
401. SUMMARY	421
402. REFERENCES	422
403. APPENDIX	423
404. INDEX	424
405. SUMMARY	425
406. REFERENCES	426
407. APPENDIX	427
408. INDEX	428
409. SUMMARY	429
410. REFERENCES	430
411. APPENDIX	431
412. INDEX	432
413. SUMMARY	433
414. REFERENCES	434
415. APPENDIX	435
416. INDEX	436
417. SUMMARY	437
418. REFERENCES	438
419. APPENDIX	439
420. INDEX	440
421. SUMMARY	441
422. REFERENCES	442
423. APPENDIX	443
424. INDEX	444
425. SUMMARY	445
426. REFERENCES	446
427. APPENDIX	447
428. INDEX	448
429. SUMMARY	449
430. REFERENCES	450
431. APPENDIX	451
432. INDEX	452
433. SUMMARY	453
434. REFERENCES	454
435. APPENDIX	455
436. INDEX	456
437. SUMMARY	457
438. REFERENCES	458
439. APPENDIX	459
440. INDEX	460
441. SUMMARY	461
442. REFERENCES	462
443. APPENDIX	463
444. INDEX	464
445. SUMMARY	465
446. REFERENCES	466
447. APPENDIX	467
448. INDEX	468
449. SUMMARY	469
450. REFERENCES	470
451. APPENDIX	471
452. INDEX	472
453. SUMMARY	473
454. REFERENCES	474
455. APPENDIX	475
456. INDEX	476
457. SUMMARY	477
458. REFERENCES	478
459. APPENDIX	479
460. INDEX	480
461. SUMMARY	481
462. REFERENCES	482
463. APPENDIX	483
464. INDEX	484
465. SUMMARY	485
466. REFERENCES	486
467. APPENDIX	487
468. INDEX	488
469. SUMMARY	489
470. REFERENCES	490
471. APPENDIX	491
472. INDEX	492
473. SUMMARY	493
474. REFERENCES	494
475. APPENDIX	495
476. INDEX	496
477. SUMMARY	497
478. REFERENCES	498
479. APPENDIX	499
480. INDEX	500
481. SUMMARY	501
482. REFERENCES	502
483. APPENDIX	503
484. INDEX	504
485. SUMMARY	505
486. REFERENCES	506
487. APPENDIX	507
488. INDEX	508
489. SUMMARY	509
490. REFERENCES	510
491. APPENDIX	511
492. INDEX	512
493. SUMMARY	513
494. REFERENCES	514
495. APPENDIX	515
496. INDEX	516
497. SUMMARY	517
498. REFERENCES	518
499. APPENDIX	519
500. INDEX	520
501. SUMMARY	521
502. REFERENCES	522
503. APPENDIX	523
504. INDEX	524
505. SUMMARY	525
506. REFERENCES	526
507. APPENDIX	527
508. INDEX	528
509. SUMMARY	529
510. REFERENCES	530
511. APPENDIX	531
512. INDEX	532
513. SUMMARY	533
514. REFERENCES	534
515. APPENDIX	535
516. INDEX	536
517. SUMMARY	537
518. REFERENCES	538
519. APPENDIX	539
520. INDEX	540
521. SUMMARY	541
522. REFERENCES	542
523. APPENDIX	543
524. INDEX	544
525. SUMMARY	545
526. REFERENCES	546
527. APPENDIX	547
528. INDEX	548
529. SUMMARY	549
530. REFERENCES	550
531. APPENDIX	551
532. INDEX	552
533. SUMMARY	553
534. REFERENCES	554
535. APPENDIX	555
536. INDEX	556
537. SUMMARY	557
538. REFERENCES	558
539. APPENDIX	559
540. INDEX	560
541. SUMMARY	561
542. REFERENCES	562
543. APPENDIX	563
544. INDEX	564
545. SUMMARY	565
546. REFERENCES	566
547. APPENDIX	567
548. INDEX	568
549. SUMMARY	569

CONTENTS

	page
PREFACE	1
CHAPTER I. MODIFIED BIELSCHOWSKY AND IMMUNOHISTOCHEMICAL STUDIES ON SENILE PLAQUES AND CEREBROVASCULAR AMYLOIDOSIS IN THE CENTRAL NERVOUS SYSTEM OF AGED DOGS	12
I. INTRODUCTION	13
II. MATERIALS AND METHODS	15
III. RESULTS	17
IV. DISCUSSION	21
V. SUMMARY	25
CHAPTER II. IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDIES ON AGE-RELATED ASTROCYTIC GLIOSIS IN THE CENTRAL NERVOUS SYSTEM OF DOGS	26
I. INTRODUCTION	27
II. MATERIALS AND METHODS	28
III. RESULTS	30
IV. DISCUSSION	33
V. SUMMARY	37

CHAPTER III. THE TOPOGRAPHIC RELATIONSHIPS BETWEEN ASTROCYTIC GLIOSIS AND SENILE PLAQUES, AND ASTROCYTIC GLIOSIS AND CEREBROVASCULAR AMYLOIDOSIS IN THE CEREBRAL CORTEX OF AGED DOGS	38
I. INTRODUCTION	39
II. MATERIALS AND METHODS	40
III. RESULTS	42
IV. DISCUSSION	43
V. SUMMARY	45
CONCLUSION	46
ACKNOWLEDGEMENTS	49
REFERENCES	52

PREFACE

Changes normally associated with aging in the central nervous system (CNS) of human beings include shrinkage of the brain and probable loss of neurons, gliosis, amyloid deposits such as senile plaques (SP) and cerebrovascular amyloidosis (CA), deposition of lipofuscin pigments in neurons and glial cells, and cytoskeletal abnormalities of neurons such as axonal dystrophy, neurofibrillary tangles (NFT), granulovacuolar degeneration, Hirano bodies and Lewy bodies. Corpora amylacea in the astrocytic processes are also a known form of aged changes.

The presence of numerous SP and nerve cells containing NFT in the cerebral cortex, hippocampus and amygdala is widely regarded as representing the histopathological hallmark of Alzheimer's disease (AD) [72]. It is also well recognized that these same pathological changes occur, but to a much lesser extent, in many non-demented individuals, particularly after the age of 65 years [18, 76, 80, 128, 131].

AD occurs throughout the world and accounts for one-half to two-thirds of all cases of late-life intellectual failure in many developed countries that have achieved high life expectancies. The application of biochemical and molecular approaches to AD has now led to the identification of several gene products whose alterations may underlie the progressive dysfunction and dystrophy of neurons and glia that occur in the limbic and association cortices and in certain subcortical nuclei that project to them. The greatest challenge to investigators studying AD, and the greatest source of ongoing controversy, derives from attempts to place the observed morphological and

biochemical changes into a temporal sequence of pathogenesis.

Animal models of human diseases usually bring closer the day when we can help affected people. The need for an animal model for AD is particularly pressing considering the number of people who are suffering or will suffer from this disease. Amyloid deposits similar to those observed in human brains have been described in aged non-human primates [19, 59, 60, 120, 135, 136, 139]. Numerous SP were also observed in the brains of aged bears [14]. SP and CA in the brains of dogs were observed by Wisniewski *et al.* [140] and Uchida *et al.* [130]. An immunohistochemical study revealed the presence of SP in five species of aged mammals including dogs, and showed that amyloid deposits that are a part of these SP consist of the amyloid β -protein (A β P) [112]. Recently, Kawabata *et al.* [51] produced transgenic mice carrying SP, NFT and neuronal degeneration similar to those in the AD brain.

In this thesis, data on the spectrum of morphological appearance of amyloid deposits in the CNS of aged dogs are presented. This study shows that the cerebral amyloid deposits in aged dogs are morphologically and immunohistochemically similar to those in human beings. Diffuse astrocytic gliosis, which is the change known to be associated with normal aging and AD, is also demonstrated in aged dogs. Aged dogs, therefore, may become the animal of choice for studying the mechanisms involved in CNS aging of human beings.

Current information on the pathology of cerebral amyloid deposition will be presented in this introduction, because this study attempts to get a insight into the pathogenesis of cerebral amyloid deposition by the systemic

examination of the CNS of aged dogs.

Review: the pathology of cerebral amyloid deposition

The classic SP of AD is a complex lesion of the cortical neuropil containing several abnormal elements: a central deposit of extracellular amyloid fibrils (the core) surrounded by dystrophic neurites (both dendrites and axon terminals), activated microglia, and hypertrophic astrocytes [142]. The sequence of involvement of these elements in plaque formation and the time required to generate such 'mature' plaques are poorly understood. Although plaques with these characteristics can occasionally be observed in other age-related degenerative brain diseases, they occur abundantly in three conditions: AD, trisomy 21, and, to a lesser extent, normal brain aging. The fibrils of the plaque core are ultrastructurally distinct from paired helical filaments of NFT (the core fibrils are extracellular, unpaired, and ~8 nm in diameter), but closely resemble the biochemically diverse amyloid filaments that accumulate extracellularly in nonneuronal tissues in a variety of unrelated systemic amyloidosis [33]. Amyloidosis is a general term in pathology that designates diseases in which deposits of 5-10 nm proteinaceous fibrils (amyloid) accumulate progressively in the extracellular spaces of tissues and their vasculature. The amyloid filaments are usually composed of proteolytic fragments of normal or mutant gene products; the particular polypeptide forming the filaments differs among the amyloidoses. Because the amyloid deposits in such diseases invariably lead to local tissue injury rather than accumulate as inert by-products of

the pathological process, it has long been assumed by some that this would also be the case in AD. In addition to plaque cores, amyloid deposits occur in the walls of some or many cerebral and leptomeningeal blood vessels in AD.

In 1984, Glenner and Wong [34] first reported the subunit composition of amyloid filaments isolated from meningeal vessels and solubilized in guanidine hydrochloride. The ~4 kd monomer was sequenced to residue 24 and found to be a novel peptide, designated the A β P (Fig. 1). The sequence of A β P derived from the meningovascular deposits was later shown to extend to 40 residues [46]. The purification of SP cores from AD cortex revealed an ~4 kd subunit protein of essentially identical amino acid composition [79, 111]. Antibodies to either the synthetic A β P [79, 145] or the native, purified A β P [46, 111] revealed the complete cross-reaction of vascular and plaque amyloid. Moreover, A β P immunohistochemistry of AD cortex has revealed innumerable noncompacted deposits of A β P ('diffuse' or 'preamyloid' plaques) that contain very few or no surrounding dystrophic neurites or glia [147]. Such amorphous deposits were not detected by the classic amyloid stains, Congo red and thioflavin S. These and many other recent studies indicate that there is a much greater amount of A β P in AD brain than previously believed, including many diffuse plaques in brain regions that appear to be largely unaffected clinically (for example, cerebellum, striatum, and thalamus) [49, 90].

The initial cloning of A β P cDNAs demonstrated that the isolated peptide was a proteolytic fragment of a 695 residue precursor protein (β -amyloid precursor protein: β APP), whose sequence predicted a glycosylated polypeptide spanning the membrane once near its C-terminus [50]. The

39-42 residue A β P region comprises the 28 amino acids just outside of the membrane plus the first 11-14 amino acids of the hydrophobic transmembrane domain (Fig. 2). Subsequent cloning has identified several alternatively spliced transcripts (563, 751, or 770 amino acids long), which contain an insert with $\sim$ 50% homology to the Kunitz family of serine protease inhibitors (KPI) [21, 57, 99, 124]. The β APP polypeptides themselves have been identified in brain, nonneural tissues, and cultured cells as a complex group of 135 kd membrane-associated proteins [114]. In cultured cells, β APP has been shown to undergo N- and O-glycosylation and tyrosine sulfation [93, 137], as well as constitutive proteolytic cleavage that releases the large, amino-terminal soluble protein into the medium [137] and retains an $\sim$ 10 kd hydrophobic carboxy-terminal fragment in the membrane [114]. Recently, Esch and colleagues [25] have purified and sequenced both of these fragments from cDNA-transfected cells and established that constitutive cleavage occurs at residue 16 of the A β P region (residue 687 of β APP₇₇₀) (Fig. 2), thereby precluding β -amyloid formation. Two (or more) alternative cleavages must occur in at least a subset of β APP molecules during aging and in AD to liberate the intact A β P fragment. The secreted amino-terminal fragment containing the KPI insert has been shown to be identical to the previously described serine protease inhibitor, protease nexin II (PN-II) [92, 132].

The biological functions of β APP are not yet well understood. The high degree of evolutionary conservation of β APP molecules, their expression in virtually all cells and tissues examined to date, and the characteristics of the β APP promoter region [105] all suggest that there are important,

multiple functions of this family of alternatively spliced proteins. Forms of β APP containing the KPI motif have been shown to inhibit trypsin and other serine proteases in vitro [57, 117]. Based on studies of the secreted serine protease inhibitor, protease nexin-I, it has been hypothesized that the PN-II portion of β APP may be secreted from cells in order to regulate extracellular serine proteases, following which the protease inhibitor complex may bind back to a cell surface receptor and be internalized. Another putative function for β APP based on in vitro experiments is as an autocrine or growth-promoting molecule. This hypothesis is based on the finding of a reduced rate of fibroblast proliferation following treatment of the cells with β APP antisense oligonucleotides; addition of exogenous PN-II restored normal growth [104]. PN-II has also recently been shown to be an inhibitor of coagulation factor XIa in vitro [118]. Its storage in the α granules of platelets and release following stimulation with platelet agonists such as thrombin or collagen have suggested a role in scar formation and wound repair [132]. Other vitro evidence suggests that β APP is a component of the extracellular matrix and could participate in cell adhesion [61, 109]. The primary structure of β APP based on its original cDNA sequence led to the hypothesis that it serves as a cell surface receptor [50], although no direct evidence of such a function has yet been published.

Additional studies establishing the normal biological functions of β APP will be needed to ascertain whether one or more of these functions is perturbed in AD. It may be that only a small minority of β APP molecules are alternatively cleaved to release the amyloidogenic A β P fragment and that most precursor molecules continue to be normally processed and remain

functional throughout the disease. Indeed, the lack of phenotypic abnormalities in nonneural tissues that express β APP as well as in numerous brain and spinal cord regions that contain abundant β APP-producing cells suggests that widespread loss of normal β APP function is not a fundamental event that underlies the pathogenesis of AD.

Although compositional analysis and fibril reconstitution studies suggest that A β P is the sole subunit protein of the amyloid fibrils in AD, there is growing evidence that several other distinct proteins are intimately and specifically associated with the β -amyloid deposits. These other constituents of the deposits, designated as β -amyloid-associated proteins, include α_1 -antichymotrypsin (ACT) [1], complement factors C1q, C3c, and C3d [103], the serum amyloid P protein [13], and heparan sulfate proteoglycans [119]. The latter two constituents are now unique to the β -amyloid of AD, but are found in all types of amyloid deposits occurring in a variety of human diseases. ACT has been found to be present in β -amyloid deposits in the several disorders in which these occur (brain aging in humans and monkeys, AD, and Down's syndrome) [1], but not in other types of amyloid. Moreover, ACT remains associated with the A β P fibrils following heating in sodium dodecyl sulfate [1]. Such a tight association has also recently been reported for heparin sulfate proteoglycan [119].

The presence of complement components, acute phase protein such as ACT and serum amyloid P, and activated microglia [45, 143] in β -amyloid deposits suggests that an inflammatory process is occurring within the SP. The nature of this inflammatory process, the possible role of cytokines [39], and the question of whether the process may represent an immune-mediated

reaction [82] remain issues for further study.

The discovery that amorphous, largely nonfilamentous deposits of A β P that have few or no surrounding dystrophic neurites, designated as 'diffuse plaques', are much more common in both AD and normal aged brains than are 'classic plaques' has heightened interest in the long-standing and unresolved question of the origin of β -amyloid deposits. The juxtaposition of the amyloid cores of classic plaques to degenerating neurites (including axonal terminals) and activated glial cells has led to suggestions that the extracellular amyloid may arise from β APP in altered neurons [78], astrocytes [116], or microglia [143]. The high levels of β APP expression in neurons demonstrated by in situ hybridization [5] and the finding that β APP undergoes fast axonal transport [63] have been used to support a neuronal origin for A β P deposits. However, virtually all cells in the nervous system, including astrocytes, microglia, and endothelial cells, as well as innumerable nonneural cells, express β APP. Furthermore, many diffuse A β P deposits in AD brain are now known to contain very few or no morphologically altered neurites [47, 49, 147]. Electron microscopy of these deposits reveals occasional altered cell processes, but much of the A β P-immunoreactive neuropil is ultrastructurally indistinguishable from the surrounding normal neuropil [149].

Support for the hypothesis that such amorphous A β P deposits precede rather than follow neuritic and glial alteration comes from immunocytochemical studies of Down's syndrome. Humans with trisomy 21 develop typical amyloid-bearing neuritic plaques and neurofibrillary tangles indistinguishable from those in AD if they survive into their fifth

and sixth decades. It is generally assumed that the invariant occurrence of β -amyloid lesions in middle-aged subjects with trisomy 21 is related to the increased dosage of the β APP gene, which has been localized to the mid-portion of the long arm of chromosome 21. Examining the brains of trisomic patients dying at various ages should reveal the temporal sequence of AD-type neuropathological changes. Such studies have now shown that diffuse A β P deposits can be detected in Down's subjects as early as the mid-teens and occur commonly in the twenties and thirties [30, 73]. At these ages, few or no mature neuritic plaques, dystrophic glia, or neurofibrillary tangles are observed. Since the brain lesions of older trisomic 21 subjects are indistinguishable from those of AD, it is likely that similar deposition of A β P in diffuse form precedes neuritic and glial alteration in AD as well. Indeed, both the cerebral cortex and some clinically uninvolved regions of AD brain show myriad diffuse plaques with little or no neuritic/neuronal or glial changes, even at the end stage of the disease.

SP and many cerebral microvessels in aged monkeys and dogs were shown to contain A β P immunochemically identical to that in AD and Down's syndrome [112]. Recently, cloning of the homologs of all three major human β APP isoforms in cynomolgus monkey has revealed that β APP₆₉₅ is completely homologous to the human polypeptide, and β APP₇₅₁ and β APP₇₇₀ contain only 1 and 4 amino acid substitutions, respectively [97]. The complete homology of β APP₆₉₅ between the monkey and human contrasts to the 18 and 22 amino acid substitutions found in the rat and mouse, respectively [115, 146]. Such study on β APP of dogs is not yet performed.

Studies of diffuse plaques in Down's and AD brains indicate that

degenerating neurites and glia are not a prerequisite for A β P deposition. Therefore, the β APP molecules that give rise to A β P originate either from structurally normal neurons or glia or from another source. The frequently abundant A β P deposits found in the wall of meningeal arteries outside of brain tissue suggest that cells intrinsic to the vessel wall (for example, endothelial or smooth muscle cells) or cellular elements of the blood could serve as a source of the β APP molecules that yield vascular A β P. A hematogenous origin for these deposits would be in concert with certain systemic amyloidoses known to derive from circulating precursors, for example, immunoglobulin amyloid in multiple myeloma and primary systemic amyloidosis, AA amyloid in the secondary amyloidosis of chronic inflammatory diseases, and transthyretin amyloid in familial amyloidotic polyneuropathy and senile systemic amyloidosis. Circumstantial evidence that the β -amyloid of SP might also be vessel- or blood-derived comes from the ultrastructural findings of Miyakawa *et al.* [86], which demonstrated a capillary bearing amyloid at its basement membrane in every SP subjected to serial ultrathin sectioning. In support of this hypothesis, occasional deposits of A β P immunoreactivity have been observed in and around microvessels of the dermis, subcutaneous tissue, small and large intestine, and adrenal gland in subjects with AD, familial AD, and Down's syndrome [48]. The chemical nature of the A β P deposits in nonneuronal tissues and their relationship to the much more abundant cerebral deposits remain unclear. However, the detection of extracerebral A β P deposits in and around selected microvessels supports the notion that A β P can be deposited without preceding neuronal/glial degeneration, indeed, in the

absence of local neurons and glia. Furthermore, the discovery of nonneural A β P-immunoreactive deposits strengthens the parallels of β -amyloidosis with the systemic amyloidoses and provides circumstantial support for a vascular or hematogenous origin. The resolution of the origin of cerebral β -amyloid has potentially important therapeutic implications, since a hematogenous origin should be easier to interrupt than an origin from neurons or other intrinsic brain cells. Much further study is needed before the question of whether A β P originates from β APP in neurons, glia, vascular cells, extracellular fluid (blood and cerebrospinal fluid), or from multiple sources can be settled.

CHAPTER I

MODIFIED BIELSCHOWSKY AND IMMUNOHISTOCHEMICAL STUDIES ON SENILE PLAQUES AND CEREBROVASCULAR AMYLOIDOSIS IN THE CENTRAL NERVOUS SYSTEM OF AGED DOGS

I. INTRODUCTION

Senile plaques (SP) and cerebrovascular amyloidosis (CA) are well-known forms of cerebral amyloid deposits in the brains of human beings with Alzheimer's disease (AD) [72, 138]. Amyloid deposits are composed primarily of a 42-43 amino acid fragment, designated amyloid β -protein ($A\beta$ P) of a larger amyloid precursor protein (β APP) [36, 50, 99, 102, 115, 123, 137]. Changes in the transcription, translation or post-translational processing of β APP are likely to play a role in the excessive $A\beta$ P formation in AD [12, 24, 35, 43, 94, 115]. Amyloid deposits may also occur in nondemented aged human beings and other mammals, including nonhuman primates, polar bears and dogs [18, 112, 113, 120, 128, 130, 136, 139, 140].

There are two main hypotheses, the neuronal and vascular, as to the source of β APP. The primary evidence supporting the neuronal origin is the high expression of β APP and/or β APP mRNA in neurons [5, 7, 62]. The vascular hypothesis rests mainly on the deposition of amyloid in the wall of blood vessels in AD and on the intimate association of capillaries with senile plaques [32, 85, 86]. However, the observation that CA is not always associated with AD suggests that the deposition of amyloid in the neuroparenchyma may differ in some ways from that in and around the vascular wall [136]. The resolution of the relationship of amyloid deposits to local elements of the brain tissue, such as neurites, glia and capillaries has important implications for the pathogenesis of them [47].

In the present study, the morphology of SP and the topographic relationship between SP and CA in the brains of aged dogs were

investigated using sensitive techniques for the detection of amyloid deposits. And then, histopathogenesis of SP was discussed.

II. MATERIALS AND METHODS

The brains were obtained from 33 aged dogs which were killed by euthanasia or died of a variety of disorders including heart worm disease, renal failure, and tumor of visceral or genital organs. They consisted of 15 males and 18 females, with an average age of 13.6 years (range 10 to 17 years) (Table 1). Most of the aged dogs showed symptoms known to be characteristic of aging: inactivity, sleepiness and poor mobility. No neurological signs were observed in the dogs examined.

The brains were fixed in 10% neutral buffered formalin, and processed routinely into paraffin wax embedding. Serial sections (6 μ m thick) systematically cut at four levels (frontal lobe, striatum, diencephalon and occipital lobe) (refer Fig. 6) of the left cerebral hemisphere and various levels of the brain stem and cerebellum were stained with hematoxylin and eosin, thioflavin S and modified Bielschowsky stains [152]. Selected sections were also stained with periodic acid-methenamine silver [41], Luxol Fast Blue, Bodian and Congo red stains or processed for immunohistochemistry using antibody directed against A β P (1-24 residues; rabbit polyclonal, kindly provided by Dr. N. Kitaguchi of Life Science Research Laboratories, Asahi Chemical Industry Co., Ltd.) [145]. The antibody was used at a dilution of 1 : 1000. Sections for immunohistochemistry were stained using an avidin-biotin peroxidase complex (ABC) method with diaminobenzidine as chromogen. Before the immunostaining, deparaffinized sections were immersed in formic acid (99%) for 5 min at room temperature to enhance the immunostaining of A β P [58]. Control sections were stained with nonimmune

rabbit serum IgG at a protein concentration equivalent to that of the primary antibody.

The number of SP was counted at a magnification of x100. Observations at the higher magnification (x200) were used to better differentiate plaque types. The incidence of SP was graded from - to +++ by the number of SP on four coronal sections of the cerebral hemisphere: +++; ≥ 1000 , ++; 500-999, +; 1-499, and -; absent. On thioflavin S-stained and immunostained sections, CA was assessed semiquantitatively by division into four grades of severity: -; not observed, +; some lesions confined to meningeal vessels, ++; some lesions in both meningeal and neuroparenchymal vessels, and +++; many lesions in both meninges and neuroparenchyma. The number and distribution of SP and CA were determined on the coronal sections by making plots for these lesions with the aid of the camera lucida method.

Histological examination using thioflavin S and modified Bielschowsky stains, and A β P immunostain for the presence of amyloid deposits was also made to the spinal cord and non-neural tissues including the liver, spleen, kidney, heart, lung, gut, pancreas and skin of the three dogs with the prominent CA and SP formation (Dog Nos. 10, 26 and 33).

III. RESULTS

The brains of the aged dogs showed normal gross-appearance except moderate to severe thickening of the meninges (Fig. 3) and mild dilatation of the ventricles. There appeared to be no difference in the age-related symptoms and gross findings of the central nervous system (CNS) between the dogs with SP and ones without SP. Histological examination of the CNS revealed typical aged changes, such as nerve cell loss, lipofuscin accumulation in neurons, occasional dystrophic axons and presence of corpora amylacea. A β P immunohistochemistry demonstrated SP and CA, these lesions being identically shown by the modified Bielschowsky and/or thioflavin S stains (Fig. 5). No neurofibrillary tangles were identified in neurons.

Most SP were observed in the neocortex of the cerebrum. Their densities were highest in the cingulate and temporal cortices. The lesion spared dorsal aspect of the coronal sections cut at various levels of the cerebral hemispheres. There were occasional SP in the subcortical nuclei, including the caudate nucleus and putamen, and hippocampus. No SP were detected in the cerebellum, brain stem and spinal cord.

Based on the morphological arrangement and relative proportions of amyloid and neuritic components, SP have been classified to several types [101, 121, 142, 148]. In the present study, three types of SP were identified on the basis of the modified Bielschowsky stain: diffuse plaques (diffuse amyloid deposits lacking amyloid cores and apparent neuritic elements), mature plaques (amyloid cores with or without apparent neuritic elements),

and perivascular plaques (dense amyloid deposits closely associated with blood vessels). Thioflavin S stain confirmed the presence of discrete deposits of amyloid that corresponded to the locations of CA and SP with dense amyloid deposits. The relationship between the age and morphological changes is given in Table 1. SP and CA were detected in 15 (45%) and 22 (67%) of 33 dogs examined, respectively. Of the 15 dogs with SP, 13 (87%) were accompanied by CA. No SP subtypes other than diffuse plaques were observed in the brains which had a small number of SP; the brains with advanced CA contained all subtypes of SP (Table 1).

In the dogs with mild CA, the lesions were confined to the meningeal vessels of the dorsal area of the cerebrum and, to a lesser degree, those of the cerebellum. In the dogs with moderate to severe CA, the lesions were observed in the penetrating vessels in the cerebral cortex and all over the meninges except the ventral area of the brain (Fig. 6). No CA was observed in neuroparenchyma of the other areas of the CNS. SP, which were confined to the cerebrum, were predominant in the medial and lateral areas of the cerebral hemisphere, especially in the cingulate and temporal cortices (Fig. 6).

Diffuse plaques, which predominated in the plaque subtypes, were distributed throughout the cortical layers, especially in cellular layers with no topographic relationship to CA (Figs. 4 and 6). Mature and perivascular plaques, both of which contained compact amyloid deposits, showed close topographic relationship to CA (Figs. 6 and 7). These plaques often occurred in the dogs with advanced CA (Table 1). Diffuse plaques of larger size, being as large as 200 μ m in diameter, often contained capillaries in addition to a

Table I. Pathological data of the dogs examined

Dog No.	Age (years)	Sex	SP ^{a)} subtype			CA ^{b)}
			Diffuse	Mature	Perivascular	
1	10	M	— ^{c)}	—	—	— ^{d)}
2	10	M	—	—	—	+
3	10	F	—	—	—	—
4	11	M	—	—	—	—
5	11	F	+	—	—	+
6	11	M	—	—	—	—
7	11	M	+	+	—	+
8	12	F	—	—	—	—
9	12	F	—	—	—	+
10	12	F	+	+	+	+
11	12	M	+	—	—	—
12	12	F	+	—	—	+
13	12	F	—	—	—	—
14	13	M	—	—	—	—
15	13	F	—	—	—	—
16	13	M	+	—	—	+
17	13	F	—	—	—	+
18	13	F	—	—	—	+
19	13	M	—	—	—	+
20	13	F	+	—	—	+
21	14	F	—	—	—	—
22	14	M	—	—	—	+
23	14	F	+	—	—	+
24	14	F	+	—	—	+
25	15	M	+	—	—	—
26	15	F	+	+	+	+
27	15	M	—	—	—	+
28	16	M	+	—	+	+
29	16	F	+	—	—	+
30	17	F	—	—	—	+
31	17	M	+	—	—	+
32	17	M	—	—	—	+
33	17	F	+	+	+	+

a) SP: Senile plaque.

b) CA: Cerebrovascular amyloidosis.

c) Number/four coronal sections of the cerebral hemisphere:
—; absent, +; 1-499, +; 500-999, ++; ≥1000.

d) —; not observed, +; some lesions confined to meningeal vessels, ++; some lesions in both meningeal and neuroparenchymal vessels, +++; many lesions in both meninges and neuroparenchyma.

few neurons and glial cells (Fig. 8). Whereas, those of smaller size ($\leq 50 \mu\text{m}$) seldom involved capillaries (Fig. 9). A β P immunohistochemistry demonstrated amyloid deposits on the periphery of neurons with a normal appearance in the cerebral cortex (Fig. 10). No amyloid deposits were detected in the non-neural tissues examined.

IV. DISCUSSION

This study demonstrated that all of the SP and CA, both of which were reacted with A β P antiserum, corresponded to those shown by the modified Bielschowsky and/or thioflavin S stains. Studies of aged human brains have also shown this correspondence [44, 78]. Because of restrictions such as limited availability of the antibody and required skills, few A β P immunohistochemistry has been employed on brain tissues of domestic animals. Therefore, the findings shown in this study may be helpful to the comparative study of aged animals.

Amyloid deposits have been demonstrated in SP and cerebral vessels in aged human beings, nonhuman primates and several other mammals [53, 112, 136, 148]. In the present study, using the sensitive methods for the detection of amyloid deposits, such as A β P immunohistochemistry, modified Bielschowsky and thioflavin S stains [112, 144, 147], SP and CA were demonstrated in 45% and 67% of the aged dogs, respectively. The incidence of amyloid deposits in the aged dogs is comparable to that of nondemented aged human beings [90]. Using a thioflavin S stain, Selkoe *et al.* [112] demonstrated CA in all nine 10- to 12-year-old dogs examined. In contrast, no sign of any form of amyloid deposits, SP or CA, was demonstrated in the brains of cats, including three animals over 18 years of age (unpublished data by the present author, A. Shimada). Studies on this possible species difference may be useful in elucidating the mechanism of A β P deposition in the brain.

The immunohistochemical characteristics of brain amyloid deposits in the

present aged dogs, combined with their morphological appearance, suggest a strong similarity to the human type of lesions and make it likely that, as in the human beings, the A β P [34] is the main proteinaceous constituent of the SP and CA. Large numbers of SP have been proposed as a diagnostic criterion for the neuropathological diagnosis of AD [55]. Application of this criterion to the dog brains examined in this study would yield a positive diagnosis in some cases. One point, however, has to be kept in mind: no neurofibrillary tangles were detected in the brains investigated.

Diffuse plaques show A β P-immunoreactivity with little evidence of Congophilic amyloid deposition, whereas other subtypes of SP contain dense amyloid (A β P) deposits which are birefringent and fluorescent [122]. Electron microscopy demonstrated that diffuse plaques contained only a sparse accumulation of amyloid fibrils, which might be below the threshold level that could be detected by thioflavin or Congo red staining at the light microscope level [149, 150]. Of the subtypes of SP found in this study, diffuse plaques were predominant. This finding coincides with previous observations on the brains of AD-affected and nondemented human beings [121, 131, 148]. Diffuse plaques have been suggested to be very early SP based on the analysis of the Down's syndrome 'model' [3, 30, 44, 74]; this also appears to be the case in the present aged dogs because no SP subtypes other than diffuse plaques were observed in the dogs with a small number of SP. The detailed mechanism of formation of amyloid fibrils in SP and cerebrovascular walls is still unknown.

There have been controversies as to the relationship between SP and CA because the two kinds of lesions do not invariably coexist [75, 87, 136]. In

the aged dogs, both SP and CA were mostly confined to the cerebrum. This distribution pattern is comparable to that of nondemented aged human beings and monkeys [90, 136]. In addition, the severity of both lesions appeared to increase with advancing age, indicating that the lesions are age-related. Furthermore, of the dogs with SP, all but two were accompanied by various degrees of CA. Therefore, it is conceivable that SP and CA share some triggering causes and mechanisms which lead to amyloid deposition.

As to the topographic relationship between SP and CA, a close association between capillaries with CA and all subtypes of SP except diffuse plaques were demonstrated in the present study. This finding agrees with the observations on human beings and monkeys [85, 136] and supports the concept of the vasculature origin, namely circulating source for amyloid in these subtypes of SP [52, 85, 110]. Expression of β APP and deposition of A β P in non-neural tissues further support this hypothesis [48, 114]. However, no sign of amyloid deposits was detected in the non-neural tissues examined in the present dogs. This finding has two implications. First, the negative staining is attributable to the method used in this study, such as antiserum and process of immunohistochemistry. Second, the aged dogs examined had actually no amyloid deposits in the non-neural tissues examined. This is also likely because normal aged human beings showed only equivocal results in the skin in A β P immunohistochemistry, though definite and specific staining was observed in the skin and intestine of AD patients [48].

Cell bodies of neurons and/or glia were almost always involved in diffuse plaques. In addition, diffuse plaques of smaller size, those being regarded as an initial stage of SP, often showed no association with capillaries.

Similar finding was also observed in human beings [4, 101, 148]. These findings, combined with the observation that amyloid deposits were demonstrated on the periphery of neurons in the brains of the dogs, support the hypothesis of a neuronal or glial cell origin of plaque amyloid [5, 16, 35, 40, 78, 81, 116].

Presence of two distinct modes of amyloid deposition in AD has been suggested based on the biochemical evidences, including differences in the C-terminal sequence, N-terminal blockage, and solubility of the A β P between CA and SP [83, 100]. Taken together, results of the present study suggest that different mechanisms may also be involved in the pathogenesis of diffuse plaques, other subtypes of SP and CA in the brain of aged dogs.

V. SUMMARY

The morphology of senile plaques (SP) and the distributions of SP and cerebrovascular amyloidosis (CA) were studied by employing thioflavin S and modified Bielschowsky stains, and A β P immunohistochemistry on serial sections of the brains of aged dogs older than 10 years of age. The aged dogs developed SP which were morphologically and immunohistochemically similar to those occurring in aged human beings. Based on the morphological characteristics demonstrated by the modified Bielschowsky stain, SP in the brains of the dogs were grouped into three types; diffuse, mature and perivascular. Out of them diffuse plaques were predominant. Most SP were observed in the cerebral cortex. Their densities were highest in the cingulate and temporal cortices. There were occasional SP in the subcortical nuclei, including the caudate nucleus and putamen, and hippocampus. Mature and perivascular plaques, both of which contained compact amyloid deposits, always showed close topographic relationship to blood vessels with CA. Whereas, most of diffuse plaques showed no topographic relationship to CA. Cell bodies of neurons and/or glia were almost always involved in diffuse plaques. In addition, A β P immunohistochemistry demonstrated amyloid deposits on the periphery of occasional neurons. These findings suggested that different mechanisms may be involved in the development and evolution of the different subtypes of SP in the brain of aged dogs.

CHAPTER II

IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDIES

ON AGE-RELATED ASTROCYTIC GLIOSIS

IN THE CENTRAL NERVOUS SYSTEM OF DOGS

I. INTRODUCTION

Astrocytic gliosis (AG) in the brain has been reported in aged normal human beings and in patients with Alzheimer's disease (AD) and senile dementia of the Alzheimer type [6, 23, 42, 69, 106, 127]. The phenomenon is believed to be a reaction to the loss of neurons and dendrites [6, 108]. AG as an aged change was also reported in the brains of animals, including rats, macaque monkeys and mice [2, 20, 29, 65, 68, 91]. No consistent results were shown in these studies of animals and this discrepancy was attributed to differing methods used, such as the number of animals, staining methods and counting techniques [42]. In addition, few studies on age-related ultrastructural changes in glial cells have been performed in both human beings and animals [20].

In this study, electron microscopy and immunohistochemistry for glial fibrillary acidic protein (GFAP), which is the primary constituent of glial intermediate filaments and is considered to be exclusively localized in astrocytes [8], were employed. Thus, as Mandybur *et al.* [71] stated, the term 'astrocytic gliosis' in this study refers to 'increase in GFAP-positive astrocytes (GFAP-PA)' or 'increased GFAP immunoreactivity' because not all astrocytic glia is positively stained by the GFAP immunohistochemistry. The aim of the present study is to report the pattern of age-related AG in the central nervous system (CNS) of dogs.

II. MATERIALS AND METHODS

Twenty-six dogs, 16 females and 10 males, were used in the present study (Table II). Seventeen were old dogs, ranging in age from 11 to 18 years (mean 14.5 years), and nine were young controls, 2 months to 4 years of age (Dog Nos. 10-15, 17-20, 22-25 of this Chapter correspond to Dog Nos. 7, 10, 8, 11, 16, 20, 24, 23, 27, 26, 29, 30, 32 and 33 of Chapter I, respectively). No signs of neurological disorders were observed in the dogs. The dogs were killed by euthanasia or died of a variety of disorders, including heart worm disease, renal failure, and tumor of visceral or genital organs. Four aged dogs (Dog Nos. 10, 13, 17 and 26) and four young controls (Dog Nos. 2, 3, 5 and 6) were anaesthetized with pentobarbital and perfused through the heart with 2 liters of 4% paraformaldehyde in phosphate buffer (pH 7.2). During the perfusion, the descending aorta was clamped with a hemostat to save fixative. The dogs were decapitated and the exposed brain was re-immersed in perfusate for overnight at 4°C.

Complete autopsies were performed on these dogs. For light microscopy, the CNS, including the cerebrum, cerebellum, brain stem and spinal cord was fixed in 10% neutral buffered formalin at autopsy. A 5-mm-thick coronal slice cut at various levels of the CNS was dissected. Both left and right cerebral hemispheres of three aged dogs and left cerebral hemispheres of the others were used in this study. The tissue block was then routinely processed and embedded in paraffin. Serial sections (6 μ m) were stained by hematoxylin and eosin (HE), Luxol Fast Blue, Nissl and Bodian. Serial sections were also processed for immunohistochemistry using the

peroxidase-antiperoxidase (PAP) method with diaminobenzidine as chromogen. The primary antibody was anti-human GFAP (rabbit polyclonal, Medac GmbH, West Germany). The antibody was used at a dilution of 1 : 200. The sections were counterstained with hematoxylin. Method specificity was evaluated by substituting normal rabbit serum with the primary antibody.

For electron microscopy small tissue blocks (1 mm^3) were taken from the cerebral cortex of the perfused dogs. These tissue blocks were post-fixed in phosphate-buffered 1% OsO_4 for 1 hr, dehydrated in ascending concentrations of ethanol and embedded in Epon. Semithin sections ($1\text{ }\mu\text{m}$) were stained with toluidine blue and were examined light microscopically. Thin sections were stained with lead citrate and uranyl acetate, and were examined with a JEM-100CXII.

At autopsy, tissue samples were also taken from a variety of organs for routine light microscopic studies and fixed in 10% neutral buffered formalin. Paraffin sections ($4\text{ }\mu\text{m}$) were made and stained with HE.

III. RESULTS

Gross and microscopic examinations of the CNS of the aged dogs revealed neither changes indicating ischemia nor signs of neurological disorders other than typical aged changes, such as thickening of the meninges, mild dilatation of the ventricles, occasional dystrophic axons, lipofuscin accumulation in neurons, and nerve cell loss (Fig. 11). No obvious gross atrophy was observed in the brains examined.

An increase in GFAP-PA in the aged dogs, as compared to the young controls, was the most constant histological feature observed in the GFAP immunohistochemistry (Table II). No correlation was noted between the intensity of AG and clinical findings, such as breed, sex and diseases which affected the dogs. AG consisted of hypertrophic astrocytes being characterized by either intense GFAP immunostaining in light microscopy (Fig. 12) or by thick bundles of intermediate filaments in electron microscopy (Fig. 13). These GFAP-PA were observed both interstitially and perivascularly (Fig. 14). There were occasional GFAP-PA surrounding nerve cell bodies (Fig. 15). In all 17 aged dogs, moderate to severe AG was observed with a consistent distribution pattern in the CNS. The change was symmetrical in the dogs whose CNS, including left and right cerebral hemispheres were examined.

A marked increase in GFAP-PA was seen in the subcortical and deep white matters in the cerebrum (Fig. 16a), especially at the cortico-medullary junction (Fig. 16b), corpus callosum, internal capsule, subcortical nuclei in the cerebrum, central nuclei in the cerebellum, various nuclei in the brain

Table II. Pathological data of the dogs examined

Dog No.	Age	Sex	Breed	AG ^{a)}
1	2 m ^{b)}	F	Mongrel	± ^{f)}
2	2 m	M	Mongrel	±
3	3 m	M	Mongrel	±
4	6 m	F	Mongrel	±
5	8 m	F	Mongrel	±
6	12 m	F	Mongrel	±
7	12 m	M	Mongrel	±
8	12 m	F	Mongrel	±
9	4 y ^{c)}	M	Mongrel	±
10	11 y	M	Y ^{d)} .terrier	+
11	12 y	F	Poodle	+
12	12 y	F	Mongrel	+
13	12 y	M	Mongrel	+
14	13 y	M	Mongrel	+
15	13 y	F	Mongrel	+
16	13 y	F	Maltese	+
17	14 y	F	Maltese	+
18	14 y	F	Mongrel	+
19	15 y	M	Mongrel	+
20	15 y	F	Shiba	+
21	16 y	F	Shiba	+
22	17 y	F	Mongrel	+
23	17 y	F	Mongrel	+
24	17 y	M	M ^{e)} .schnauzer	+
25	17 y	F	Mongrel	+
26	18 y	M	Mongrel	+

a) AG: Astrocytic gliosis.

b) m: months.

c) y: years.

d) Y: Yorkshire.

e) M: Miniature.

f) The neuropathological lesion is graded: -; absent, ±; slight, +; mild, ++; moderate, +++; marked.

stem, and gray matter of the spinal cord (Fig. 16c). A prominent increase of them was also seen in the cerebral and cerebellar cortices (Figs. 12 and 16d) and thalamus (Fig. 16e).

A moderate increase was observed in the hippocampus (Fig. 16f), white matter of the cerebellum and spinal cord, reticular formation, optic tract and subependymal areas (Fig. 16g) and periaqueductal zone throughout the CNS.

Young control dogs showed minimum numbers of GFAP-PA in the CNS; the molecular layer of the cerebrum and white matter throughout the CNS consistently showed a slight increase in GFAP-PA (Fig. 16h).

Light microscopy failed to demonstrate no obvious degenerative changes of the neural architecture in the area with prominent AG (Fig. 17). Electron microscopy revealed a variety of degenerative profiles of neural components, such as dark axon terminals, swollen synapse-like vesicles with dense contents, and heterogeneous dense bodies in dendrites in the cerebral cortex of the aged dogs (Fig. 18). These changes were often located in the vicinity of hypertrophic astrocytic processes (Fig. 19). Few such profiles were observed in the young controls examined (Fig. 20).

IV. DISCUSSION

In contrast to slight staining of GFAP in the young dogs, prominent GFAP-immunoreactivity was shown in astrocytes of the CNS of the aged dogs, indicating that age-related AG also occurs in dogs. There are several reports which demonstrated astrocytic hypertrophy in the aging CNS [2, 9, 29, 68]. It was not certain whether there was an increase in the volume of GFAP per astrocyte or just an increase in astrocyte size [9]. In the present study, hypertrophic astrocytes with either intense GFAP immunostaining in light microscopy or thick bundles of intermediate filaments in electron microscopy were frequently observed. These findings may suggest that the observed age-related hypertrophy of GFAP-PA be related to an increase in the volume of GFAP per astrocyte. This morphological finding appears to correspond to the results from the biochemical studies of the brains of the experimental animals, in which age-related increase in the concentration of either GFAP or GFAP mRNA was demonstrated [38, 89].

The brains of the aged dogs showed normal gross-appearance except mild dilatation of the ventricles and thickening of the meninges. There appeared to be no difference in the thickness of the cerebral cortex between the aged dogs and the young controls. Similarly, no statistical difference as to the cortical thickness was noted between the AD group and the controls in the morphometric studies of human brains [69, 106, 126]. Therefore, the increase of GFAP-PA could not be related to the shrinkage or condensation of the tissue [69, 106].

Studies of the brains of aged rats demonstrated synaptic deterioration

and functional changes in synaptic transmission mechanisms [64, 133]. Age-related alterations in neural components, including dendrites and synapses have been considered as the stimulus for AG in aging [42, 66]. In this ultrastructural study, degenerating profiles of presynaptic axon terminals and dendrites, some of which were located in the vicinity of reactive astrocytic processes, were frequently observed. These findings are in accord with those of aged human beings [108].

The neocortical neuropil of AD patients is characterized by extensive synaptic pathology, such as synaptic loss [17, 107], dilatation of presynaptic terminals [17, 107] and abnormal synapses [37]. These changes are accompanied by focal amyloid deposition [78, 122], dystrophic neurite formation [31, 125], glial cell hypertrophy in plaques [22], and massive accumulation of abnormal cytoskeletal filaments in dendrites [11]. Recent studies in aged monkeys [15] and Down's syndrome cases [30] have suggested that abnormal neurites and synapse alterations scattered in neuropil precede amyloid deposition. In this regard, it is important to establish the origin and nature of the dystrophic neurites and their relation to synaptic pathology in the cerebral cortex. Electron microscopic studies in AD and aged animals [140, 141] have supported various theories about the origin of the abnormal neurites, including (1) Wallerian degeneration, (2) 'dying back' axonal degeneration, (3) 'dying back' of dendrites [31], and (4) a primary pathological process in the presynaptic terminal. Using synaptophysin-immunoelectron microscopy, Masliah *et al.* [77] suggested that the dystrophic neurites in the AD neocortex are distorted presynaptic terminal. Changes of the neural components in the present study may, at

least in part, originate from the presynaptic terminal, because of the similarity of these changes in the conventional electron microscopy to those in the AD neocortex.

Recent studies suggested that astrocytes have a phagocytic function, which may contribute to remove degenerative debris in the CNS [2, 88]. However, no dense bodies suggestive of ingestion of degenerative neural components were observed even in the cytoplasm of reactive astrocytes occurring among the profiles of degenerating axon terminals. Recent studies demonstrated evidence of new roles for astrocytes, including neurotransmitter uptake [10, 56], synthesis and secretion of trophic factors [27, 28, 54, 98], targets for hormones and immune system cytokines [26, 67, 134], and regulation of cerebral blood flow [96]. Thus, astrocytes may play important roles in the regulation of the microenvironment in the CNS. Therefore, the possibility exists that astrocytic hypertrophy represents a primary effect of aging rather than a mere secondary response to degenerative events in the CNS [89].

As to the patterns of age-related AG, increased numbers of GFAP-PA have been reported in the cerebral cortex and hippocampus in AD and normal aging brains of human beings [6, 23, 42, 69, 106, 127]. The pattern of AG observed in the present study is similar to that demonstrated in the recent study of the AD and aging cerebrums, in which extensive AG was observed in the deep cerebral white matter, cortico-medullary junction and subcortical gray matter in addition to the cerebral cortex [6]. Using GFAP immunohistochemistry, Mandybur *et al.* [71] systematically examined the brains of old nude mice, in which an increase in the number of GFAP-PA and

their hypertrophy were also observed in a pattern similar to that found in the dogs except no evidence of AG in the cerebral and cerebellar cortices in the nude mice. This discrepancy may be attributable to the species difference in neuroanatomical and functional specificity of the CNS between the two species.

In animals, occurrence of age-related specific changes, such as senile plaques and cerebrovascular amyloidosis is restricted to some species and in small quantity [71]. In Chapter I, both two changes, which were morphologically and immunohistochemically similar to those of human beings, were demonstrated to occur in the brains of aged dogs with a high incidence. In addition, the present study demonstrated the occurrence of age-related AG with a consistent pattern similar to that of human beings. It thus appears that aged dogs may be suitable as an animal model for the study of the mechanisms involved in CNS aging of human beings.

V. SUMMARY

The pattern of astrocytic gliosis (AG) was examined in 26 2-month-old to 18-year-old dogs using glial fibrillary acidic protein (GFAP) immunohistochemistry and electron microscopy. Coronal sections from various levels of the central nervous system (CNS) were stained with hematoxylin and eosin, Luxol Fast Blue, Nissl, and Bodian in addition to GFAP immunohistochemistry. A consistent pattern of age-related AG was observed in the dogs; the pattern was similar to that of aged human beings. The white matter, cortico-medullary junction, and subcortical nuclei in the cerebrum, central nuclei in the cerebellum, various nuclei in the brain stem, and grey matter of the spinal cord showed even and intense GFAP staining. AG was also prominent in the cerebral and cerebellar cortices and thalamus. Moderate AG was observed in the hippocampus and white matter of the cerebellum and spinal cord. Electron microscopy demonstrated increased numbers of profiles of degenerative neural components in the vicinity of hypertrophic astrocytes in the cerebral cortex of the aged dogs. Moderate to severe AG was consistently shown in the CNS of the aged dogs. In contrast, young normal dogs showed minimum numbers of GFAP-positive astrocytes in the CNS. These findings suggest that the observed AG in the CNS of the dogs may be a morphological expression of aging.

CHAPTER III

THE TOPOGRAPHIC RELATIONSHIPS BETWEEN ASTROCYTIC GLIOSIS AND SENILE PLAQUES, AND ASTROCYTIC GLIOSIS AND CEREBROVASCULAR AMYLOIDOSIS IN THE CEREBRAL CORTEX OF AGED DOGS

I. INTRODUCTION

Astrocytic gliosis (AG) as an aged change was demonstrated in the brains of dogs in Chapter II. AG has been believed to be a reaction to the loss of neural components [6, 106]. However, recent studies of the brains from patients affected with Alzheimer's disease (AD) and senile dementia of the Alzheimer type (SDAT) suggested the possible association of astrocytes in the process of senile plaque (SP) formation [6, 95, 129, 150, 151]. Furthermore, following the observation of β -amyloid precursor protein (β APP) in reactive astrocytes in the experimentally induced lesions of the brains of rats, Siman *et al.* [116] suggested that astrocytes may be a source of amyloid β -protein ($A\beta$ P) for SP and cerebrovascular amyloidosis (CA). In Chapter I, SP and CA were demonstrated in the brains of aged dogs, both of which were morphologically and immunohistochemically similar to those occurring in aged humans. In the present study, the topographic relationships between AG and SP, and AG and CA in the cerebral cortex of aged dogs were examined and the possibility of the astrocytic involvement in the evolution of SP was discussed.

II. MATERIALS AND METHODS

Six dogs, three females and three males, ranging in age from 11 to 18 years, were used in this study (Dog Nos. 1-6 of this Chapter correspond to Dog Nos. 7, 11, 24, 31, and 33 of Chapter I, and Dog No. 26 of Chapter II, respectively). All the dogs were demonstrated to have prominent CA and SP formation. The dogs had no history of neurological disorders. Four of the 6 dogs were anaesthetized with sodium pentobarbital and perfused through the heart with 2 liters of 4% paraformaldehyde in phosphate buffer (pH 7.2). During the perfusion, the descending aorta was clamped with a hemostat to save fixative. The dogs were decapitated and the exposed brain was re-immersed in perfusate for overnight at 4°C. The left cerebral hemisphere was used in this study.

For light microscopy a 5-mm-thick coronal slice cut at a level of the optic chiasma was dissected from the left hemisphere. The slice contained the cingulate and temporal cortices, where the highest density of SP was observed in the previous study (see Chapter I). The tissue block was then routinely processed and embedded in paraffin. Serial sections (6 μ m) were stained with hematoxylin and eosin (HE), Nissl, Bodian, modified Bielschowsky [152] and thioflavin S. In the previous study on the brain amyloid deposits in aged dogs, SP and CA were well shown by the latter two stains (see Chapter I). Serial sections were also processed for immunohistochemistry using the peroxidase-antiperoxidase (PAP) method with diaminobenzidine as chromogen. The primary antibody was anti-human GFAP (rabbit polyclonal, Medac GmbH, West Germany). The sections were

counterstained with hematoxylin. Method specificity was evaluated by substituting normal rabbit serum with the primary antibody.

For electron microscopy small tissue blocks (1 mm^3) were taken from the cerebral cortex of the perfused dogs. These tissue blocks were post-fixed in phosphate-buffered 1% OsO_4 for 1 hr, dehydrated in ascending concentrations of ethanol and embedded in Epon. Semithin sections ($1\text{ }\mu\text{m}$) were stained with toluidine blue and were examined light microscopically. Thin sections were stained with lead citrate and uranyl acetate, and were examined in a JEM-100CXII.

At autopsy, tissue samples were taken from a variety of organs for routine light microscopic studies and fixed in 10% neutral buffered formalin. Paraffin sections ($4\text{ }\mu\text{m}$) were made and stained with HE.

III. RESULTS

The brains of the aged dogs showed normal gross-appearance except moderate to severe thickening of the meninges. The dogs were affected with a variety of disorders, including heart worm disease and tumor of visceral or genital organs.

The cerebral cortex of the aged dogs showed diffuse AG, in which prominent astrocytic reaction was shown around vessels irrespective of the intensity of amyloid deposition in the involved vessels (Fig. 21). In addition, such pattern of AG was consistent throughout the cerebral cortex, where CA was not always accompanied. As shown in the previous study, the majority of SP observed in the aged dogs were of diffuse type (see Chapter I). Diffuse AG was consistently observed throughout the cortex regardless of the intensity of SP formation in these lesions (Fig. 22). Serial sections stained with GFAP and modified Bielschowsky showed no sign of GFAP-positive astrocytes (GFAP-PA) surrounding diffuse SP; many mature SP were demonstrated to contain processes of GFAP-PA (Fig. 23). Electron microscopy frequently showed normal-looking capillaries wrapped with hypertrophic processes of astrocytes (Fig. 24).

IV. DISCUSSION

The findings obtained here showed that diffuse AG as well as perivascular AG appears not to be associated with amyloid deposits in vessels. Perivascular gliosis was also reported in normal aging and AD-affected human beings, and aged mice [6, 69-71]. It has been suggested that perivascular gliosis may be associated with either leakage of blood derived substances or thickening of the vascular wall, which may induce an impairment of exchange of oxygen and metabolites [69, 84]. In the present study, however, there were no signs of vascular pathology, including hemorrhage and ischemic changes. Thus, the cause of age-related perivascular AG is not fully understood at the present time.

The reactive astrocytes around SP have been reported in both humans and animals, although the significance of the relationship between them is still uncertain [6, 22, 23, 70, 106, 136]. Most of the astrocyte-associated SP observed in these studies might have been mature SP, because conventional staining methods used in the previous studies could not have demonstrated diffuse SP, which contain no compact amyloid deposits.

Diffuse SP can be visualized when they are stained with A β P immunohistochemistry or improved silver staining methods (see Chapter I).

There are reports which suggest that astrocytes react simply to SP [22, 23]. Recent studies revealed that astrocytes express α_1 -antichymotrypsin (ACT) mRNA and β APP in the AD-affected brains of humans and the injured brains of mice, respectively [95, 116]. Both ACT and β APP are considered to be associated with the pathogenesis of the core amyloid deposits in SP [1, 50,

57]. In addition, using A β P immunoelectron microscopy Yamaguchi *et al.* [150, 151] demonstrated astrocytic processes surrounding amyloid fibrils in diffuse SP and subpial A β P deposits in the AD-affected and SDAT-affected brains, respectively. Furthermore, acidic fibroblast growth factor, which has potent neurotrophic actions, was demonstrated in astrocytes surrounding SP in AD cases [129]. These findings may imply that astrocytes are involved in the evolution of SP. In the present study, mature plaques accompanied by a substantial number of GFAP-PA were often observed. In contrast, no obvious accumulation of GFAP-PA was detected around diffuse plaques. This may support the hypothesis that diffuse plaques are of early form of SP [150], because astrocytes respond to brain injuries in a timely fashion. Nevertheless, the possibility that astrocytes are associated with diffuse SP could exist because not all astrocytes necessarily reveal themselves by GFAP staining method [71]. Whether astrocytes react simply to degenerative neuritic components and/or deposited amyloid in neuropil as suggested by Mandybur [70] or actually participate in the process of SP formation should not be determined until the fine structural as well as physiological relationships between SP and astrocytes are elucidated.

V. SUMMARY

The topographic relationships between astrocytic gliosis (AG) and senile plaques (SP), and AG and cerebrovascular amyloidosis (CA) were studied in the cerebral cortex of six aged dogs using glial fibrillary acidic protein (GFAP) immunohistochemistry and electron microscopy. Coronal sections containing the cingulate and temporal cortices were stained with hematoxylin and eosin, Nissl, Bodian, modified Bielschowsky and thioflavin S in addition to GFAP immunohistochemistry. The cerebral cortex of the aged dogs showed diffuse AG, with which prominent astrocytic reaction around vessels was associated irrespective of the intensity of amyloid deposition in the involved vessels. Such pattern of AG was consistent throughout the cerebral cortex, where CA was not always accompanied. In addition, there was no difference in the pattern of AG between the area with SP and the area without SP, except occasional involvement of the processes of GFAP-positive astrocytes (GFAP-PA) in mature SP in the former area. It appears that an increase in GFAP-PA may not necessarily occur secondary to SP formation and CA.

CONCLUSION

1. The aged dogs developed senile plaques (SP) which were morphologically and immunohistochemically similar to those occurring in normal aged human beings and patients with Alzheimer's disease (AD). Based on the morphological characteristics demonstrated by the modified Bielschowsky stain, SP in the brains of the dogs were grouped into three types; diffuse, mature and perivascular plaques, of which diffuse plaques were predominant.
2. The broad similarities in staining properties between the SP of human beings and those of the dogs suggest a common mode of the formation and the evolution of the structure in these different species.
3. Most SP were observed in the cerebral cortex. Their densities were highest in the cingulate and temporal cortices. There were occasional SP in the subcortical nuclei, including the caudate nucleus and putamen, and hippocampus.
4. Mature and perivascular plaques, both of which contained compact amyloid deposits, always showed a close topographic relationship to blood vessels with cerebrovascular amyloidosis (CA). Whereas, most of the diffuse plaques showed no topographic relationship to CA. Cell bodies of neurons and/or glia were almost always involved in diffuse plaques. In addition, β -protein immunohistochemistry demonstrated

amyloid deposits on the periphery of occasional neurons. These findings suggest that different mechanisms may be involved in the development and evolution of the different subtypes of SP in the brain of aged dogs.

5. Age-related astrocytic gliosis (AG) was observed in the dogs; the pattern was similar to that of aged human beings. The white matter, cortico-medullary junction, and subcortical nuclei in the cerebrum, central nuclei in the cerebellum, various nuclei in the brain stem, and grey matter of the spinal cord showed even and intense GFAP staining. AG was also prominent in the cerebral and cerebellar cortices and thalamus. Moderate AG was observed in the hippocampus and white matter of the cerebellum and spinal cord.
6. Electron microscopy demonstrated increased numbers of profiles of degenerative neural components in the vicinity of hypertrophic astrocytes in the cerebral cortex of the aged dogs. These findings imply that synaptic pathology may, like aged human beings, be also involved in the course of central nervous system (CNS) aging of dogs.
7. Moderate to severe AG was consistently shown in the CNS of the aged dogs. In contrast, young normal dogs showed minimum numbers of GFAP-positive astrocytes (GFAP-PA) in the CNS. These findings suggest that the observed AG in the CNS of the dogs may be a morphological expression of aging.

8. The cerebral cortex of the aged dogs showed diffuse AG, with which prominent astrocytic reactions around vessels were associated irrespective of the intensity of amyloid deposition in the involved vessels. Such pattern of AG was consistent throughout the cerebral cortex, where CA was not always accompanied. In addition, there was no difference in the pattern of AG between the area with SP and the area without SP, except occasional involvement of the processes of GFAP-PA in mature SP in the former area. It appears that an increase of GFAP-PA may not necessarily occur secondary to SP formation and CA.
9. Based on these findings, it is concluded that aged dogs may be suitable as an animal model for the study of the mechanisms involved in CNS aging of human beings.

ACKNOWLEDGEMENTS

This study was performed using the facilities of the Department of Veterinary Pathology, Faculty of Agriculture at Tottori University. I would like to thank the following people whose contribution to this thesis is sincerely appreciated:

Professor C. Itakura of the Department of Comparative Pathology, Faculty of Veterinary Medicine at Hokkaido University, and professor T. Umemura of the Department of Veterinary Pathology, Faculty of Agriculture at Tottori University for providing me with the opportunity to undertake this project and their continuous help and encouragement throughout the study.

Professor M. Sugimura and professor Y. Maede of Faculty of Veterinary Medicine, and professor K. Nagashima of School of Medicine at Hokkaido University for advice and help in the preparation of this manuscript.

Professor M. Uehara and assistant professor H. Kitagawa of the Department of Veterinary Anatomy, Faculty of Agriculture at Tottori University, and professor E. Ohama and assistant professor K. Takada of the Division of Neuropathology, Institute of Neurological Science, School of Medicine at Tottori University for advice and help in immunohistochemistry and electron microscopy of the study.

I also wish to thank Dr. N. Kitaguchi of Life Science Research Laboratories,

Asahi Chemical Industry Co. Ltd. for his kind gift of β -protein polyclonal antibody.

Ms. E. Kawahara of Faculty of Agriculture at Tottori University for her advice and technical assistance in electron microscopy.

I am indebted to the following for providing aged dogs:

Dr. H. Eguchi

Dr. E. Kobayashi

Dr. N. Machida

Dr. K. Yoshida

Drs. K. & Y. Sawashima

Dr. S. Mizushima

Dr. M. Yao

Dr. A. Haruna

Dr. J. Taguchi

Dr. T. Kono

Dr. N. Ichida

Dr. K. Koide

To the students of the Department of Veterinary Pathology, Faculty of Agriculture at Tottori University, in particular Mr. M. Kuwamura and Mr. K. Shiraki for providing assistance in histology.

I would also like to express thankfulness to my family for their continuous encouragement, patience and help.

To the Ministry of Education of Japan for the provision of a Scientific Research Grant.

REFERENCES

1. Abraham, C.R., Selkoe, D.J., and Potter, H. 1988. Immunochemical identification of the serine protease inhibitor, α_1 -antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. Cell 52: 487-501.
2. Adams, I. and Jones, D.G. 1983. Synaptic remodelling and astrocytic hypertrophy in rat cerebral cortex from early to late adulthood. Neurobiol. Aging 3: 179-186.
3. Allsop, D., Haga, S., Haga, C., Ikeda, S., Mann, D.M.A., and Ishii, T. 1989. Early senile plaques in Down's syndrome brains show a close relationship with cell bodies of neurons. Neuropathol. Appl. Neurobiol. 15: 531-542.
4. Allsop, D., Landon, M., Kidd, M., Lowe, J.S., Reynolds, G.P., and Gardner, A. 1986. Monoclonal antibodies raised against a subsequence of senile plaque core protein react with plaque cores, plaque periphery and cerebrovascular amyloid in Alzheimer's disease. Neurosci. Lett. 68: 252-256.
5. Bahmanyar, S., Higgins, G.A., Goldgaber, D., Lewis, D.A., Morrison, J.H., Wilson, M.C., Shanker, S.K., and Gajdusek, D.C. 1987. Localization of amyloid β protein messenger RNA in brains from patients with Alzheimer's disease. Science 237: 77-80.
6. Beach, T.G., Walker, R., and McGeer, E.G. 1989. Patterns of gliosis in Alzheimer's disease and aging cerebrum. Glia 2: 420-436.
7. Bendotti, C., Forloni, G.L., Morgan, R.A., O'Hara, B.F., Oster-Granite, M.L., Reeves, R.H., Gearhart, J.D., and Coyle, J.T. 1988. Neuroanatomical

- localization and quantification of amyloid precursor protein mRNA by in situ hybridization in the brains of normal, aneuploid and lesioned mice. Proc. Natl. Acad. Sci. U.S.A. 85: 3628-3632.
8. Bignami, A., Eng, L.F., Dahl, D., and Uyeda, C.T. 1972. Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence. Brain Res. 43: 429-435.
 9. Bjorklund, H., Eriksdotter-Nilsson, M., Dahl, D., Rose, G., Hoffer, B., and Olson, L. 1985. Image analysis of GFA-positive astrocytes from adolescence to senescence. Exp. Brain Res. 58: 163-170.
 10. Bowman, C.L. and Kimelberg, H.K. 1984. Excitatory amino acids directly depolarize rat brain astrocytes in primary culture. Nature (Lond.) 311: 656-659.
 11. Braak, H., Braak, E., Grundke-Iqbal, I., and Iqbal, K. 1986. Occurrence of neuropil threads in the senile brain and in Alzheimer's disease: a third location of paired helical filaments outside the neurofibrillary tangles and neuritic plaques. Neurosci. Lett. 65: 351-355.
 12. Card, J.P., Meade, R.P., and Davis, L.G. 1988. Immunocytochemical localization of the precursor protein for β -amyloid in the rat central nervous system. Neuron 1: 835-846.
 13. Coria, F., Castano, E., Prelli, F., Larrondo-Lillo, M., van Duinen, S., Shelanski, M.L., and Frangione, B. 1988. Isolation and characterization of amyloid P component from Alzheimer's disease and other types of cerebral amyloidosis. Lab. Invest. 58: 454-458.
 14. Cork, L.C., Powers, R.E., Selkoe, D.J., Davies, P., Geyer, J.J., and Price, D.L. 1988. Neurofibrillary tangles and senile plaques in aged bears. J.

Neuropathol. Exp. Neurol. 47: 629-641.

15. Cork, L.C. and Price, D.L. 1990. Relationships of abnormal neuronal processes and amyloid in plaques in aged monkeys. J. Neuropathol. Exp. Neurol. 49: 309.
16. Cras, P., Kawai, M., Siedlak, S., Mulvihill, P., Gambetti, P., Lowery, D., Gonzalez-DeWhitt, P., Greenberg, B., and Perry, G. 1990. Neuronal and microglial involvement in β -amyloid protein deposition in Alzheimer's disease. Am. J. Pathol. 137: 241-246.
17. Davies, C.A., Mann, D.M.A., Sumpter, P.Q., and Yates, P.O. 1987. A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease: correlation with cognitive severity. Ann. Neurol. 27: 457-464.
18. Dayan, A.D. 1970. Quantitative histological studies in the aged human brain. I. senile plaques and neurofibrillary tangles in 'normal' patients. Acta Neuropathol. (Berl.) 16: 85-94.
19. Dayan, A.D. 1971. Comparative neuropathology of ageing. Brain 94: 91-42.
20. de la Roza, C., Cano, J., and Reinoso-Suarez, F. 1985. An electron microscopic study of astroglia and oligodendroglia in the lateral geniculate nucleus of aged rats. Mech. Ageing Dev. 29: 267-281.
21. de Sauvage, F. and Octave, J.-N. 1989. A novel mRNA of the A4 amyloid precursor gene coding for a possibly secreted protein. Science 245: 651-653.
22. Dickson, D.W., Farlo, J., Davies, P., Crystal, H., Fuld, P., and Yen, S.C. 1988. Alzheimer disease. A double immunohistochemical study of senile

- plaques. Am. J. Pathol. 132: 86-101.
23. Duffy, P.E., Rapport, M., and Graf, L. 1980. Glial fibrillary acidic protein and Alzheimer-type senile dementia. Neurology 30: 778-782.
24. Dyrks, T., Weidemann, A., Multhaup, G., Salbaum, J.M., Lemaire, H.-G., Kang, J., Muller-Hill, B., Masters, C.L., and Beyreuther, K. 1988. Identification, transmembrane orientation and biogenesis of the amyloid A4 precursor of Alzheimer's disease. EMBO J. 7: 949-957.
25. Esch, F.S., Keim, P.S., Beattie, E.C., Blacher, R.W., Cuwell, A.R., Oltersdorf, T., McClure, D., and Ward, P.J. 1990. Cleavage of amyloid β peptide during constitutive processing of its precursor. Science 248: 1122-1124.
26. Fierz, W., Endler, B., Reske, K., Wekerle, H., and Fontana, A. 1985. Astrocytes as antigen-presenting cells: I. Induction of Ia antigen expression on astrocytes by T cells via immune interferon and its effect on antigen presentation. J. Immunol. 134: 3785-3793.
27. Frei, K., Bodmer, S., Schwerdel, C., and Fontana, A. 1985. Astrocytes of the brain synthesize interleukin 3-like factors. J. Immunol. 135: 4044-4047.
28. Furukawa, S., Furukawa, Y., Satoyoshi, E., and Hayashi, K. 1986. Synthesis and secretion of nerve growth factor by mouse astroglial cells in culture. Biochem. Biophys. Res. Commun. 136: 57-63.
29. Geinisman, Y., Bondareff, W., and Dodge, J.T. 1978. Hypertrophy of astroglial processes in the dentate gyrus of the senescent rat. Am. J. Anat. 153: 537-544.
30. Giaccone, G., Tagliavini, F., Linoli, G., Bouras, C., Frigerio, L.,

- Frangione, B., and Bugiani, O. 1989. Down's patients: extracellular preamyloid deposits precede neuritic degeneration and senile plaques. Neurosci. Lett. 97: 232-238.
31. Gibson, P.H. 1987. Ultrastructural abnormalities in the cerebral neocortex and hippocampus associated with Alzheimer's disease and aging. Acta Neuropathol. (Berl.) 73: 86-91.
 32. Glenner, G.G. 1979. Congophilic microangiopathy in the pathogenesis of Alzheimer's syndrome (presenile dementia). Med. Hypotheses 5: 1231-1236.
 33. Glenner, G.G. 1980. Amyloid deposits and amyloidosis. N. Engl. J. Med. 302: 1283-1291.
 34. Glenner, G.G. and Wong, C.W. 1984. Alzheimer disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem. Biophys. Res. Commun. 120: 885-890.
 35. Goedert, M. 1987. Neuronal localization of amyloid beta protein precursor mRNA in normal human brain and in Alzheimer's disease. EMBO J. 6: 3627-3632.
 36. Goldgaber, D., Lerman, M.I., McBride, O.W., Saffiotti, U., and Gajdusek, D.C. 1987. Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. Science 235: 877-880.
 37. Gonatas, N.K., Anderson, W.V., and Evangelista, I. 1967. The contribution of altered synapses in the senile plaque: an electron microscopic study in Alzheimer's disease. J. Neuropathol. Exp. Neurol. 26: 25-39.
 38. Goss, J.R., Finch, C.E., and Morgan, D.G. 1991. Age-related changes in

- glial fibrillary acidic protein mRNA in the mouse brain. Neurobiol. Aging 12: 165-170.
39. Griffin, W.S.T., Stanley, L.C., Ling, C., White, L., MacLeod, V., Perrot, L.J., White, C.L.III, and Araoz, C. 1989: Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc. Natl. Acad. Sci. U.S.A. 86: 7611-7615.
40. Haga, S., Akai, K., and Ishii, T. 1989. Demonstration of microglial cells in and around senile (neuritic) plaques in the Alzheimer brain. Acta Neuropathol. 77: 569-575.
41. Haga, C., Yamaguchi, H., Ikeda, K., and Kosaka, K. 1989. PAM-modified methenamine silver stain for senile plaques: comparison with β -protein immunostaining. Dementia 3: 417-422.
42. Hansen, L.A., Armstrong, D.M., and Terry, R.D. 1987. An immunohistochemical quantification of fibrous astrocytes in the aging human cerebral cortex. Neurobiol. Aging 8: 1-6.
43. Higgins, G.A., Lewis, D.A., Bahmanyar, S., Goldgaber, D., Gajdusek, D.C., Young, W.G., Morrison, J.H., and Wilson, M.C. 1988. Differential regulation of amyloid- β -protein mRNA expression within hippocampal neuronal subpopulations in Alzheimer disease. Proc. Natl. Acad. Sci. U.S.A. 85: 1297-1301.
44. Ikeda, S., Yanagisawa, N., Allsop, D., and Glenner, G.G. 1989. Evidence of amyloid β protein immunoreactive early plaque lesions in Down's syndrome brains. Lab. Invest. 61:133-137.
45. Itagaki, S., McGeer, P.L., Akiyama, H., Zhu, S., and Selkoe, D.J. 1989. Relationship of microglia and astrocytes to amyloid deposits of

- Alzheimer disease. J. Neuroimmunol. 24: 173-182.
46. Joachim, C.L., Duffy, L.K., Morris, J.H., and Selkoe, D.J. 1988. Protein chemical and immunocytochemical studies of meningovascular β -amyloid protein in Alzheimer's disease and normal aging. Brain Res. 474: 100-111.
 47. Joachim, C., Games, D., Morris, J., Ward, P., Frenkel, D., and Selkoe, D. 1991. Antibodies to non-beta regions of the beta-amyloid precursor protein detected a subset of senile plaques. Am. J. Pathol. 138: 373-384.
 48. Joachim, C.L., Mori, H., and Selkoe, D.L. 1989. Amyloid β -protein deposition in tissues other than brain in Alzheimer's disease. Nature (Lond.) 341: 226-230.
 49. Joachim, C.L., Morris, J.H., and Selkoe, D.J. 1989. Diffuse amyloid plaques occur commonly in the cerebellum in Alzheimer's disease. Am. J. Pathol. 5: 309-319.
 50. Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K.-H., Multhaup, G., Beyreuther, K., and Muller-Hill, B. 1987. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature (Lond.) 325: 733-736.
 51. Kawabata, S., Higgins, G.A., and Gordon, J.W. 1991. Amyloid plaques, neurofibrillary tangles and neuronal loss in brains of transgenic mice overexpressing a C-terminal fragment of human amyloid precursor protein. Nature (Lond.) 354: 476-478.
 52. Kawai, M., Gras, P., Siedlak, S., Lowery, D., Gonzalez-DeWhitt, P., Greenberg, B., Skelton, E., Gambetti, P., and Perry, G. 1990. Role of vascular smooth muscle cells in amyloid deposition in cerebral amyloid

- angiopathy. J. Neuropathol. Exp. Neurol. 49: 339.
53. Kemper, T. 1984. Neuroanatomical and neuropathological changes in normal aging and in dementia. pp. 9-52. In: Clinical Neurology of Aging (Albert, M.L. ed.), Oxford University Press, New York.
 54. Kesslak, J.P., Nieto-Sampedro, M., Globus, J., and Cotman, C.W. 1986. Transplants of purified astrocytes promote behavioral recovery after frontal cortex ablation. Exp. Neurol. 93: 377-390.
 55. Khachaturian, Z.S. 1985. Diagnosis of Alzheimer's disease. Arch. Neurol. 42: 1097-1105.
 56. Kimelberg, H.K. and Katz, D.M. 1985. High-affinity uptake of serotonin into immunocytochemically identified astrocytes. Science 228: 889-891.
 57. Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S., and Ito, H. 1988. Novel precursor of Alzheimer's disease amyloid protein shows protease inhibitory activity. Nature (Lond.) 331: 530-532.
 58. Kitamoto, T., Ogomori, K., Tateishi, J., and Prusiner, S.B. 1987. Formic acid pretreatment enhances immunostaining of cerebral and systemic amyloids. Lab. Invest. 57: 230-236.
 59. Kitt, C.A., Price, D.L., Struble, R.G., Cork, L.C., Wainer, B.H., Becher, M.W. and Mobley, W.C. 1984. Evidence for cholinergic neurites in senile plaques. Science 226: 1443-1445.
 60. Kitt, C.A., Struble, R.G., Cork, L.C., Mobley, W.C., Walker, L.C., Joh, T.H., and Price, D.L. 1985. Catecholaminergic neurites in senile plaques in prefrontal cortex of aged nonhuman primates. Neuroscience 16: 105-115.
 61. Klier, F.G., Cole, G., Stallcup, W., and Schubert, D. 1990. Amyloid β -protein precursor is associated with extracellular matrix. Brain Res.

- 515: 336-342.
62. Koo, E.H., Sisodia, S.S., Archer, D.R., Martin, L.J., Beyreuther, K., Weidemann, A., and Price, D.L. 1989. Amyloid precursor protein (APP) undergoes fast anterograde transport. Soc. Neurosci. Abstr. 15: 23.
 63. Koo, E.H., Sisodia, S.S., Archer, D.A., Martin, L.J., Weidemann, A., Beyreuther, K., Masters, C.L., Fischer, P., and Price, D.L. 1990. Precursor of amyloid protein in Alzheimer's disease undergoes fast anterograde axonal transport. Proc. Natl. Acad. Sci. U.S.A. 87: 1561-1565.
 64. Landfield, P.W., McGaugh, J.L., and Lynch, G. 1978. Impaired synaptic potentiation processes in the hippocampus of aged, memory-deficient rats. Brain Res. 150: 85-101.
 65. Landfield, P.W., Rose, G., Sandles, L., Wohlstadter, T.C., and Lynch, G. 1977. Patterns of astroglial hypertrophy and neuronal degeneration in the hippocampus of aged, memory-deficient rats. J. Gerontol. 32: 3-12.
 66. Leuba, G. 1983. Aging of dendrites in the cerebral cortex of the mouse. Neuropathol. Appl. Neurobiol. 9: 467-475.
 67. Lindholm, D., Heumann, R., Meyer, M., and Thoenen, H. 1987. Interleukin-1 regulates synthesis of nerve growth factor in nonneuronal cells of rat sciatic nerve. Nature (Lond.) 330: 658-659.
 68. Lindsey, J.D., Landfield, P.W., and Lynch, G. 1979. Early onset and topographical distribution of hypertrophied astrocytes in hippocampus of aging rats: a quantitative study. J. Gerontol. 34: 661-671.
 69. Mancardi, G.L., Liwnicz, B.H., and Mandybur, T.I. 1983. Fibrous astrocytes in Alzheimer's disease and senile dementia of Alzheimer's type. Acta Neuropathol. 61: 76-80.

70. Mandybur, T.I. 1989. Cerebral amyloid angiopathy and astrocytic gliosis in Alzheimer's disease. Acta Neuropathol. 78: 329-331.
71. Mandybur, T.I., Ormsby, I., and Zemlan, F.P. 1989. Cerebral aging: a quantitative study of gliosis in old nude mice. Acta Neuropathol. 77: 507-513.
72. Mann, D.M.A. 1985. The neuropathology of Alzheimer's disease: a review with pathogenetic, aetiological and therapeutic considerations. Mech. Ageing Dev. 31: 213-255.
73. Mann, D.M.A. 1989. Cerebral amyloidosis, aging and Alzheimer's disease: a contribution from studies on Down's syndrome. Neurobiol. Aging 10: 397-399.
74. Mann, D.M.A. and Esiri, M.M. 1989. The pattern of acquisition of plaques and tangles in the brains of patient under 50 years of age with Down's syndrome. J. Neurol. Sci. 89: 169-179.
75. Mann, D.M.A., Jones, D., Prinja, D., and Purkiss, M.S. 1990. The prevalence of amyloid (A4) protein deposits within the cerebral and cerebellar cortex in Down's syndrome and Alzheimer's disease. Acta Neuropathol. 80: 318-327.
76. Mann, D.M.A., Tucker, C.M., and Yates, P.O. 1987. The topographic distribution of senile plaques and neurofibrillary tangles in the brains of non-demented persons of different ages. Neuropathol. Appl. Neurobiol. 13: 123-139.
77. Masliah, E., Hansen, L., Albright, T., Mallory, M., and Terry, R.D. 1991. Immunoelectron microscopic study of synaptic pathology in Alzheimer's disease. Acta Neuropathol. 81: 428-433.

78. Masters, C.L., Multhaup, G., Simms, G., Pottgiesser, J., Martins, R.N., and Beyreuther, K. 1985. Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. EMBO J. 4: 2757-2763.
79. Masters, C.L., Simms, G., Weinman, N.A., Multhaup, G., McDonald, B.L., and Beyreuther, K. 1985. Amyloid plaque core protein in Alzheimer's disease and Down's syndrome. Proc. Natl. Acad. Sci. U.S.A. 82: 4245-4249.
80. Matsuyama, H. and Nakamura, S. 1978. Senile changes in the brain in the Japanese: Incidences of Alzheimer neurofibrillary changes and senile plaques. pp. 287-297. In: Alzheimer's Disease: Senile Dementia and Related Disorders (Katzman, R., Terry, R.D., and Bick, K.L. eds.), Raven Press, New York.
81. Mattice, L.A., Davies, P., Yen, S.-H., and Dickson, D.W. 1990. Microglia in cerebellar plaques in Alzheimer's disease. Acta Neuropathol. 80: 493-498.
82. McGeer, P.L., Akiyama, H., Itagaki, S., and McGeer, E.G. 1989. Immune system response in Alzheimer's disease. Can. J. Neurol. Sci. 16: 516-527.
83. Miller, D.L., Currie, J.R., Iqbal, K., Potempska, A., and Styles, J. 1988. Relationships among the cerebral amyloid peptides and their precursors. Alzheimer Disease and Associated Disorders 2: 253.
84. Miquel, J., Johnson, J.E., Jr., and Cervous-Navarros, J. 1983. Comparison of CNS aging in humans and experimental animals. pp. 231-258. In: Brain Aging: Neuropathology and Neuropharmacology, Aging, vol. 21 (Cervous-Navarro, J. and Sarkander, J.-I. eds.), Ravan Press, New York.
85. Miyakawa, T., Katsuragi, S., Watanabe, K., Shimoji, A., and Ikeuchi, Y. 1986. Ultrastructural studies of amyloid fibrils and senile plaques in

- human brain. Acta Neuropathol. (Berl.) 70: 202-208.
86. Miyakawa, T., Shimoji, A., Kuramoto, R., and Higuchi, Y. 1982. The relationship between senile plaques and cerebral blood vessels in Alzheimer's disease and senile dementia. Morphological mechanism of senile plaque production. Virchows Arch. [B] 40: 121-129.
87. Mountjoy, C.Q., Tomlinson, B.E., and Gibson, R.H. 1982. Amyloid and senile plaques and cerebral blood vessels. A semi-quantitative investigation of a possible relationship. J. Neurol. Sci. 57: 89-103.
88. Nolan, C.C. and Brown, A.W. 1989. Reversible neuronal damage in hippocampal pyramidal cells with triethyllead: the role of astrocytes. Neuropathol. Appl. Neurobiol. 15: 441-457.
89. O'Callaghan, J.P. and Miller, D.B. 1991. The concentration of glial fibrillary acidic protein increases with age in the mouse and rat brain. Neurobiol. Aging 12: 171-174.
90. Ogomori, K., Kitamoto, T., Tateishi, J., Sato, Y., Suetsugu, M., and Abe, M. 1989. β -Protein amyloid is widely distributed in the central nervous system of patients with Alzheimer's disease. Am. J. Pathol. 134: 243-251.
91. O'Kusky, J. and Colonnier, M. 1982. Postnatal changes in the number of astrocytes, oligodendrocytes, and microglia in the visual cortex (area 17) of the macaque monkey: a stereological analysis in normal and monocularly deprived animals. J. Comp. Neurol. 210: 307-315.
92. Oltersdorf, T., Fritz, L.C., Schenk, D.B., Lieberburg, I., Johnson-Wood, K.L., Beattie, E.C., Ward, P.J., Blacher, R.W., Dovey, H.F., and Sinha, S. 1989. The secreted form of the Alzheimer's amyloid precursor protein with Kunitz domain is protease nexin-II. Nature (Lond.) 341: 144-147.

93. Oltersdorf, T., Ward, P.J., Henriksson, T., Beattie, E.C., Neve, R., Lieberberg, I., and Fritz, L.C. 1990. The Alzheimer amyloid precursor protein. Identification of a stable intermediate in the biosynthetic/degradative pathway. J. Biol. Chem. 265: 4492-4497.
94. Palmert, M.R., Golde, T.E., Cohen, M.L., Kovacs, D.M., Tanzi, R.E., Gusella, J.F., Usiak, M.F., Younkin, L.H., and Younkin, S.G. 1988. Amyloid protein precursor messenger RNAs: differential expression in Alzheimer's disease. Science 241: 1080-1084.
95. Pasternack, J.M., Abraham, C.R., Van Dyke, B.J., Potter, H., and Younkin, S.G. 1989. Astrocytes in Alzheimer's disease gray matter express α_1 -antichymotrypsin mRNA. Am. J. Pathol. 135: 827-834.
96. Paulson, O.B. and Newman, E.A. 1987. Does the release of potassium from astrocyte endfeet regulate cerebral blood flow? Science 237: 896-898.
97. Podlisny, M.B., Tolan, D., and Selkoe, D.J. 1991. Homology of the amyloid β -protein precursor in monkey and human supports a primate model for β -amyloidosis in Alzheimer's disease. Am. J. Pathol. 138: 1423-1435.
98. Politis, M.J. and Miller, J.E. 1985. The roles of reactive gliosis and mitosis on tropic factor production in traumatized nervous system tissue. Brain Res. 346: 186-189.
99. Ponte, P., Gonzalez-DeWhitt, P., Schilling, J., Miller, J., Hsu, D., Greenberg, B., Davis, K., Wallace, W., Lieberburg, I., Fuller, F., and Cordell, B. 1988. A new A4 amyloid mRNA contains a domain homologous to serine proteinase inhibitors. Nature (Lond.) 331: 525-527.
100. Prelli, F., Castano, E., Glenner, G.G., and Frangione, B. 1988. Differences between vascular and plaque core amyloid in Alzheimer's

- disease. J. Neurochem. 51: 648-651.
101. Probst, A., Brunnschweiler, H., Lautenschlager, C., and Ulrich, J. 1987. A special type of senile plaque, possibly an initial stage. Acta Neuropathol. (Berl.) 74: 133-141.
 102. Robakis, N.K., Ramakrishna, N., Wolfe, G., and Wisniewski, H.M. 1987. Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neuritic plaque amyloid peptides. Proc. Natl. Acad. Sci. U.S.A. 84: 4190-4194.
 103. Rozemuller, J.M., Eikelenboom, P., Stam, F.C., Beyreuther, K., and Masters, C.L. 1989. A4 protein in Alzheimer's disease: primary and secondary cellular events in extracellular amyloid deposition. J. Neuropathol. Exp. Neurol. 48: 674-691.
 104. Saitoh, T., Sundsmo, M., Roch, J.-M., Kimura, N., Cole, G., Schubert, D., Oltersdorf, T., and Schenk, D.B. 1989. Secreted form of amyloid β protein precursor is involved in the growth regulation of fibrosis. Cell 58: 615-622.
 105. Salbaum, J.M., Weidemann, A., Lemaire, H.G., Masters, C.L., and Beyreuther, K. 1988. The promoter of Alzheimer's disease amyloid A4 precursor gene. EMBO J. 7: 2807-2813.
 106. Schechter, R., Yen, S.-H.C., and Terry, R.D. 1981. Fibrous astrocytes in senile dementia of the Alzheimer type. J. Neuropathol. Exp. Neurol. 40: 95-101.
 107. Scheff, S.W., De Kosky, S.T., and Price D.A. 1990. Quantitative assessment of cortical synaptic density in Alzheimer's disease. Neurobiol. Aging 11: 29-37.

108. Scheibel, M.E. and Scheibel, A.B. 1975. Structural changes in the aging brain. pp. 11-37. In: Clinical, Morphologic, and Neurochemical Aspects in the Aging Central Nervous System, vol. 1 (Brody, H., Harman, D., and Ord, J.M. eds), Raven Press, New York.
109. Schubert, D., Jin, L.-W., Saitoh, T., and Cole, G. 1989. The regulation of amyloid β protein precursor secretion and its modulatory role in cell adhesion. Neuron 3: 689-694.
110. Selkoe, D.J. 1988. Molecular pathology of amyloidogenic proteins and the role of vascular amyloidosis in Alzheimer's disease. Neurobiol. Aging 10: 387-395.
111. Selkoe, D.J., Abraham, C.R., Podlisny, M.B., and Duffy, L.K. 1986. Isolation of low-molecular-weight proteins from amyloid plaque fibers in Alzheimer's disease. J. Neurochem. 146: 1820-1834.
112. Selkoe, D.J., Bell, D.S., Podlisny, M.B., Price, D.L., and Cork, L.C. 1987. Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease. Science 235: 873-877.
113. Selkoe, D.J., Davies, P., Geyer, J.J., and Price, D.L. 1988. Neurofibrillary tangles and senile plaques in aged bears. J. Neuropathol. Exp. Neurol. 47: 629-641.
114. Selkoe, D.J., Podlisny, M.B., Joachim, C.L., Vickers, E.A., Lee, G., Fritz, L.C., and Oltersdorf, T. 1988. β -amyloid precursor protein of Alzheimer disease occurs as 110- to 135-kilodalton membrane-associated proteins in neural and nonneural tissues. Proc. Natl. Acad. Sci. U.S.A. 85: 7341-7345.
115. Shivers, B.D., Hilbich, C., Multhaup, G., Salbaum, M., Beyreuther, K.,

- and Seeburg, P.H. 1988. Alzheimer's disease amyloidogenic glycoprotein: expression pattern in rat brain suggests a role in cell contact. EMBO J. 7: 1365-1370.
116. Siman, R., Card, J.P., Nelson, R.B., and Davis, L.G. 1989. Expression of beta-amyloid precursor protein in reactive astrocytes following neuronal damage. Neuron 3: 275-285.
117. Sinha, S., Dovey, H.F., Seubert, P., Ward, P.J., Blacher, R.W., Blaber, M., Bradshaw, R.A., Arici, M., Mobley, W.C., and Lieberburg, I. 1990. The protease inhibitory properties of the Alzheimer's β -amyloid precursor protein. J. Biol. Chem. 265: 8983-8985.
118. Smith, R.P., Higuchi, D.A., and Broze, G.J., Jr. 1990. Platelet coagulation factor XIa-inhibitor, a form of Alzheimer amyloid precursor protein. Science 248: 1126-1128.
119. Snow, A.D., Mar, H., Nochlin, D., Kimata, K., Kato, M., Suzuki, S., Hassell, J., and Wight, T.N. 1988. The presence of heparan sulfate proteoglycans in the neuritic plaques and congophilic angiopathy in Alzheimer's disease. Am. J. Pathol. 133: 456-463.
120. Struble, R.G., Price, D.L. Jr., Cork, L.C., and Price, D.L. 1985. Senile plaques in cortex of aged normal monkeys. Brain Res. 361: 267-275.
121. Suenaga, T., Hirano, A., Llena, J.F., Yen, S.-H., and Dickson, D.W. 1990. Modified Bielschowsky stain and immunohistochemical studies on striatal plaques in Alzheimer's disease. Acta Neuropathol. 80: 280-286.
122. Tagliavini, F., Giaccone, G., Frangione, B., and Bugiani, O. 1988. Preamyloid deposits in cerebral cortex of patients with Alzheimer's disease and non demented individuals. Neurosci. Lett. 93: 191-196.

123. Tanzi, R.E., Gusella, J.F., Watkins, P.C., Bruns, G.A.P., St. George-Hyslop, P., Van Keuren, M.L., Patterson, D., Pagan, S., Kurnit, D.M., and Neve, R.L. 1987. Amyloid β protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. Science 235: 880-884.
124. Tanzi, R.E., McClatchey, A.I., Lamperti, E.D., Villa-Komaroff, L.L., Gusella, J.F., and Neve, R.L. 1988. Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. Nature (Lond.) 331: 528-530.
125. Terry, R.D., Gonatas, N.K., and Weiss, M. 1964. Ultrastructural studies in Alzheimer's presenile dementia. Am. J. Pathol. 44: 269-297.
126. Terry, R.D., Peck, A., Deteresa, R., Schechter, R., and Horoupian, D.S. 1981. Some morphometric aspects of the brain in senile dementia of the Alzheimer type. Ann. Neurol. 10: 184-192.
127. Tobo, M. 1984. Immunohistochemical study of gliosis in the brain of aging and dementia. Fukuoka Igaku Zasshi 75: 72-88. (in Japanese with English abstract)
128. Tomlinson, B.E., Blessed, G., and Roth, M. 1968. Observations on the brains of non-demented elderly persons. J. Neurol. Sci. 7: 331-356.
129. Tooyama, I., Akiyama, H., McGeer, P.L., Hara, Y., Yasuhara, O., and Kimura, H. 1991. Acidic fibroblast growth factor-like immunoreactivity in brain of Alzheimer patients. Neurosci. Lett. 121: 155-158.
130. Uchida, K., Miyauchi, Y., Nakayama, H., and Goto, N. 1990. Amyloid angiopathy with cerebral hemorrhage and senile plaque in aged dogs. Jpn. J. Vet. Sci. 52: 605-611.

131. Ulrich, J. 1985. Alzheimer changes in non-demented patients younger than sixty-five: possible early stages of Alzheimer's disease and senile dementia of Alzheimer type. Ann. Neurol. 17: 273-277.
132. Van Nostrand, W.E., Wagner, S.L., Suzuki, M., Choi, B.H., Farrow, J.S., Geddes, J.W., Cotman, C.W., and Cunningham, D.D. 1989. Protease nexin-II, a potent anti-chymotrypsin, shows identity to amyloid β -protein precursor. Nature (Lond.) 341: 546-549.
133. Vaughan, D.W. 1977. Age-related deterioration of pyramidal cell basal dendrites in rat auditory cortex. J. Comp. Neurol. 171: 501-516.
134. Vijayan, V.K. and Cotman, C.W. 1987. Hydrocortisone administration alters glial reaction to entorhinal lesion in the rat dentate gyrus. Exp. Neurol. 96: 307-320.
135. Walker, L.C., Kitt, C.A., Schwam, E., Buckwald, B., Garcia, F., Sepinwall, J., and Price, D.L. 1987. Senile plaques in aged squirrel monkeys. Neurobiol. Aging 8: 291-296.
136. Walker, L.C., Masters, C., Beyreuther, K., and Price, D.L. 1990. Amyloid in the brains of aged squirrel monkeys. Acta Neuropathol. 80: 381-387.
137. Weidemann, A., Konig, G., Bunke, D., Fischer, P., Salbaum, J.M., Masters, C.L., and Beyreuther, K. 1989. Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein. Cell 57: 115-126.
138. Wisniewski, H.M. 1983. Neuritic (senile) and amyloid plaques. pp. 57-61. In: Alzheimer's Disease (Reisberg, B. ed.), The Free Press, New York.
139. Wisniewski, H.M., Ghetti, B., and Terry, R.D. 1973. Neuritic (senile) plaques and filamentous changes in aged rhesus monkeys. J.

- Neuropathol. Exp. Neurol. 32: 566-584.
140. Wisniewski, H.M., Jonson, A.B., Raine, C.S., Kay, W.J., and Terry, R.D. 1970. Senile plaques and cerebral amyloidosis in aged dogs. Lab. Invest. 23: 287-296.
 141. Wisniewski, H. and Terry, R.D. 1970. An experimental approach to the morphogenesis of neurofibrillary degeneration and the argyrophilic plaque. pp. 223-248. In: Ciba Foundation Symposium on Alzheimer's Disease and Related Conditions (Wolstenholme, G.E.W. and O'Connor, M. eds.), J. & A. Churchill, London.
 142. Wisniewski, H.M. and Terry, R.D. 1973. Reexamination of the pathogenesis of the senile plaque. Prog. Neuropathol. 2: 1-26.
 143. Wisniewski, H.M., Weigel, J., Wang, K.C., Kujawa, M., and Lach, B. 1989. Ultrastructural studies of the cells forming amyloid fibers in classical plaques. Can. J. Neurol. Sci. 16: 535-542.
 144. Wisniewski, H.M., Wen, G.Y., and Kim, K.S. 1989. Comparison of four staining methods on the detection of neuritic plaques. Acta Neuropathol. 78: 22-27.
 145. Wong, C.W., Quaranta, V., and Glenner, G.G. 1985. Neuritic plaques and cerebrovascular amyloid in Alzheimer disease are antigenically related. Proc. Natl. Acad. Sci. U.S.A. 82: 8729-8732.
 146. Yamada, T., Sasaki, H., Furuya, H., Miyata, T., Goto, I., and Sakaki, Y. 1987. Complementary DNA for the mouse homolog of the human amyloid beta protein precursor. Biochem. Biophys. Res. Commun. 149: 665-671.
 147. Yamaguchi, H., Hirai, S., Morimatsu, M., Shoji, M., and Harigaya, Y. 1988. Diffuse type of senile plaques in the brains of Alzheimer-type dementia.

Acta Neuropathol. 77: 113-119.

148. Yamaguchi, H., Hirai, S., Morimatsu, M., Shoji, M., and Ihara, Y. 1988. A variety of cerebral amyloid deposits in the brain of the Alzheimer-type dementia demonstrated by β protein immunostaining. Acta Neuropathol. 76: 541-549.
149. Yamaguchi, H., Nakazato, Y., Hirai, S., and Shoji, M. 1990. Immunoelectron microscopic localization of amyloid β protein in the diffuse plaques of Alzheimer-type dementia. Brain Res. 508: 320-324.
150. Yamaguchi, H., Nakazato, Y., Hirai, S., Shoji, M., and Harigaya, Y. 1989. Electron micrograph of diffuse plaques: initial stage of senile plaque formation in the Alzheimer brain. Am. J. Pathol. 135: 593-597.
151. Yamaguchi, H., Nakazato, Y., Yamazaki, T., Shoji, M., Kwarabayashi, T., and Hirai, S. 1991. Subpial β /A4 amyloid deposition occurs between astroglial processes in Alzheimer-type dementia. Neurosci. Lett. 223: 217-220.
152. Yamamoto, T. and Hirano, A. 1986. A comparative study of modified Bielschowsky, Bodian and thioflavin S stains on Alzheimer's neurofibrillary tangles. Neuropathol. Appl. Neurobiol. 12: 3-9.

NH₂-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Gln-Val-

His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val---COOH

Fig. 1 Sequence of cerebrovascular amyloid protein (Glenner & Wong, 1984) [34]

- a*
- A Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-
 1 5 10
 Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe
 11 15 20
 Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala
 21 25 30
 Ile-Xxx-Xxx-Leu-Met-Val
 31 35 36
- B Gly-Ala-Ile-Xxx-Xxx-Leu-Met-Val
 29 36
- C Val-Gly-Gly-Val-Val-Ile-Ala
 36 42
- D Val-Gly-Gly-Val-Val-Ile-Ala-Thr
 36 43

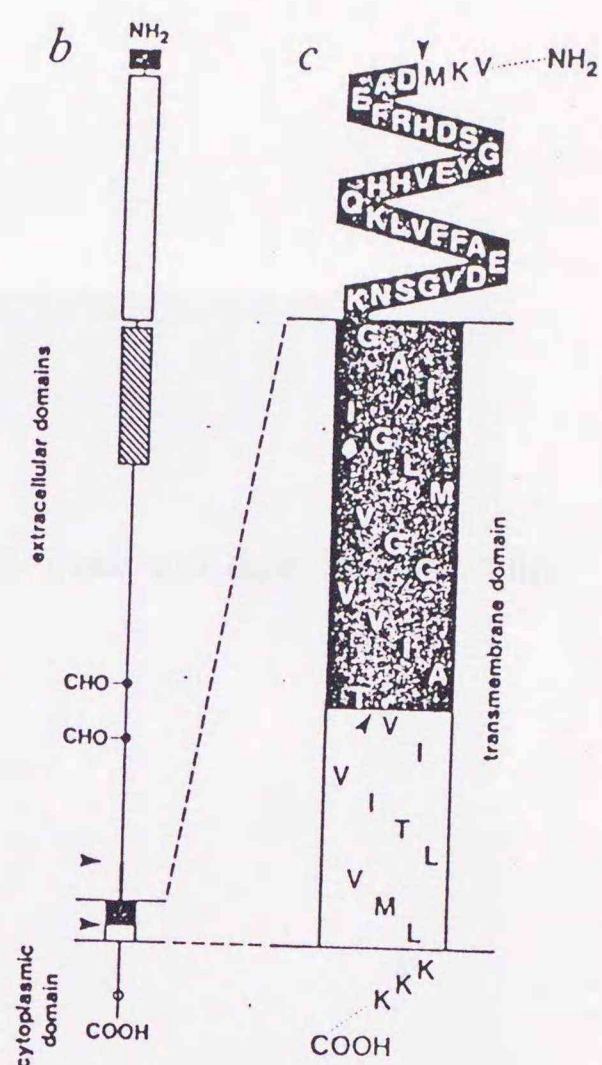
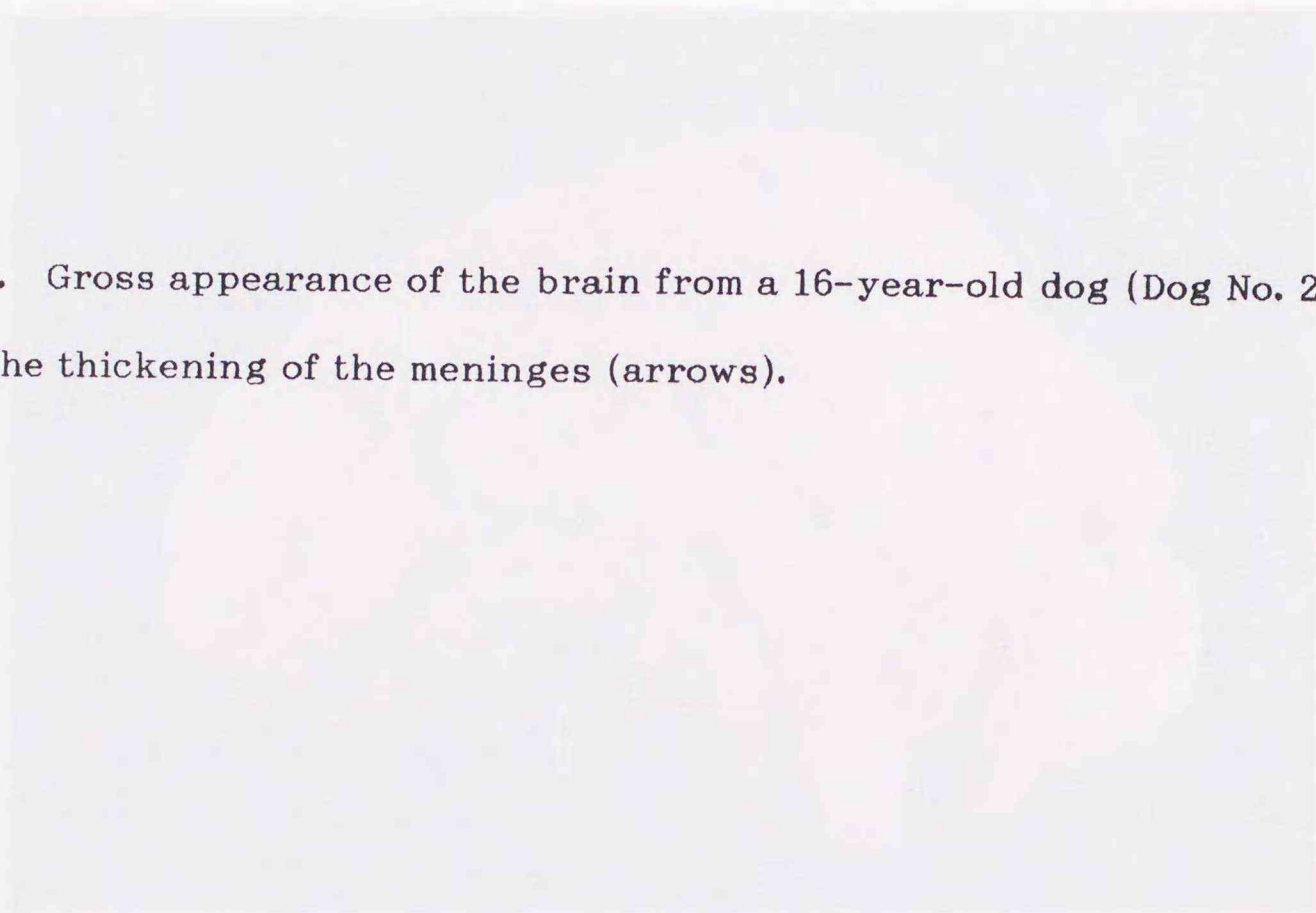


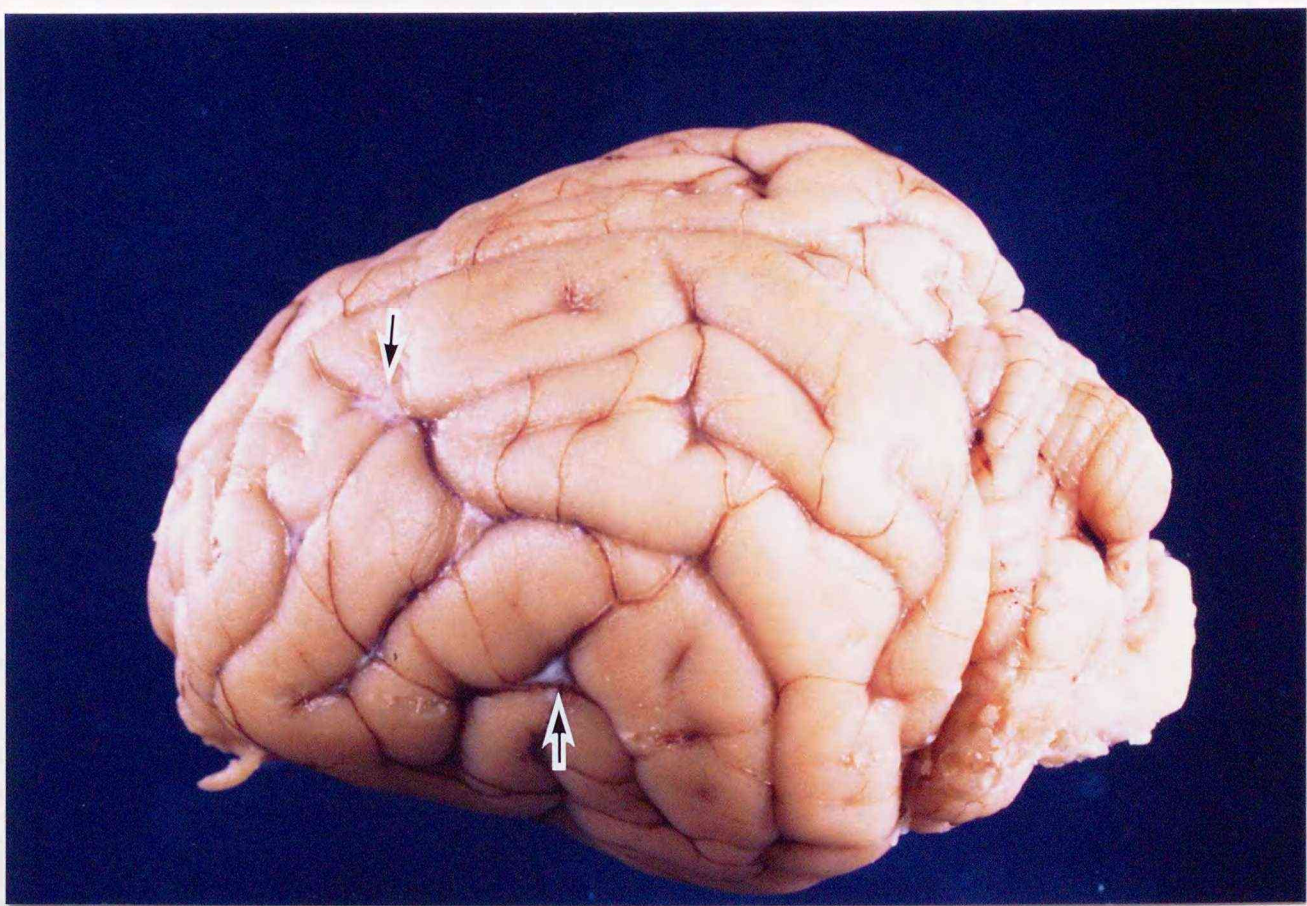
Fig. 2. Schematic diagram of β APP as a cell-surface glycoprotein.

(Kang et al. 1987) [50]

Fig. 3. Gross appearance of the brain from a 16-year-old dog (Dog No. 29).

Note the thickening of the meninges (arrows).





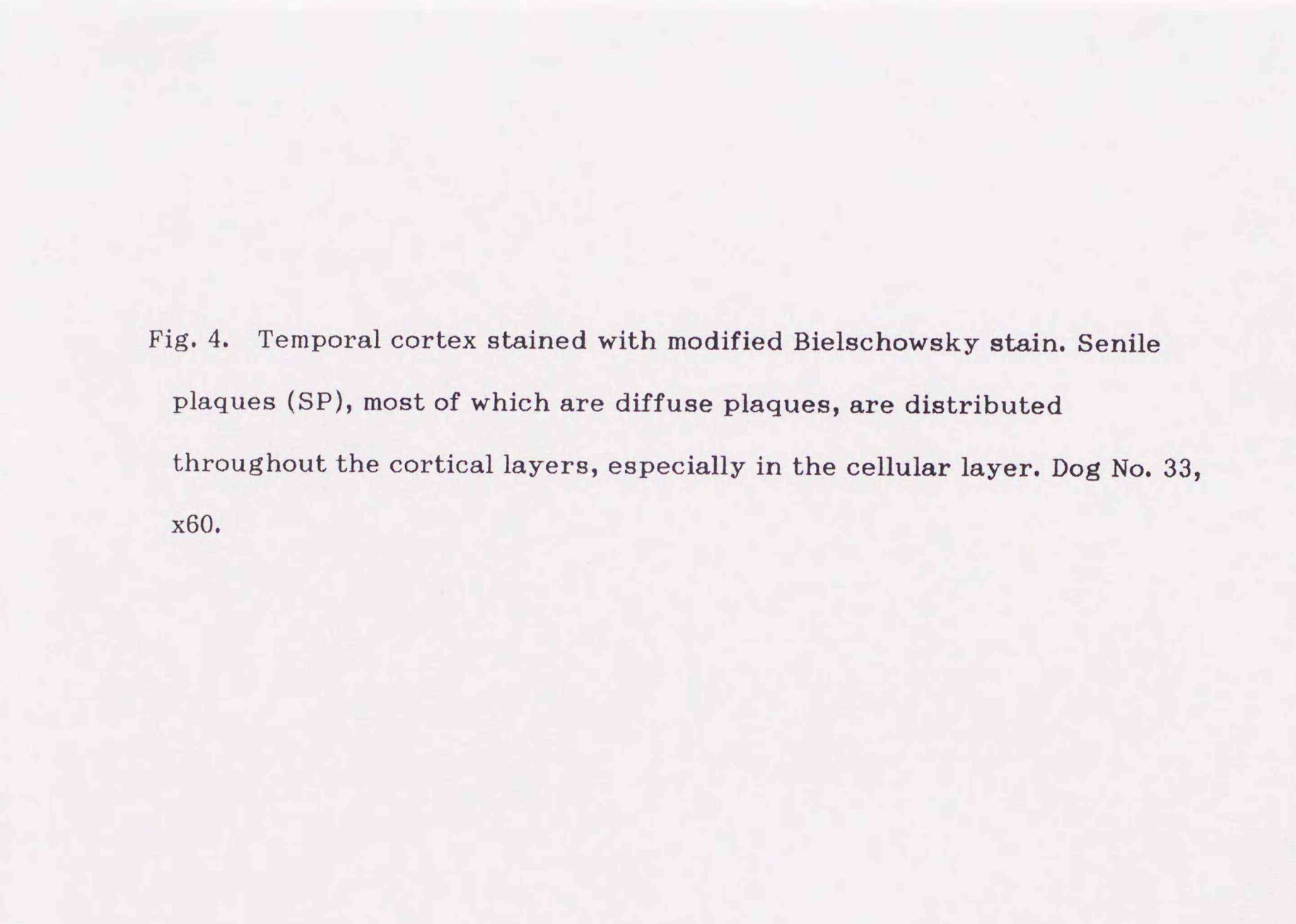
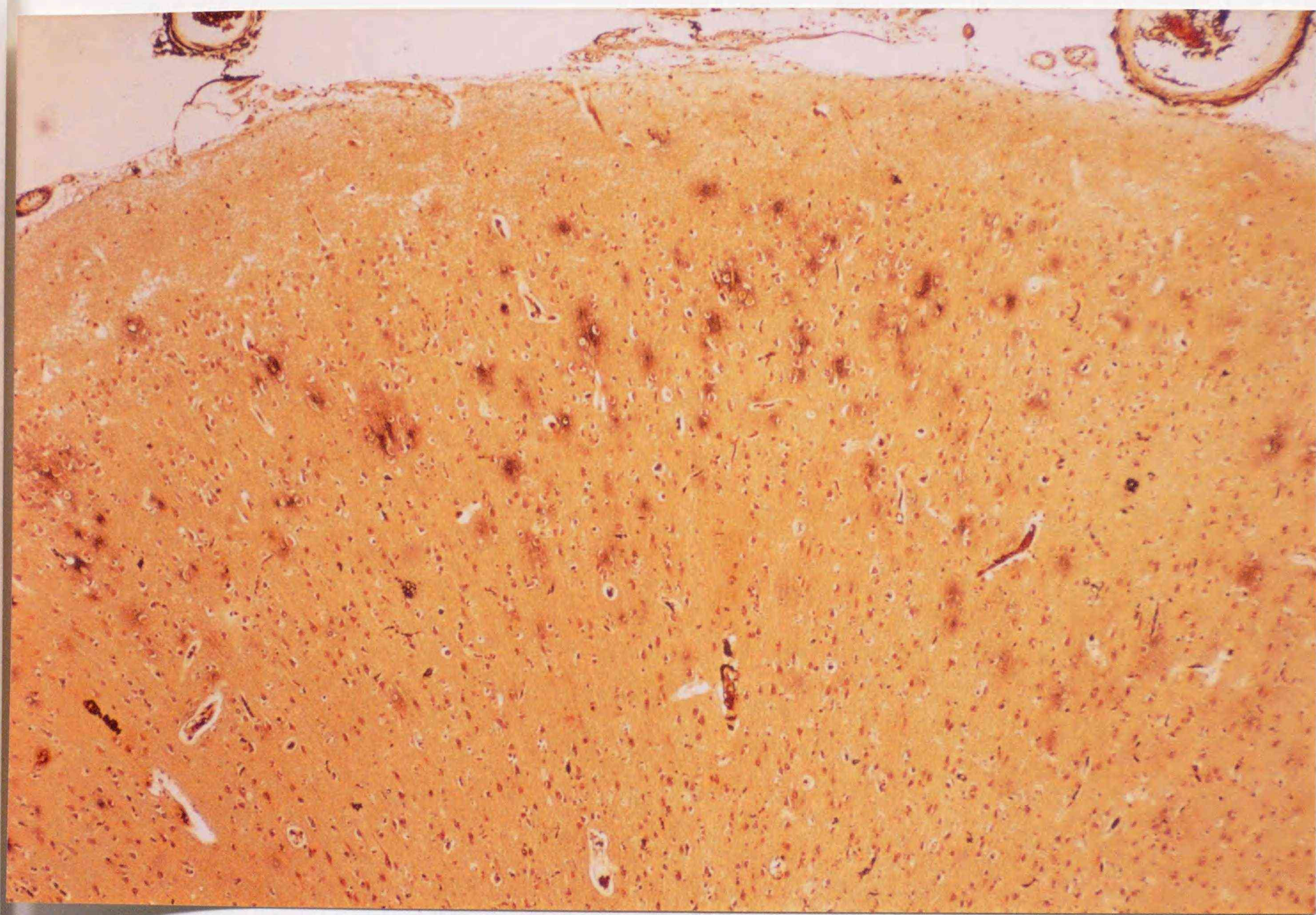


Fig. 4. Temporal cortex stained with modified Bielschowsky stain. Senile plaques (SP), most of which are diffuse plaques, are distributed throughout the cortical layers, especially in the cellular layer. Dog No. 33, x60.



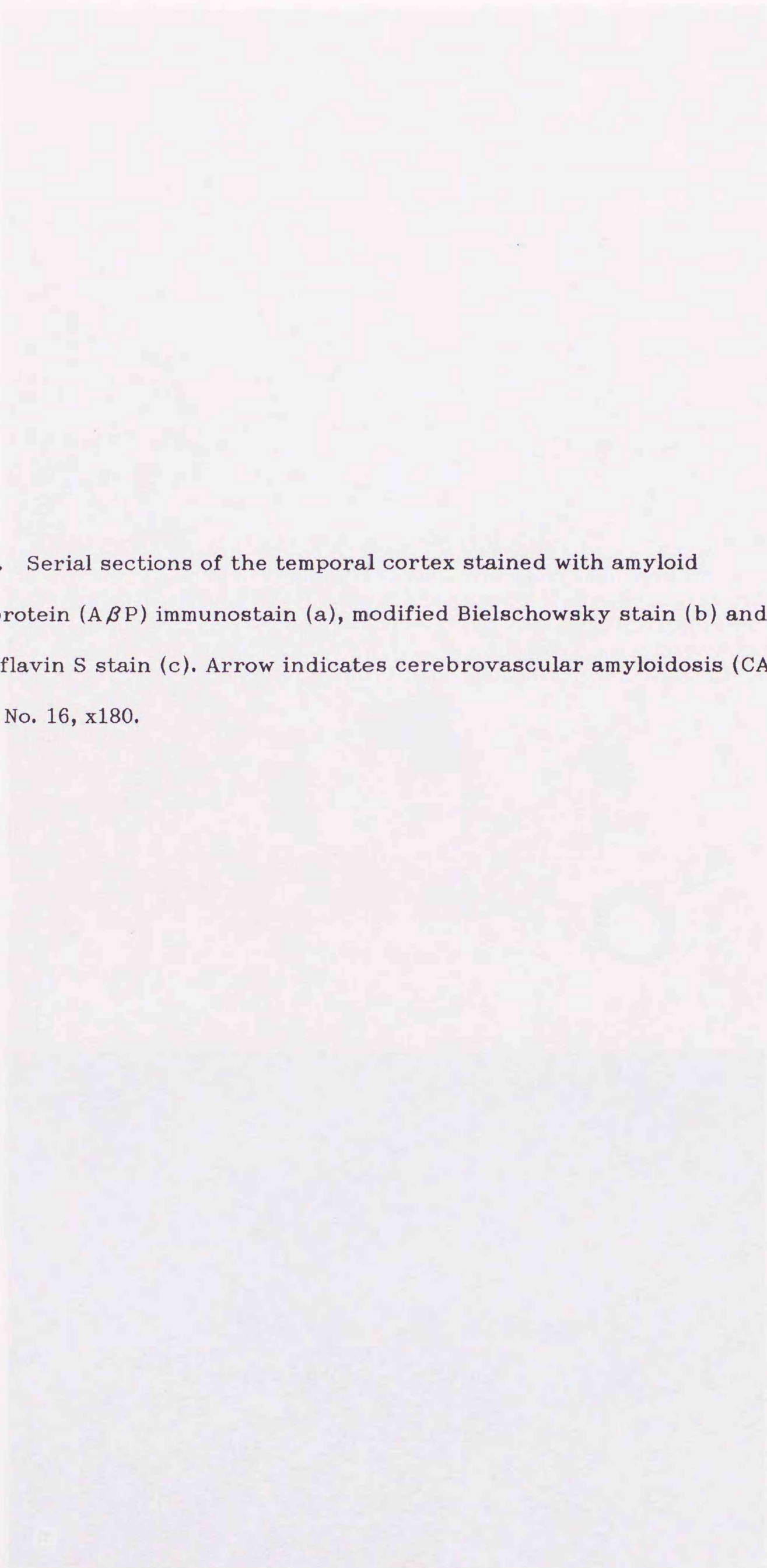
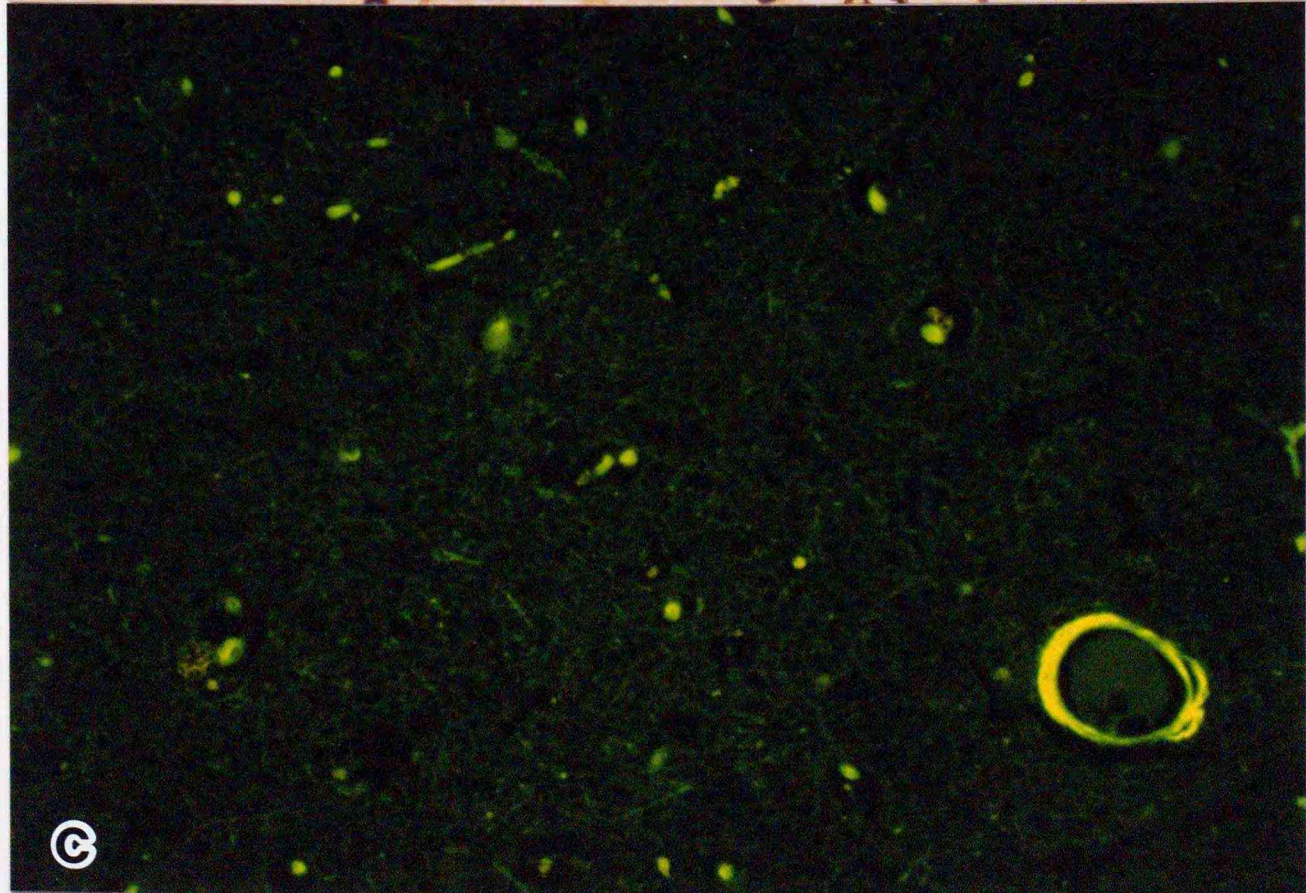
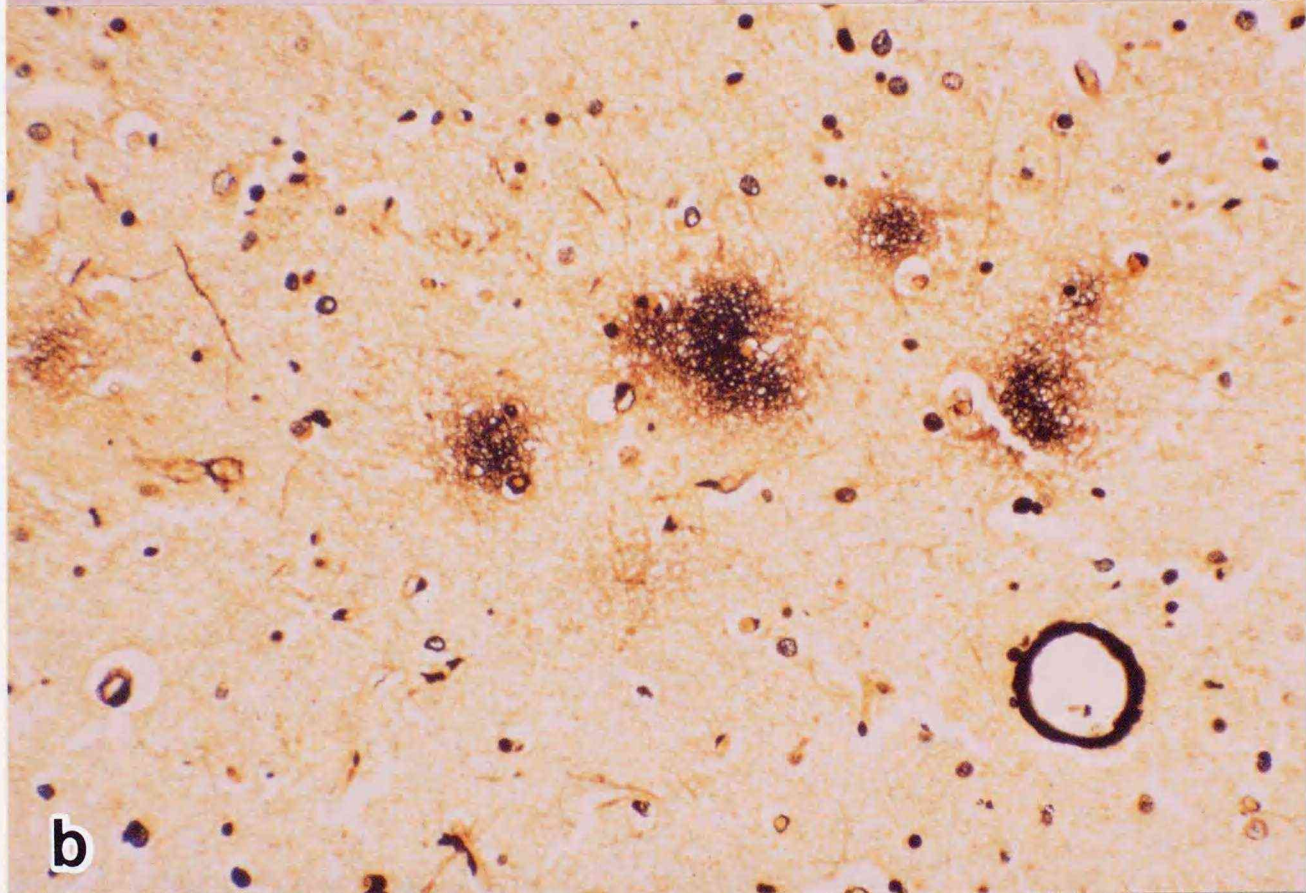
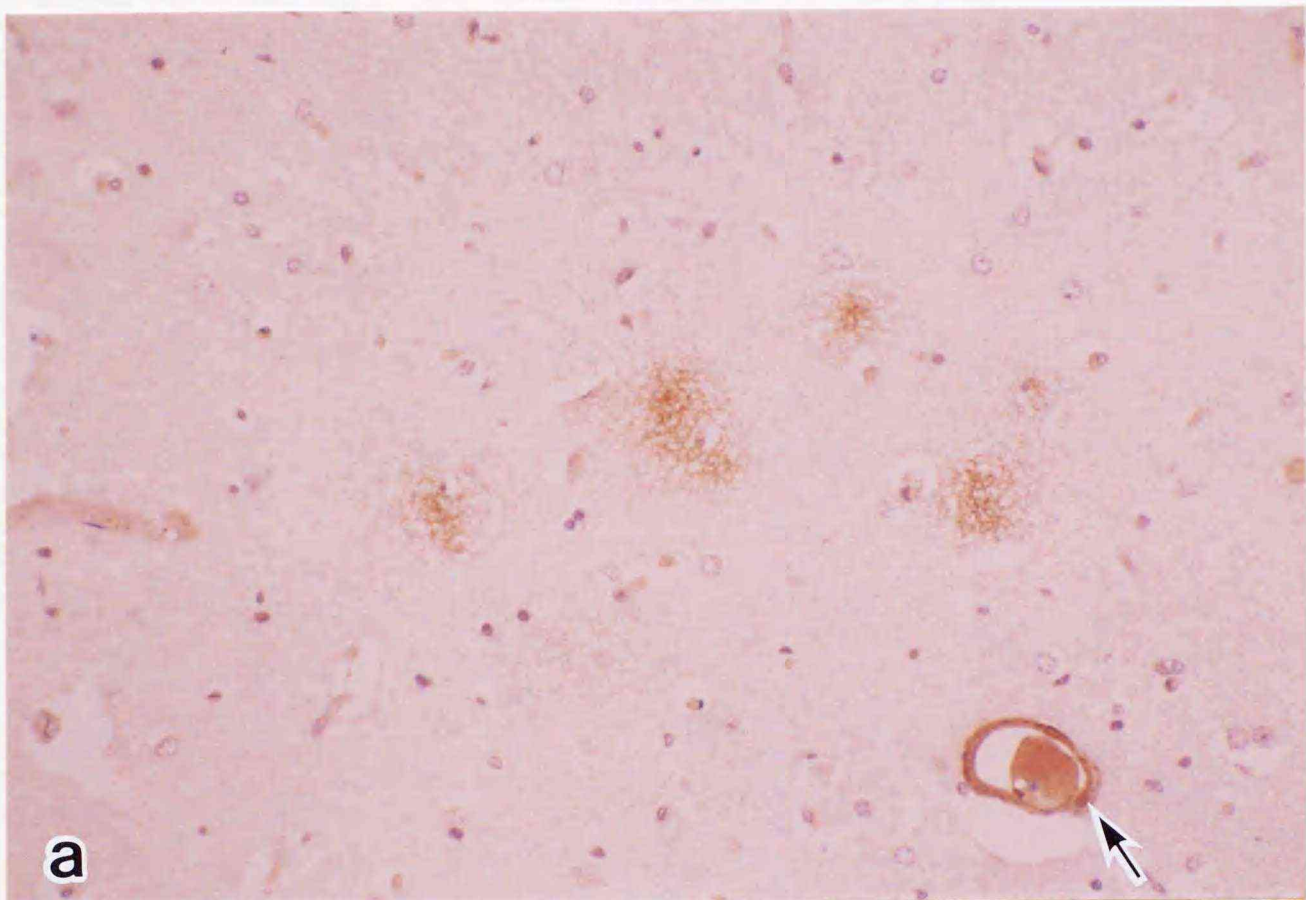


Fig. 5. Serial sections of the temporal cortex stained with amyloid β -protein (A β P) immunostain (a), modified Bielschowsky stain (b) and thioflavin S stain (c). Arrow indicates cerebrovascular amyloidosis (CA). Dog No. 16, x180.



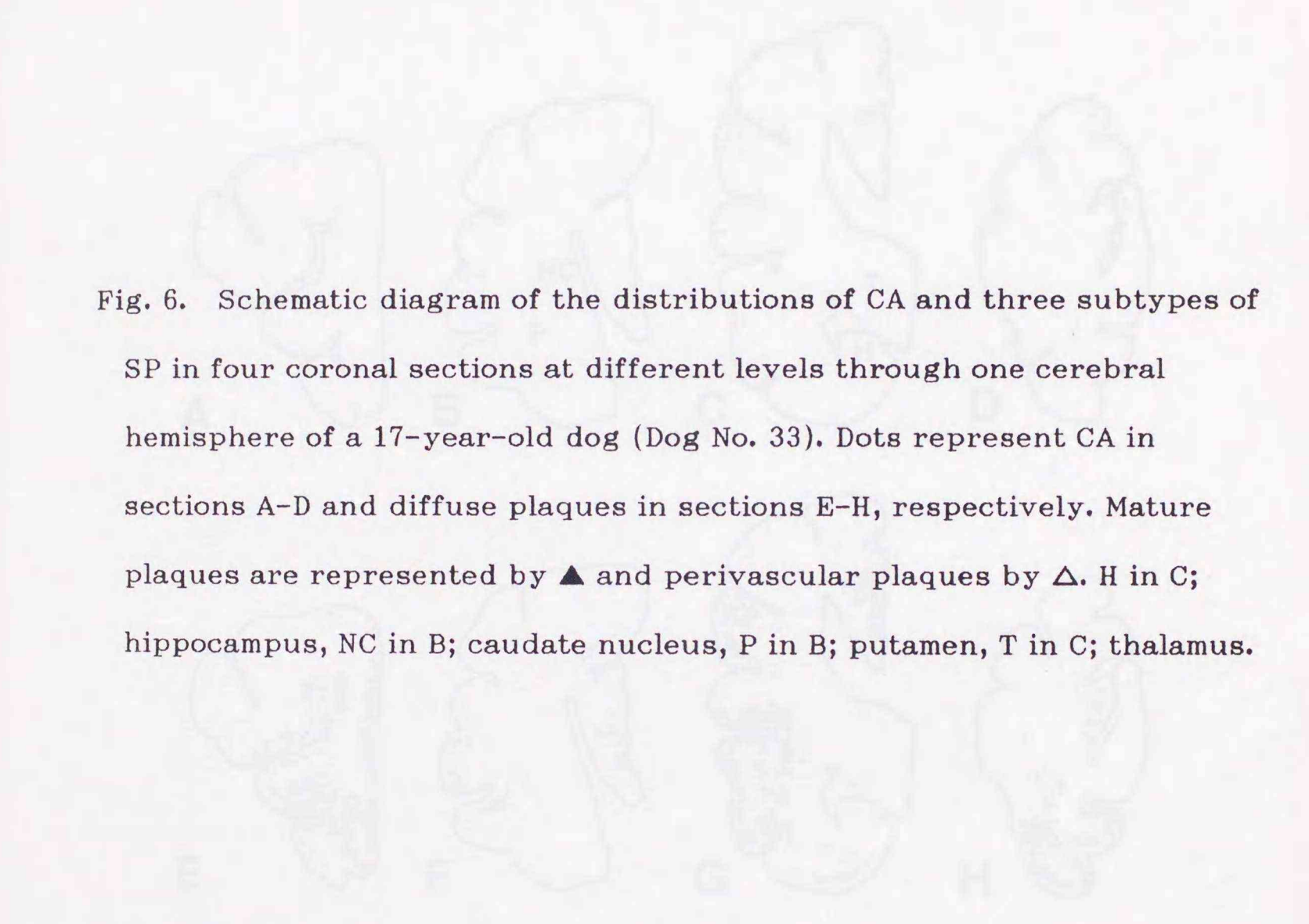
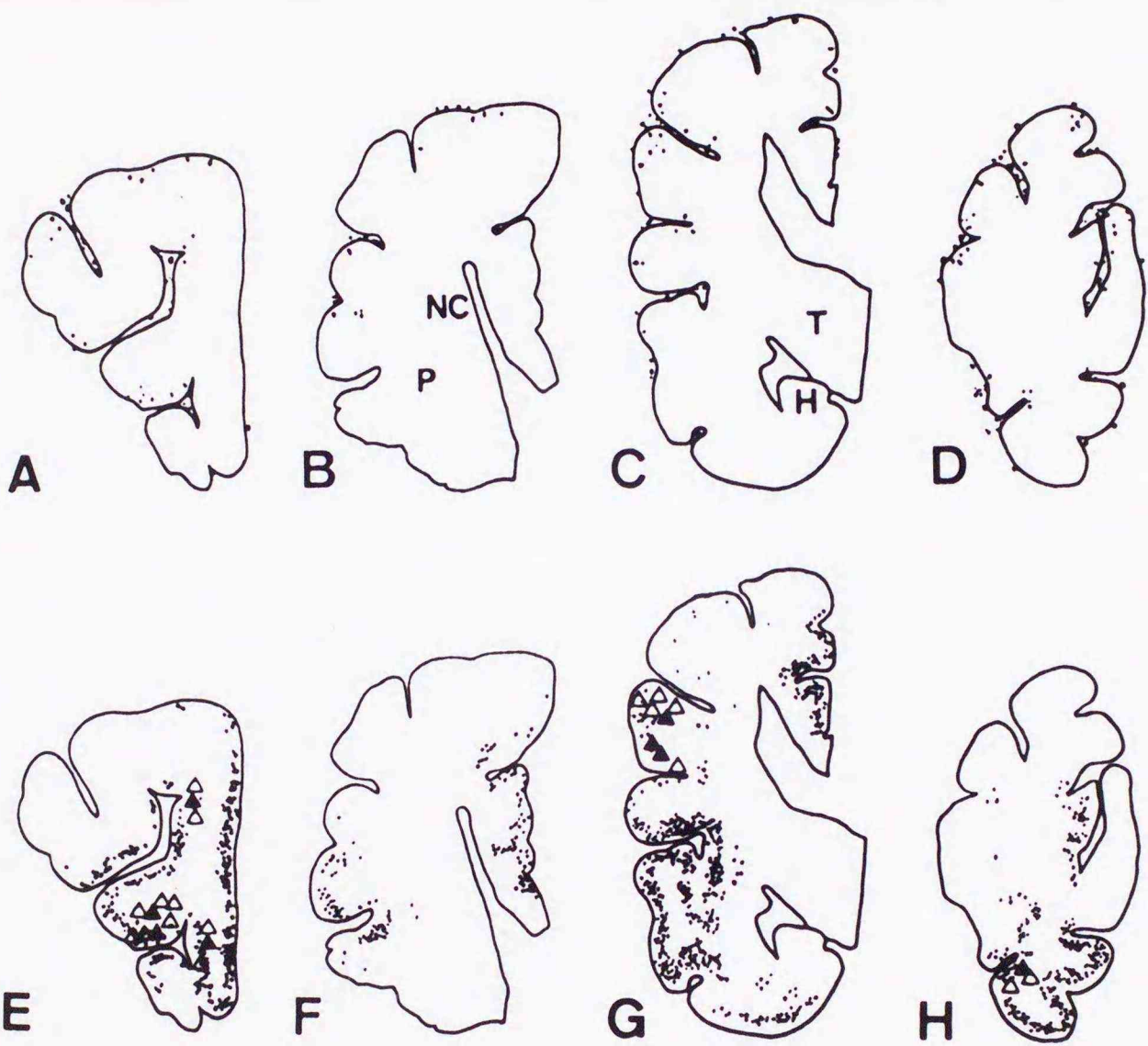


Fig. 6. Schematic diagram of the distributions of CA and three subtypes of SP in four coronal sections at different levels through one cerebral hemisphere of a 17-year-old dog (Dog No. 33). Dots represent CA in sections A-D and diffuse plaques in sections E-H, respectively. Mature plaques are represented by ▲ and perivascular plaques by Δ. H in C; hippocampus, NC in B; caudate nucleus, P in B; putamen, T in C; thalamus.



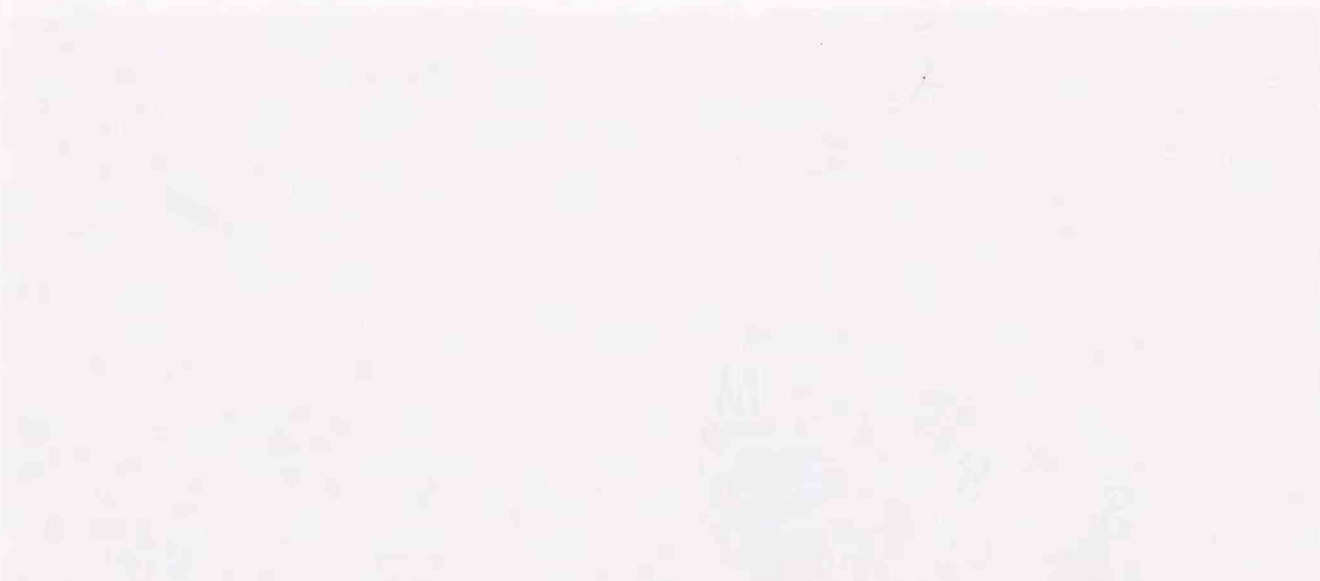
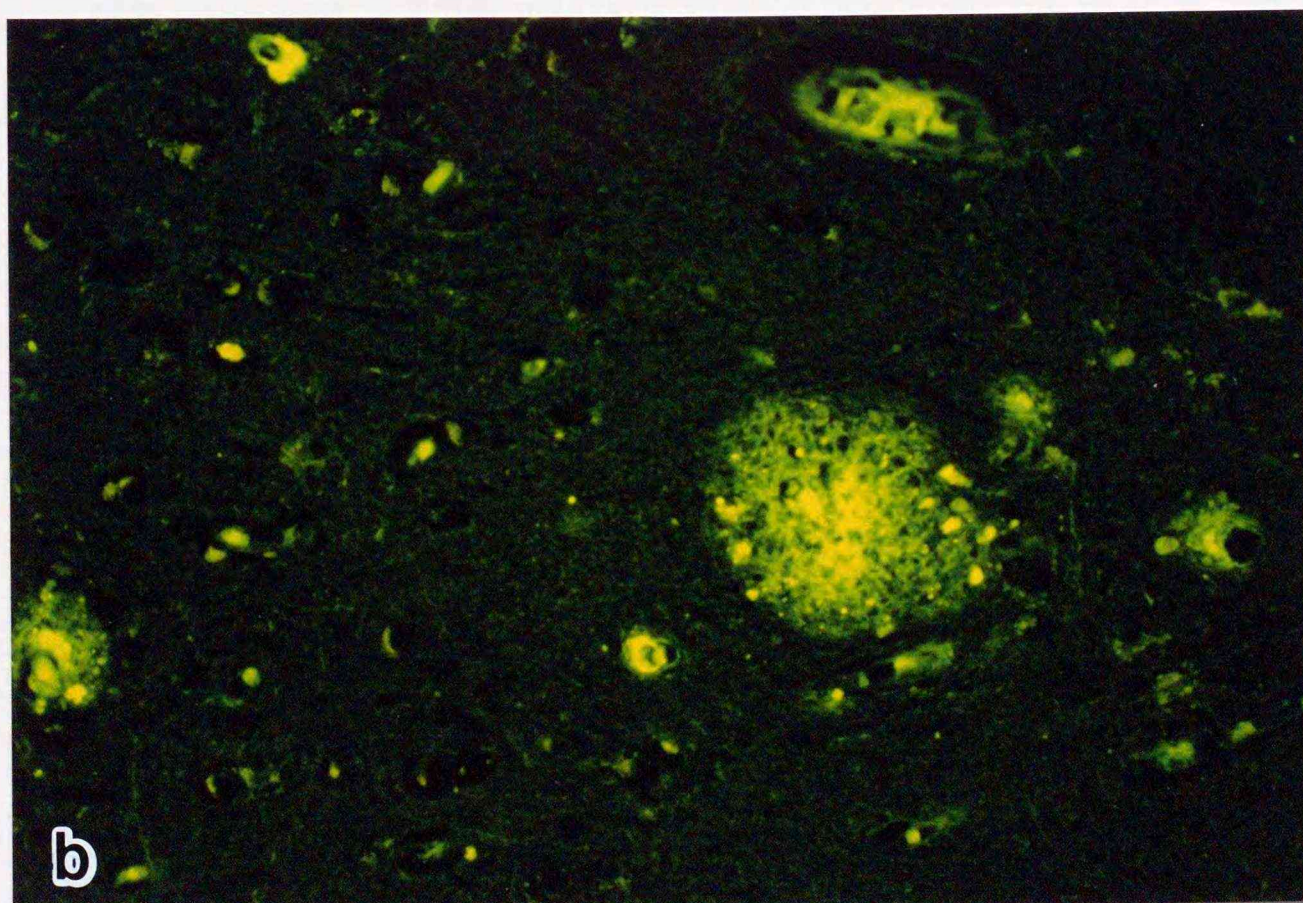
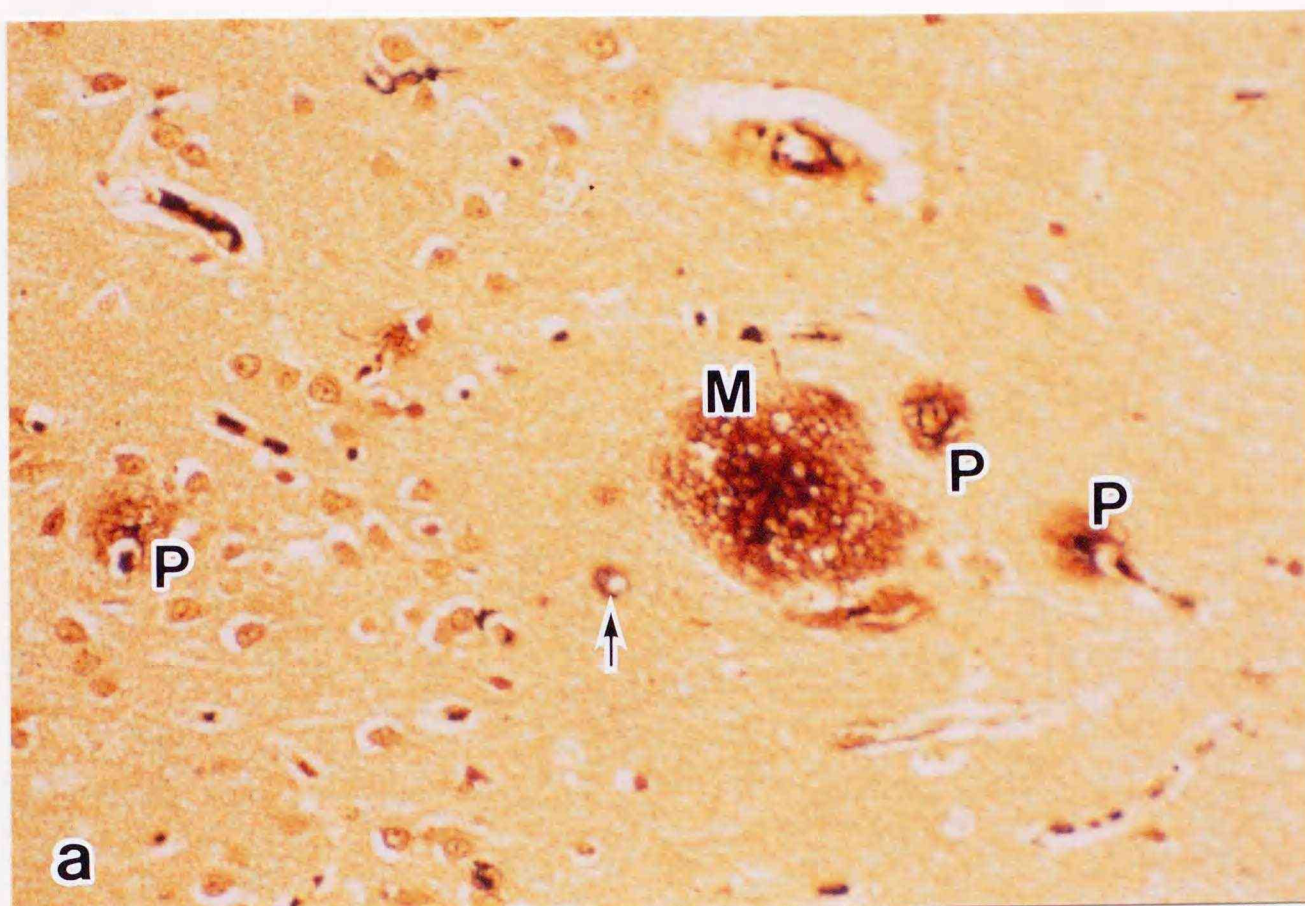


Fig. 7. Serial sections of the frontal cortex stained with modified Bielschowsky stain (a) and thioflavin S stain (b). The mature plaque (M), perivascular plaques (P) and CA (arrow) are topographically related. Dog No. 33, x180.





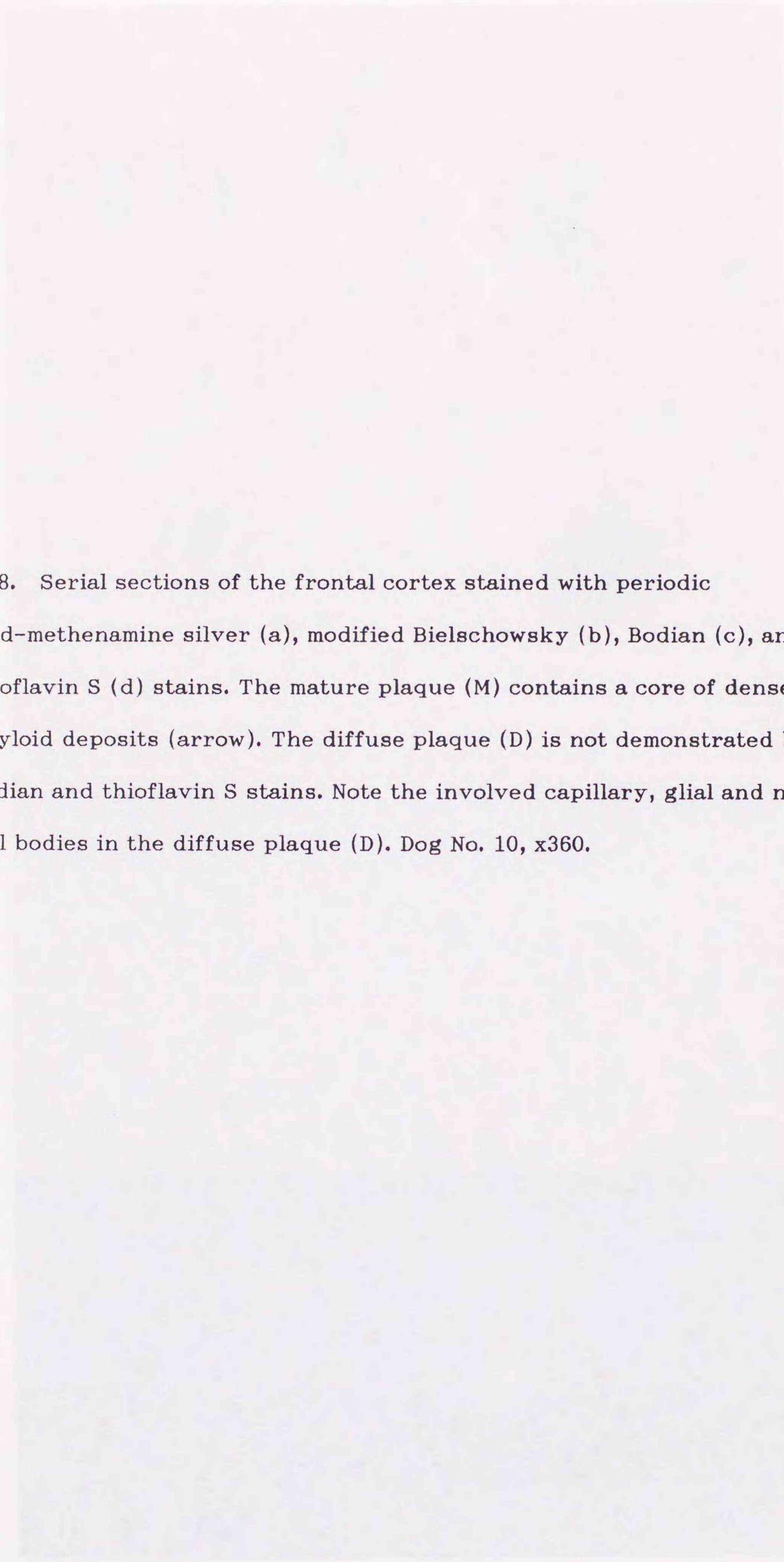
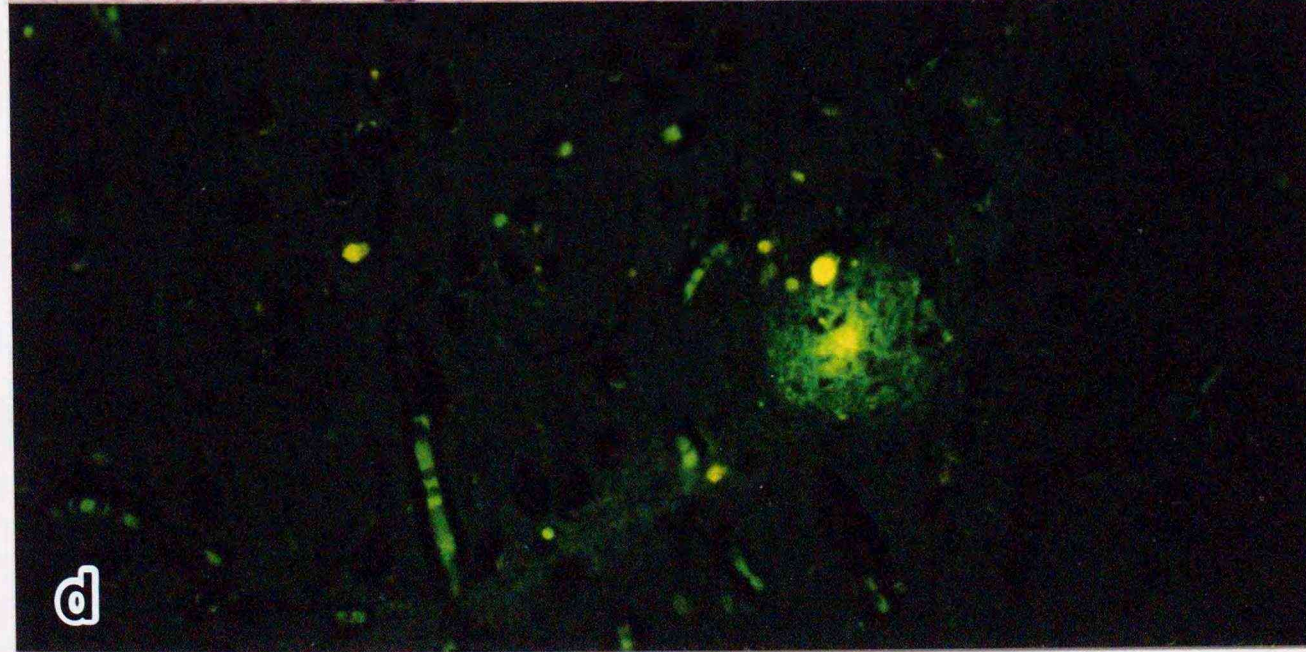
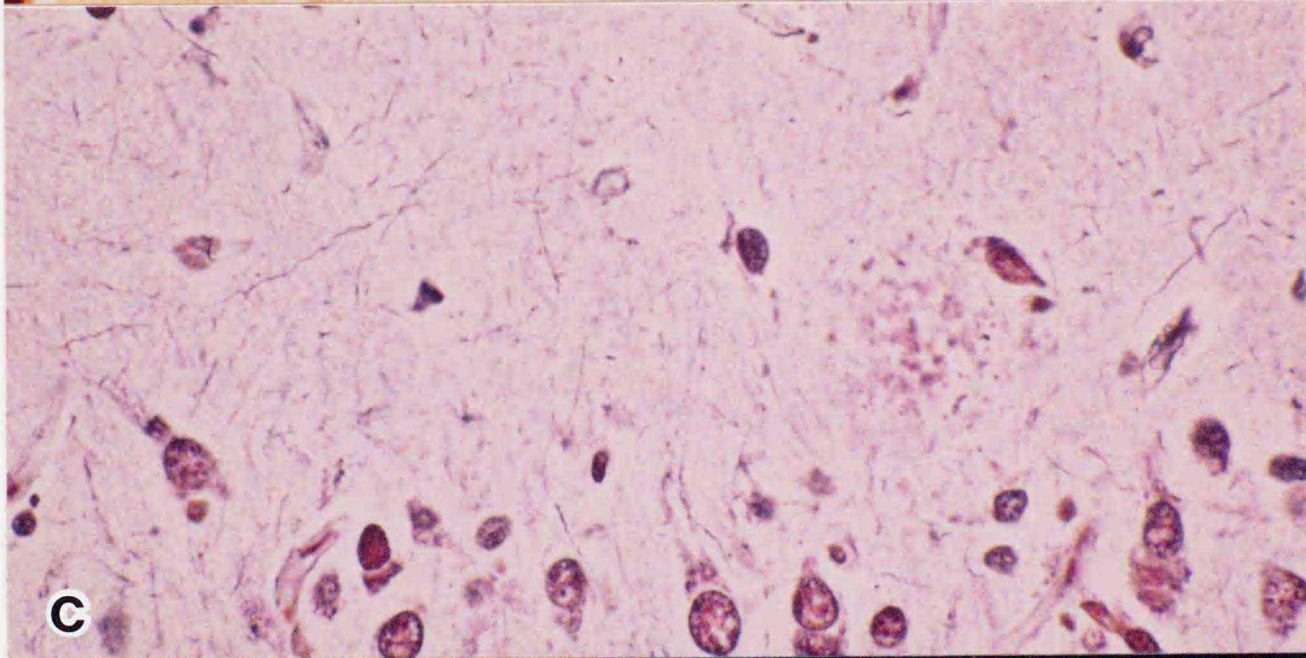
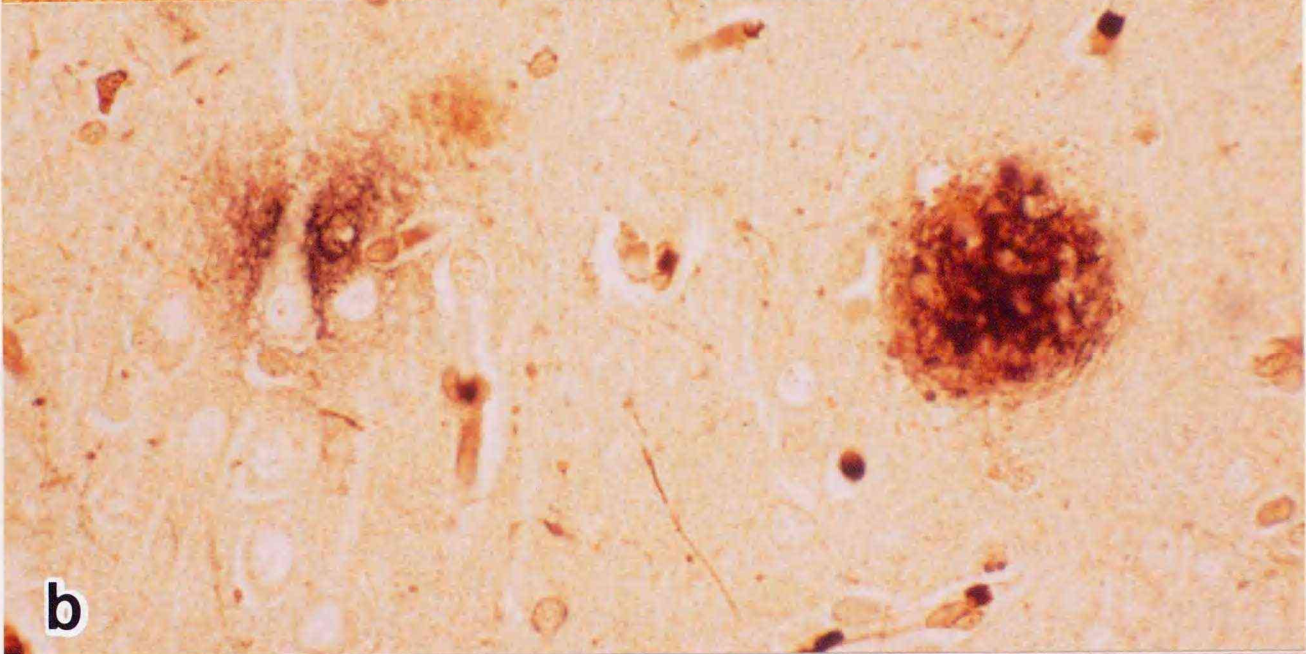
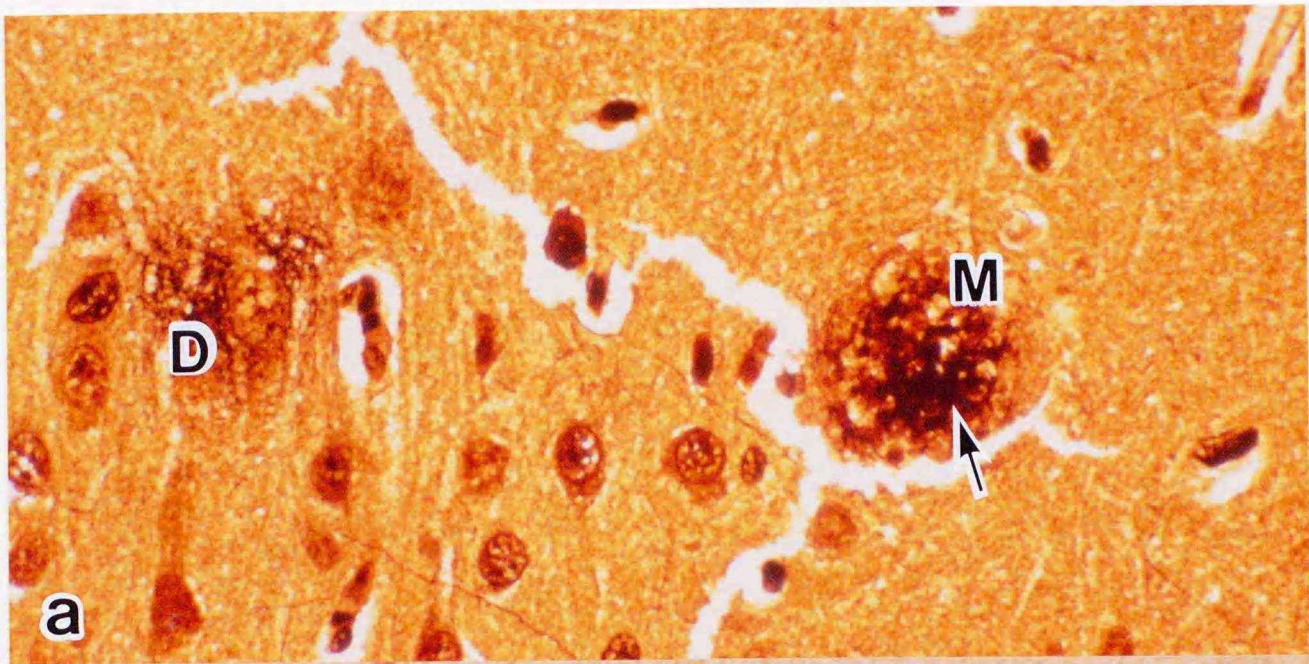


Fig. 8. Serial sections of the frontal cortex stained with periodic acid-methenamine silver (a), modified Bielschowsky (b), Bodian (c), and thioflavin S (d) stains. The mature plaque (M) contains a core of dense amyloid deposits (arrow). The diffuse plaque (D) is not demonstrated by Bodian and thioflavin S stains. Note the involved capillary, glial and nerve cell bodies in the diffuse plaque (D). Dog No. 10, x360.



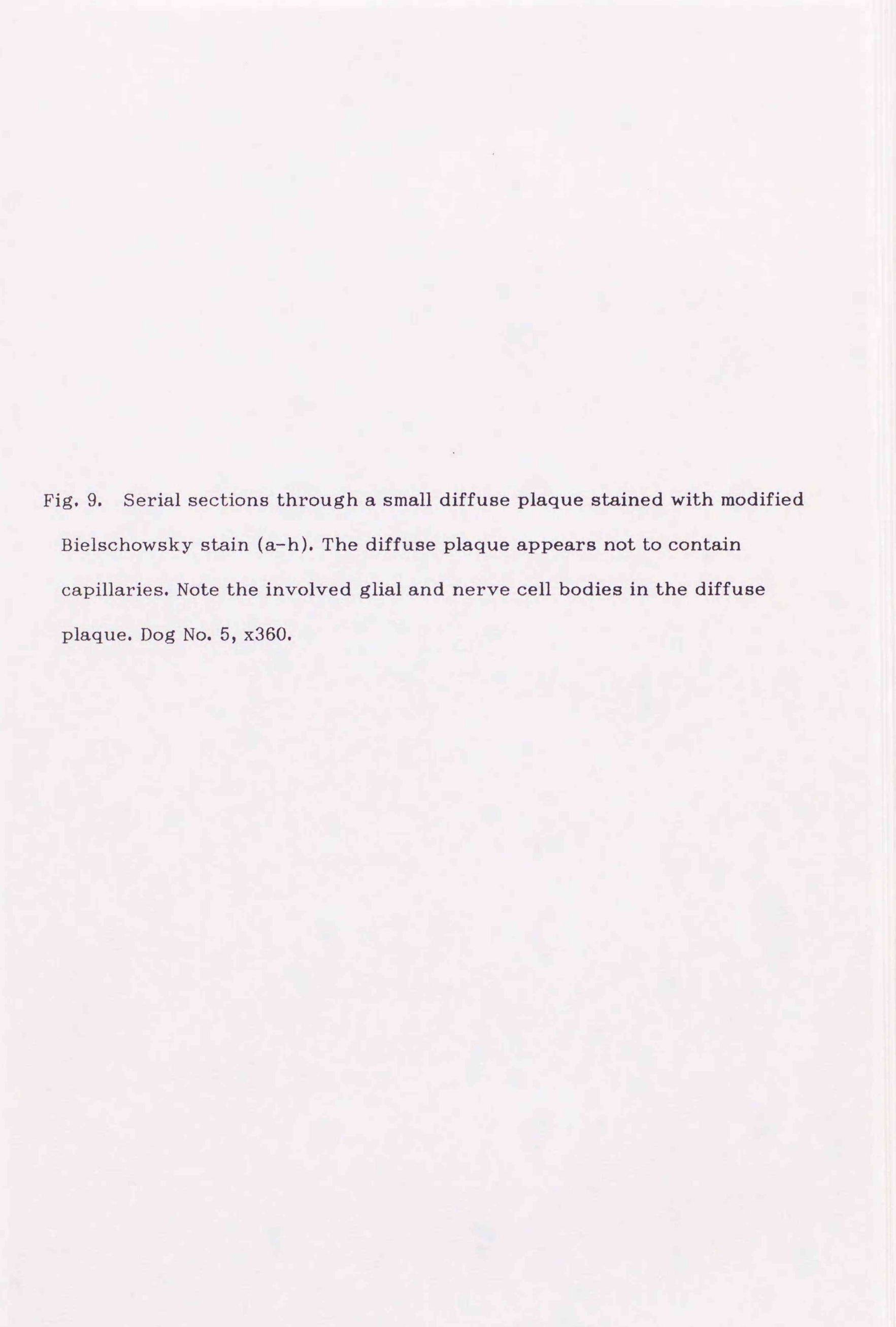
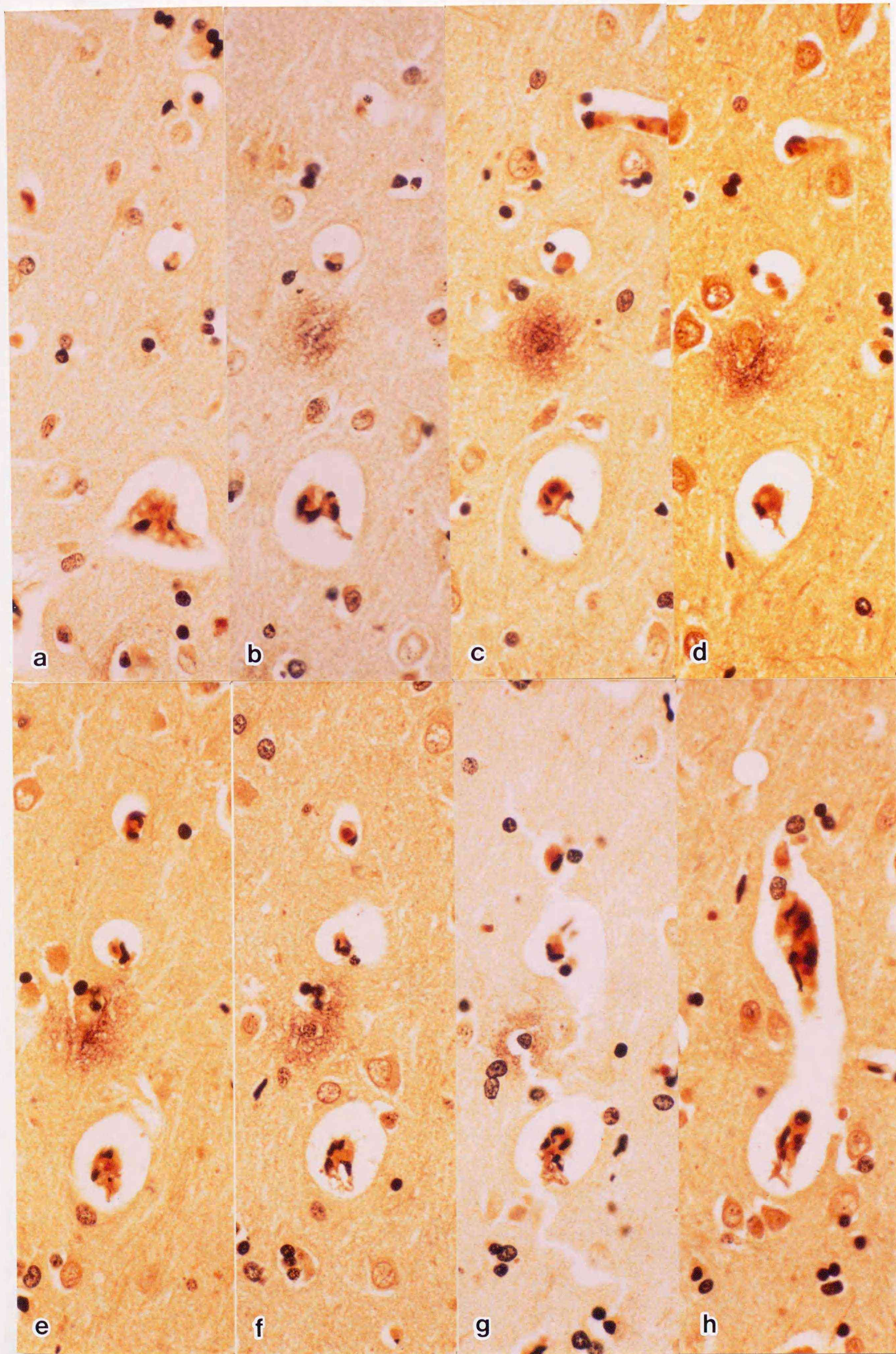


Fig. 9. Serial sections through a small diffuse plaque stained with modified Bielschowsky stain (a-h). The diffuse plaque appears not to contain capillaries. Note the involved glial and nerve cell bodies in the diffuse plaque. Dog No. 5, x360.



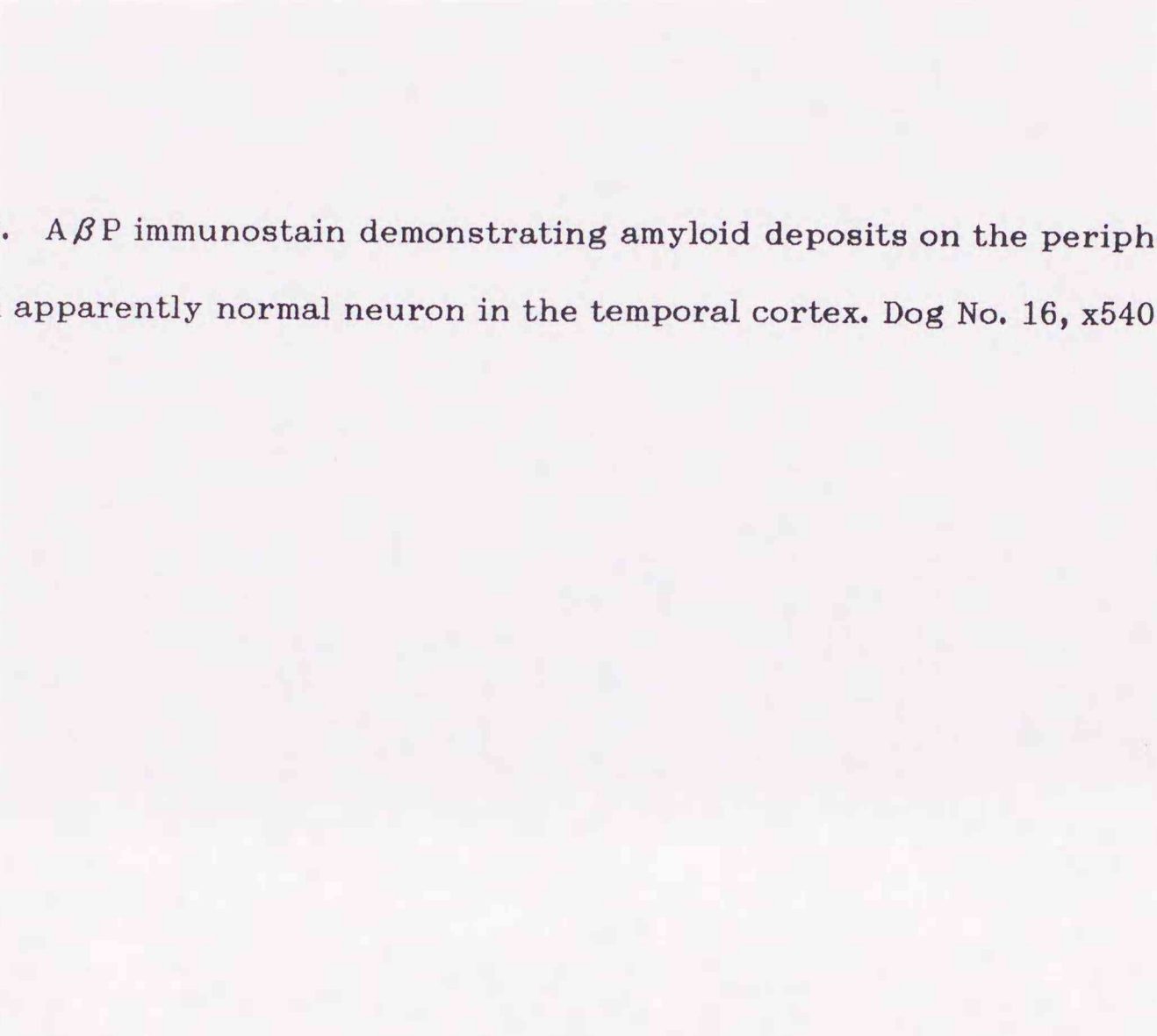


Fig. 10. A β P immunostain demonstrating amyloid deposits on the periphery of an apparently normal neuron in the temporal cortex. Dog No. 16, x540.

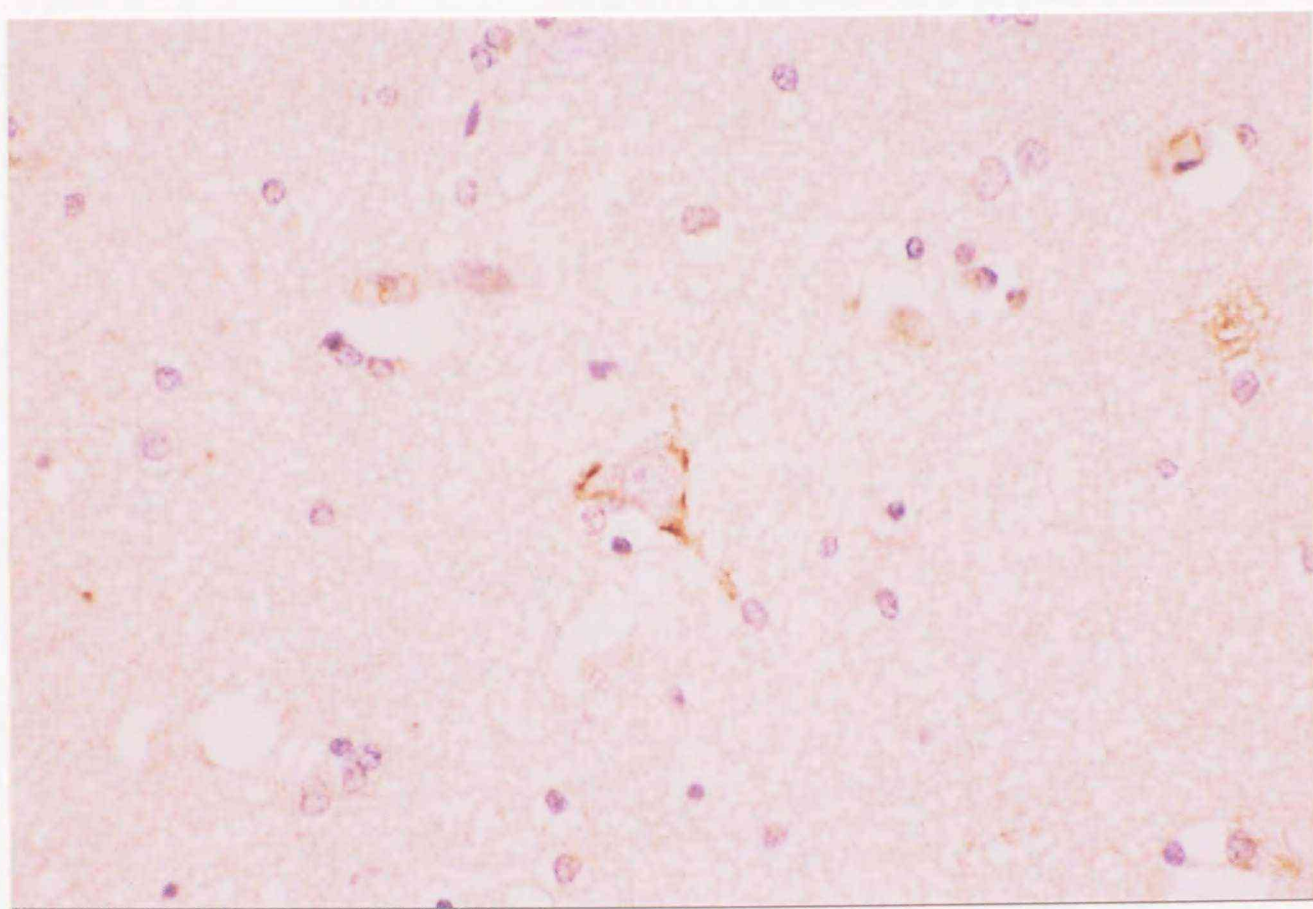


Fig. 11. Cerebral cortices from a 8-month-old dog (Dog No. 5) (a) and a 17-year-old dog (Dog No. 25) (b). Note the apparent neuronal loss in b. Nissl stain, x75.

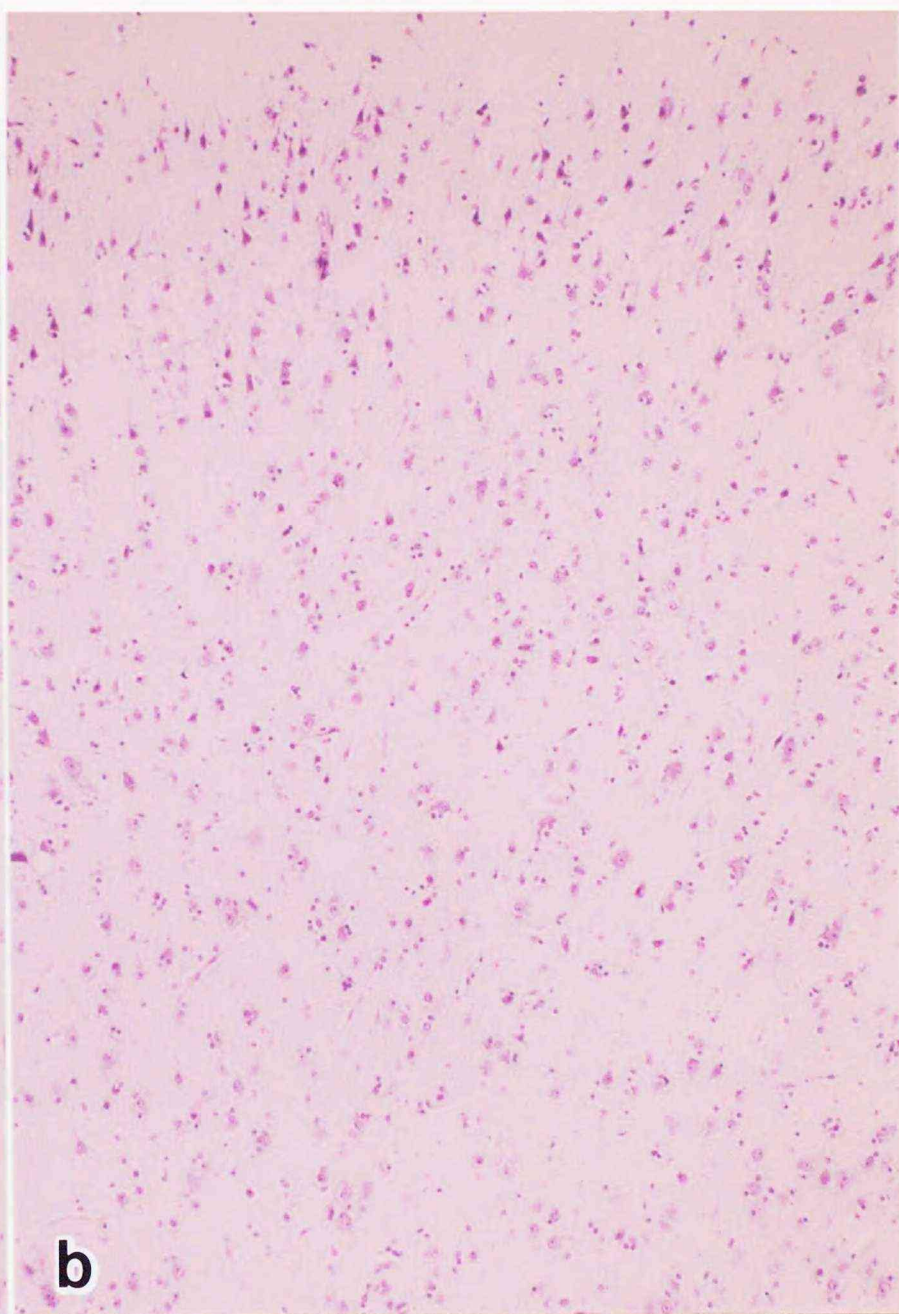
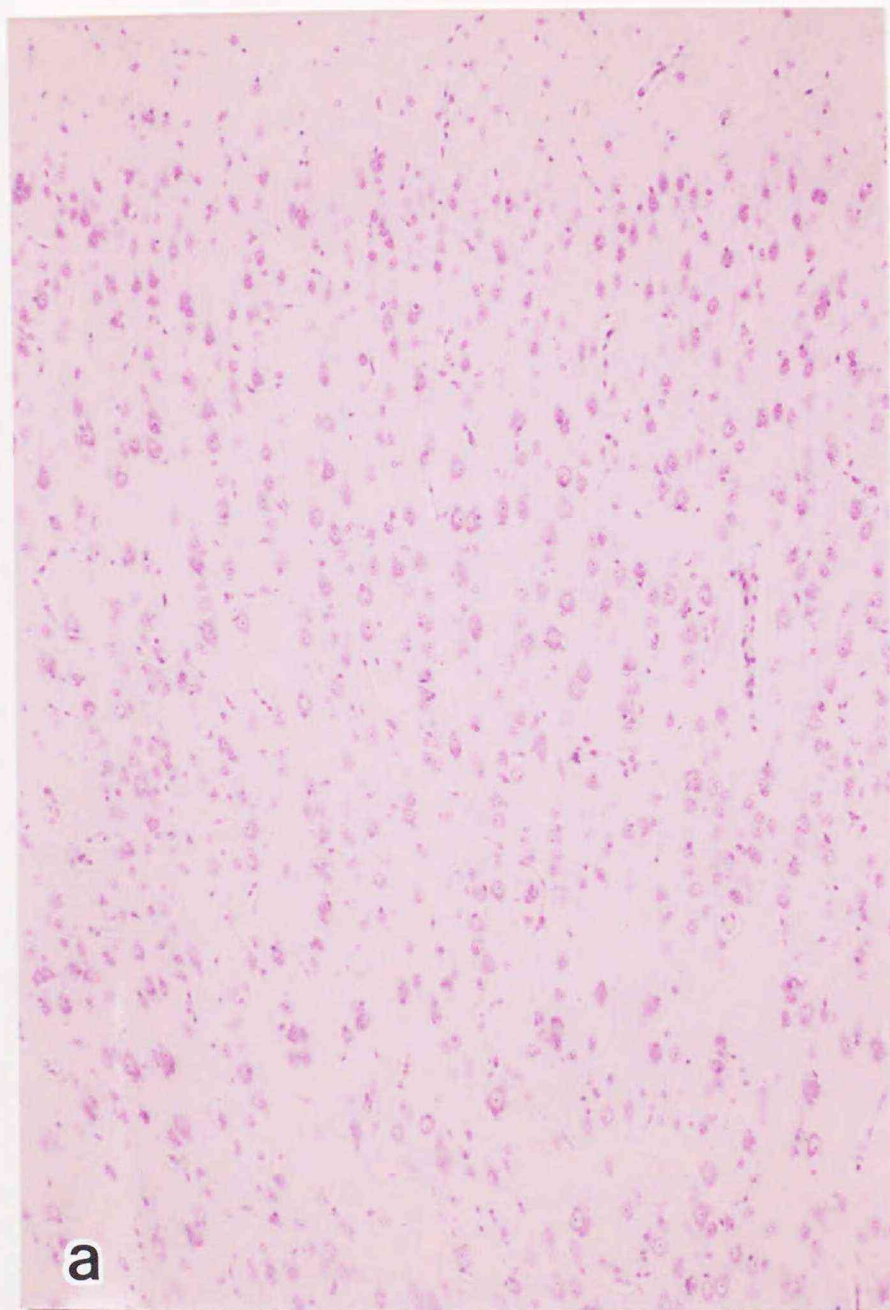
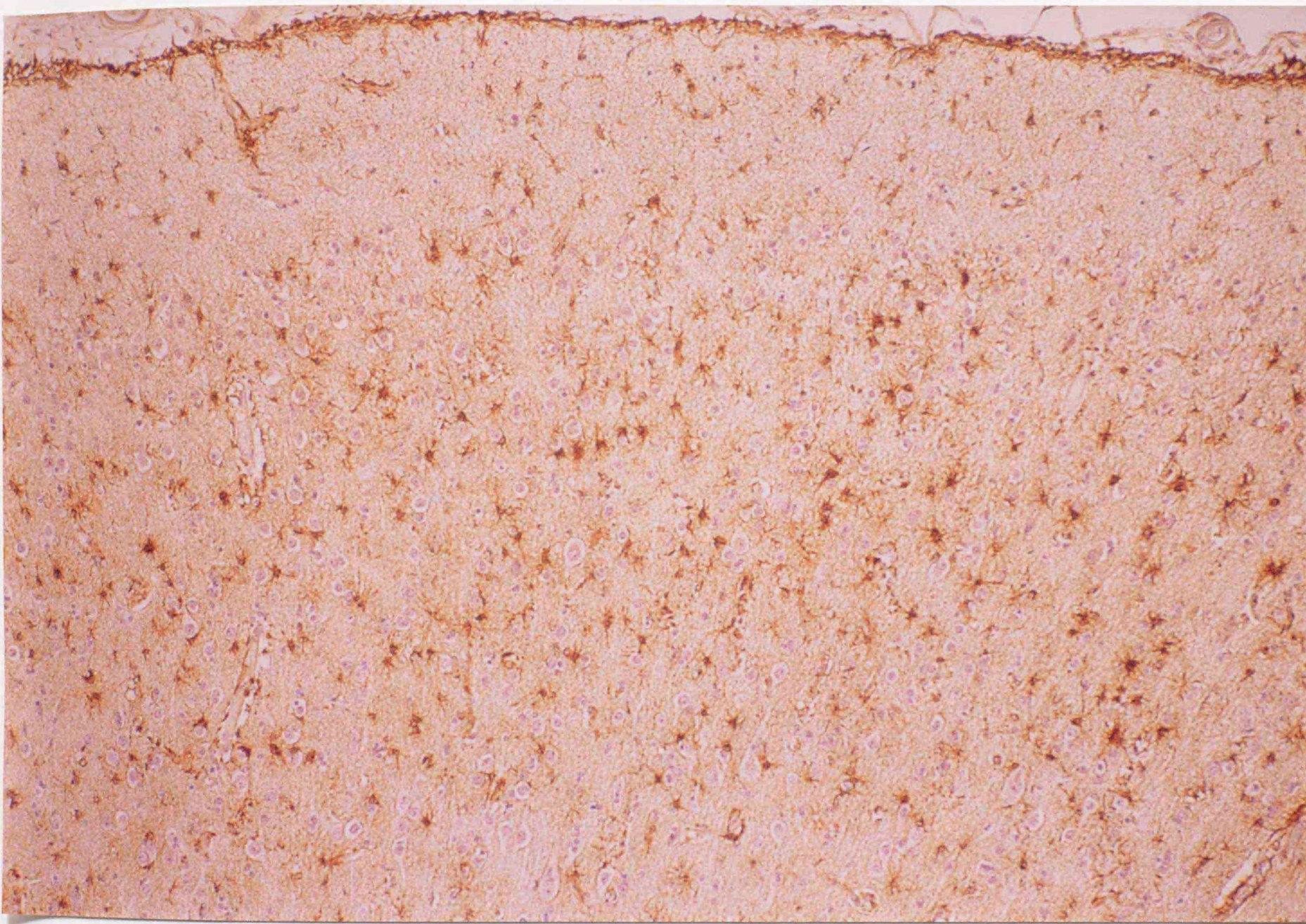




Fig. 12. Cerebral cortex from a 17-year-old dog (Dog No. 25), showing a considerable increase in glial fibrillary acidic protein (GFAP)-positive astrocytes (PA). GFAP immunostain, x120.



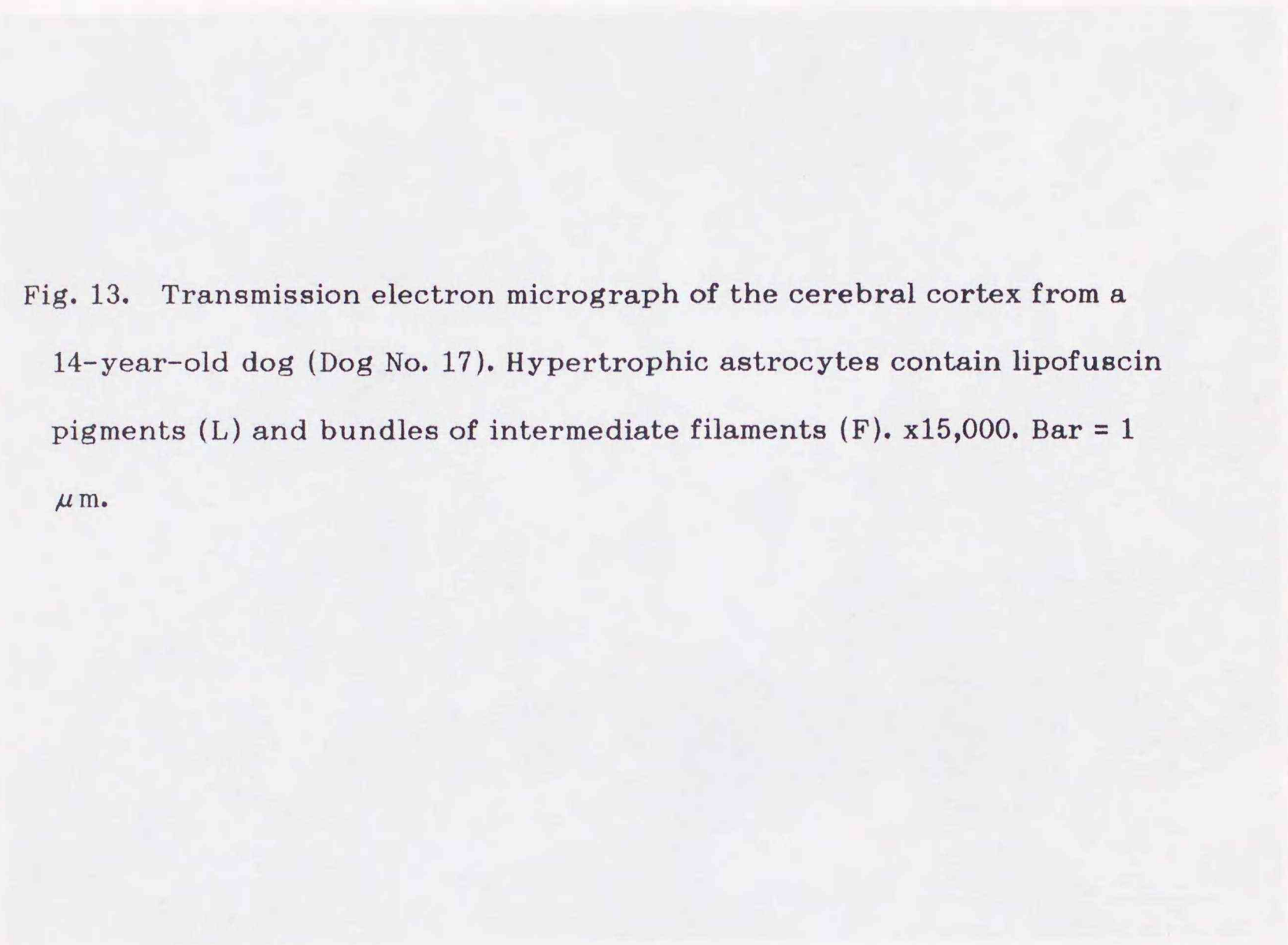
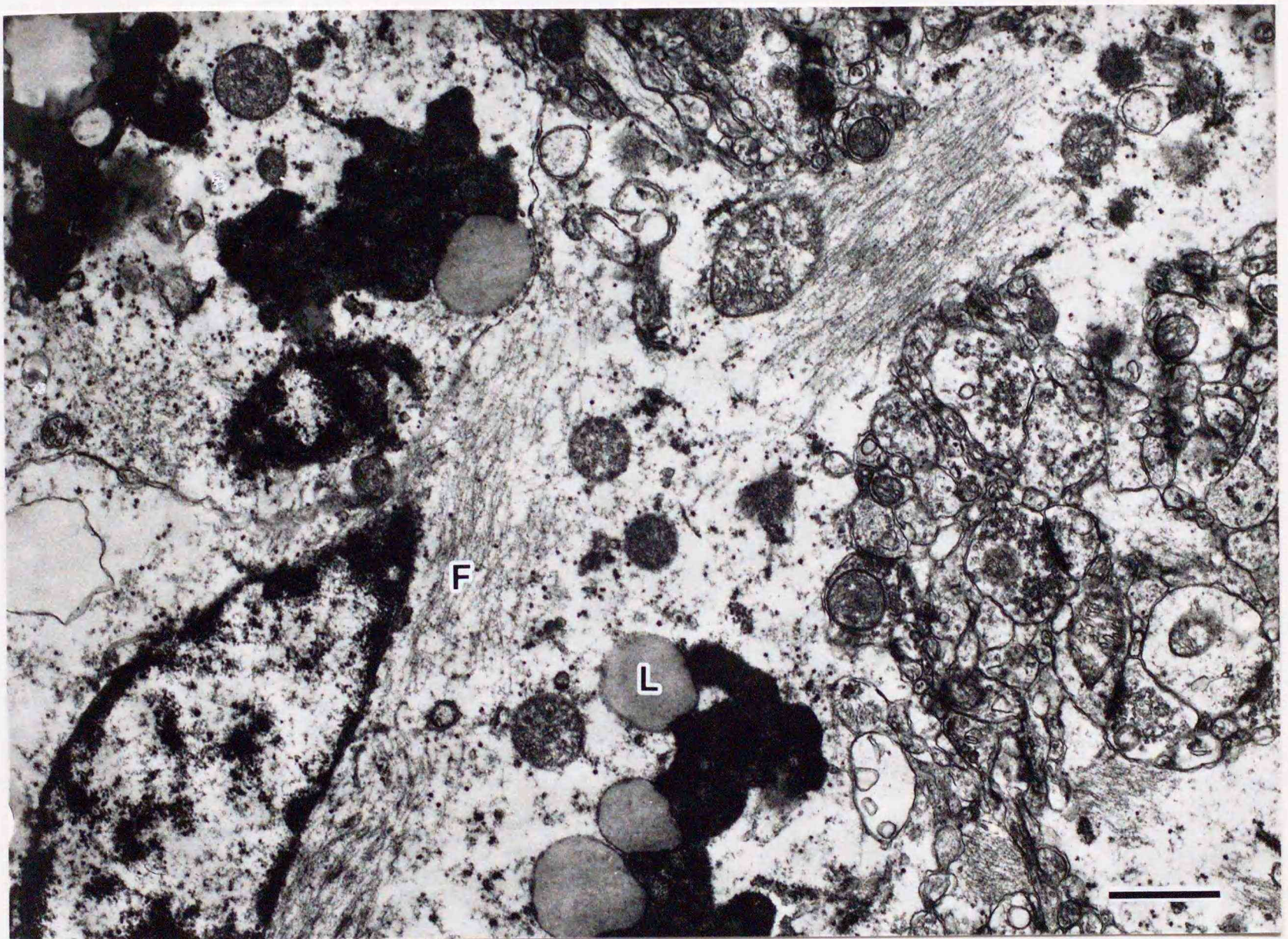


Fig. 13. Transmission electron micrograph of the cerebral cortex from a 14-year-old dog (Dog No. 17). Hypertrophic astrocytes contain lipofuscin pigments (L) and bundles of intermediate filaments (F). x15,000. Bar = 1 μ m.



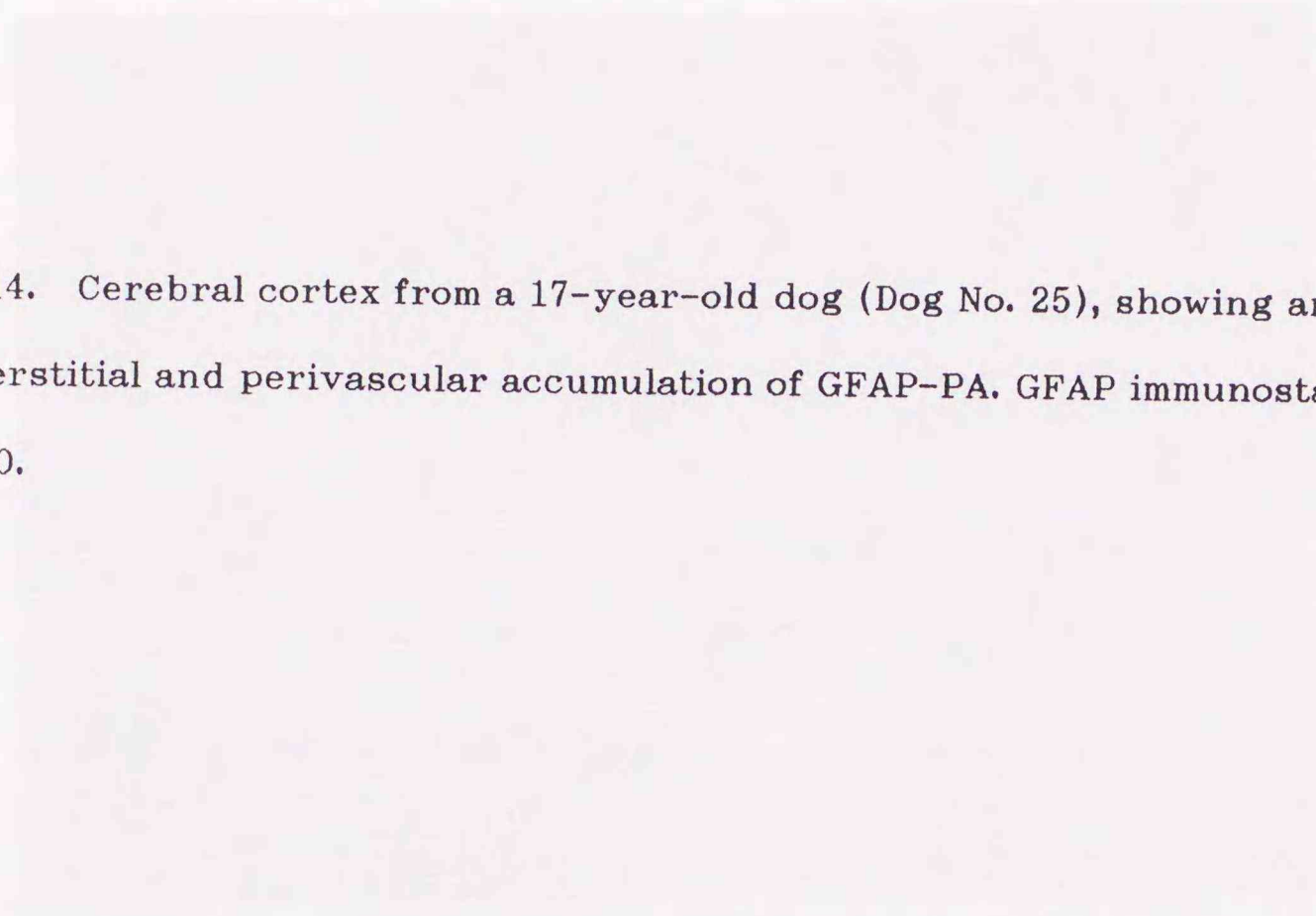


Fig. 14. Cerebral cortex from a 17-year-old dog (Dog No. 25), showing an interstitial and perivascular accumulation of GFAP-PA. GFAP immunostain, x240.

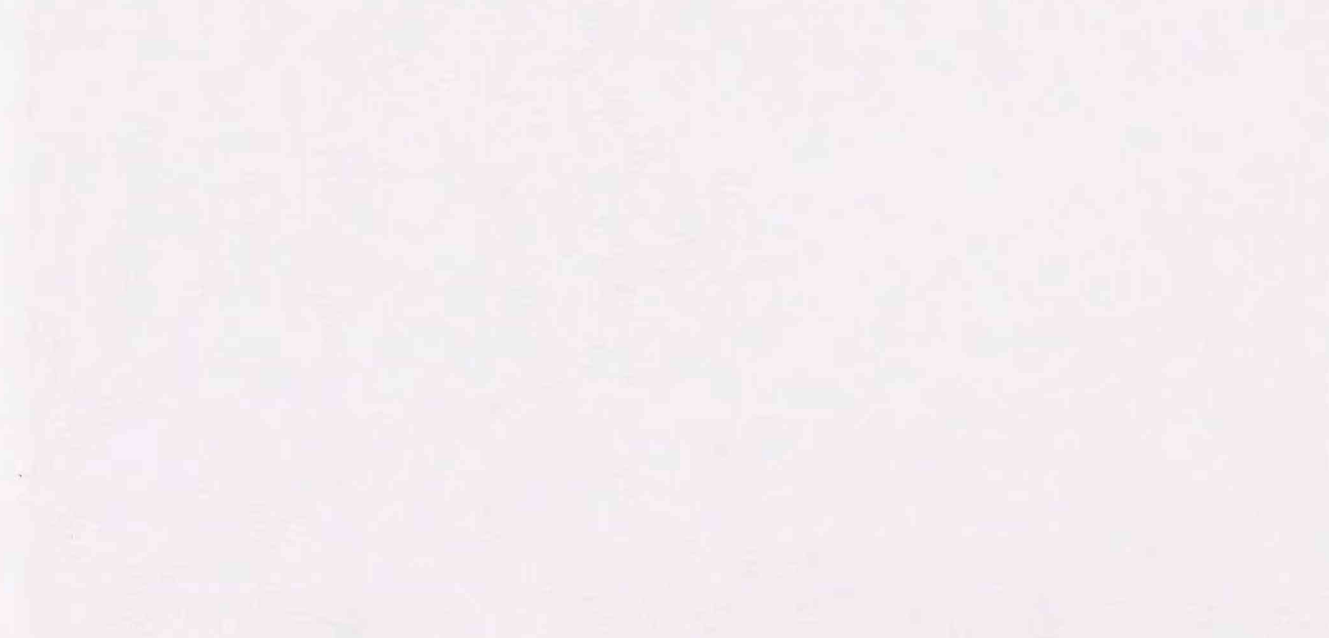
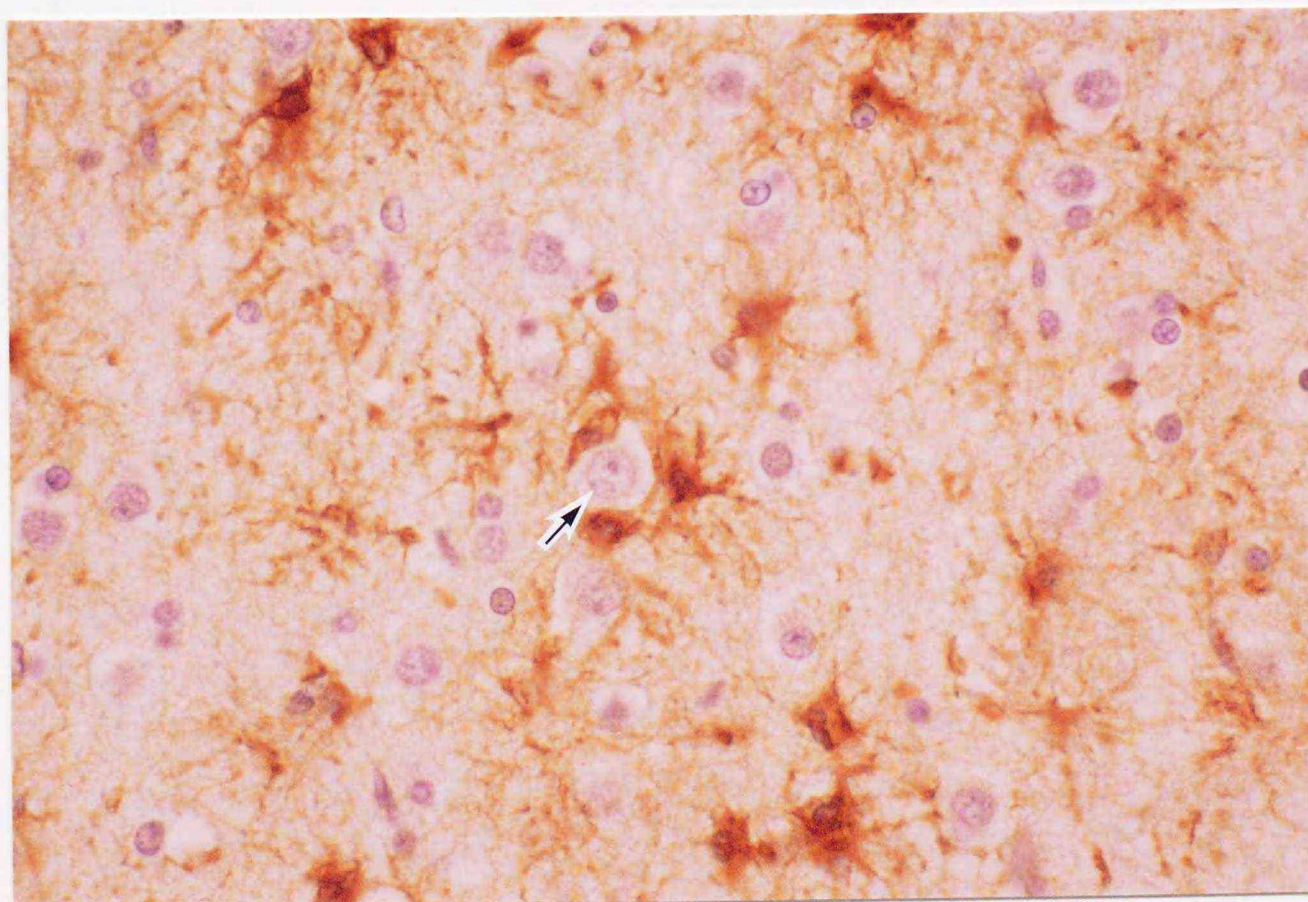
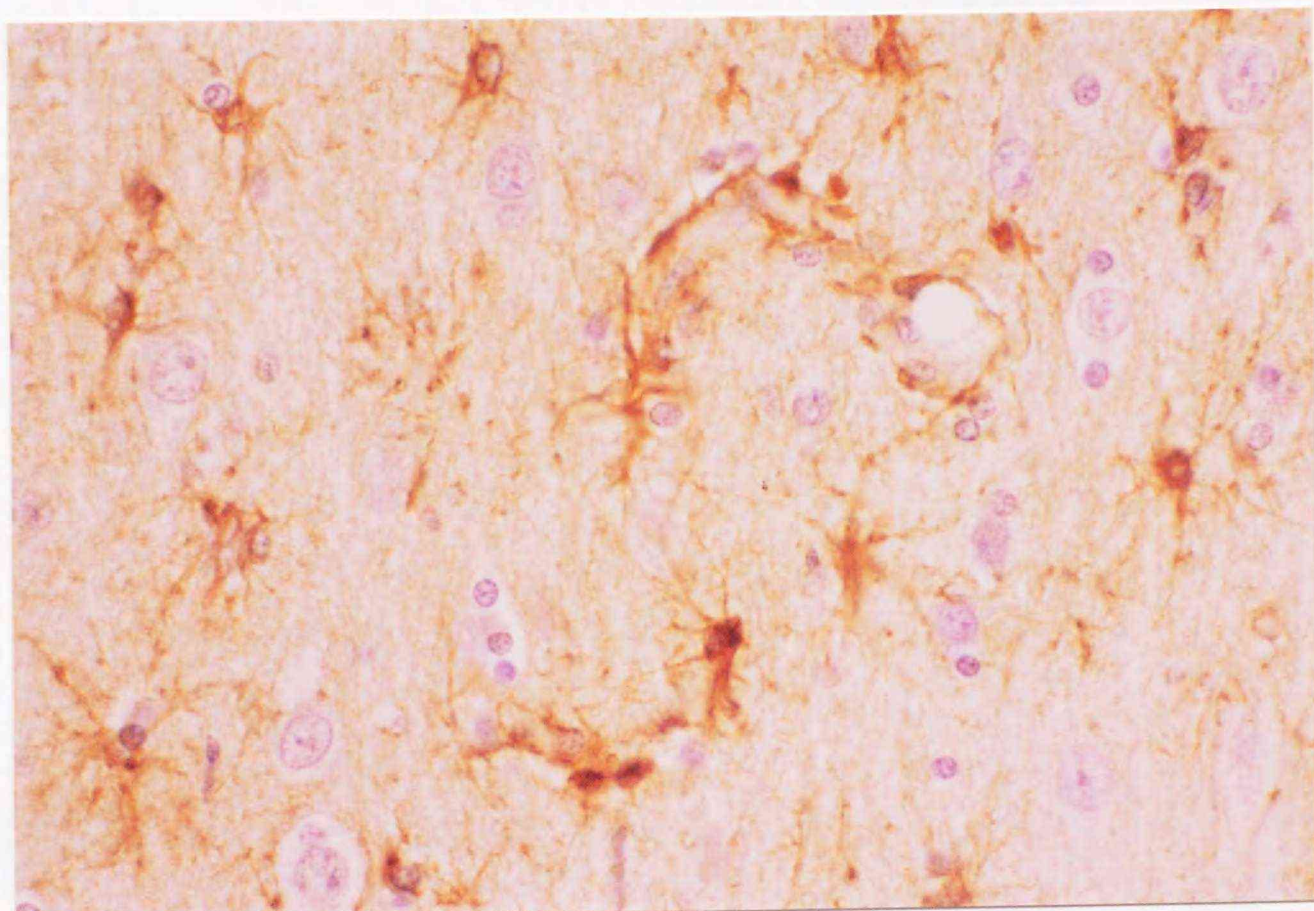


Fig. 15. Cerebral cortex from a 17-year-old dog (Dog No. 25), showing hypertrophic GFAP-PA. Note the GFAP-PA surrounding the nerve cell body (arrow). GFAP immunostain, x240.



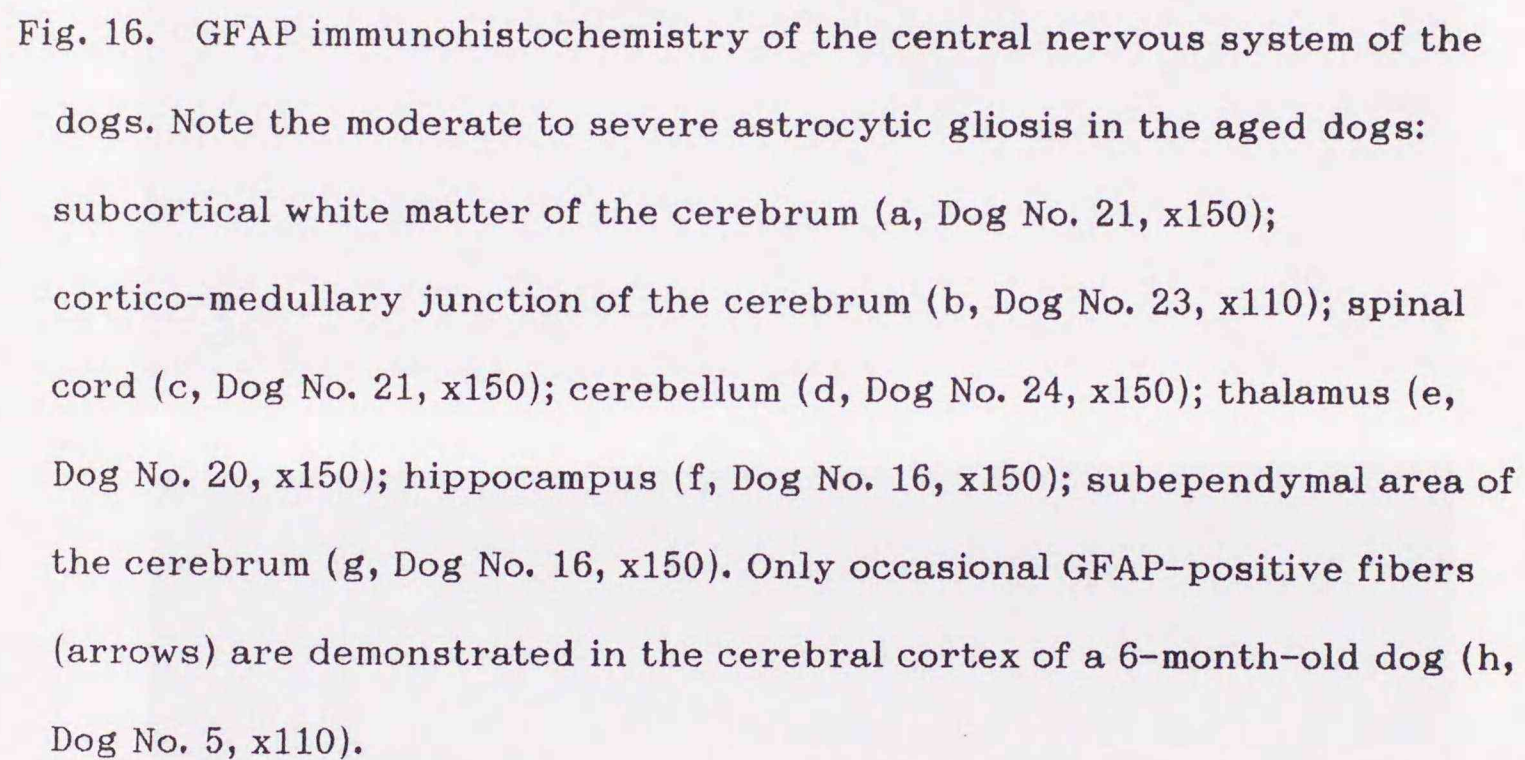
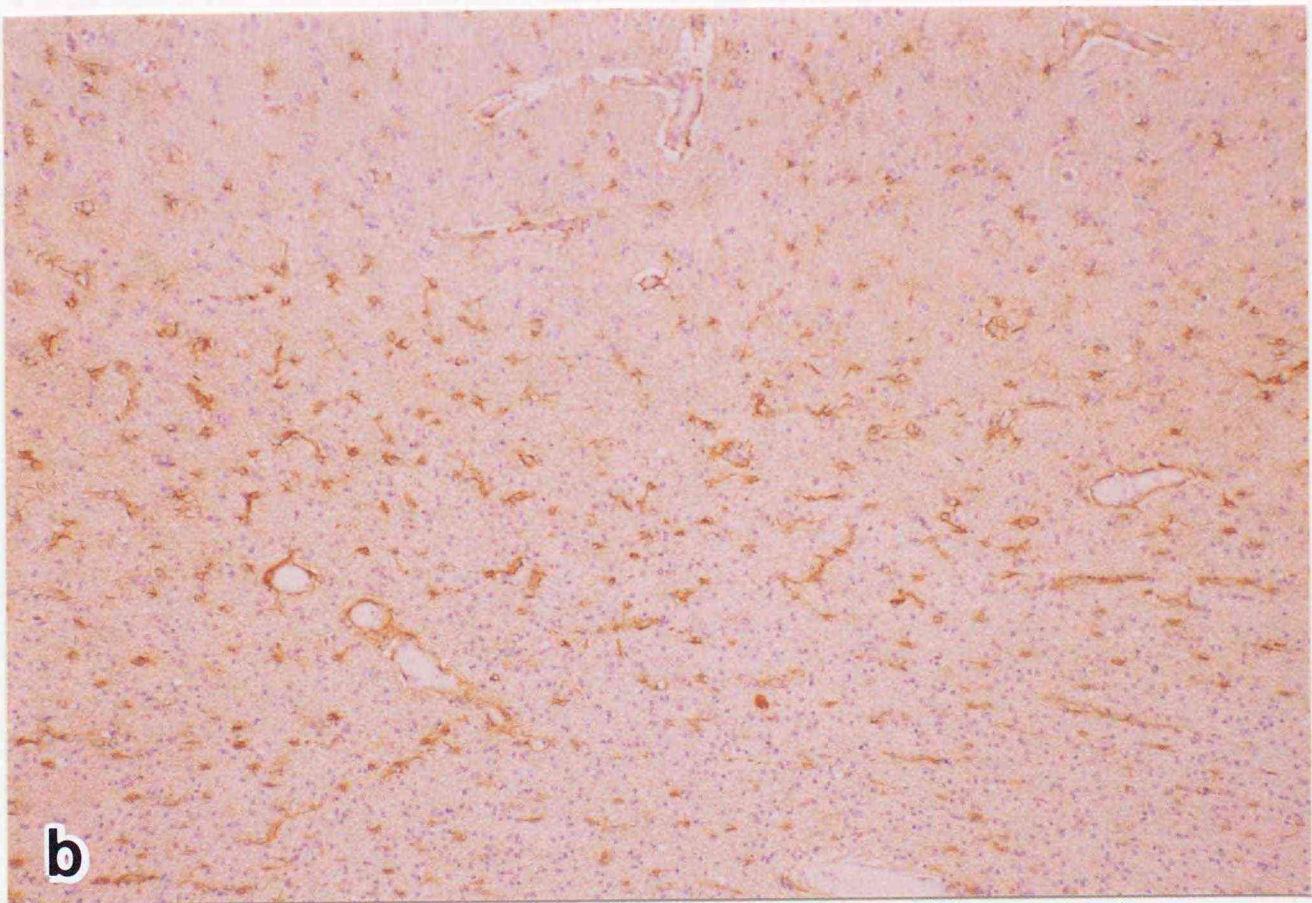
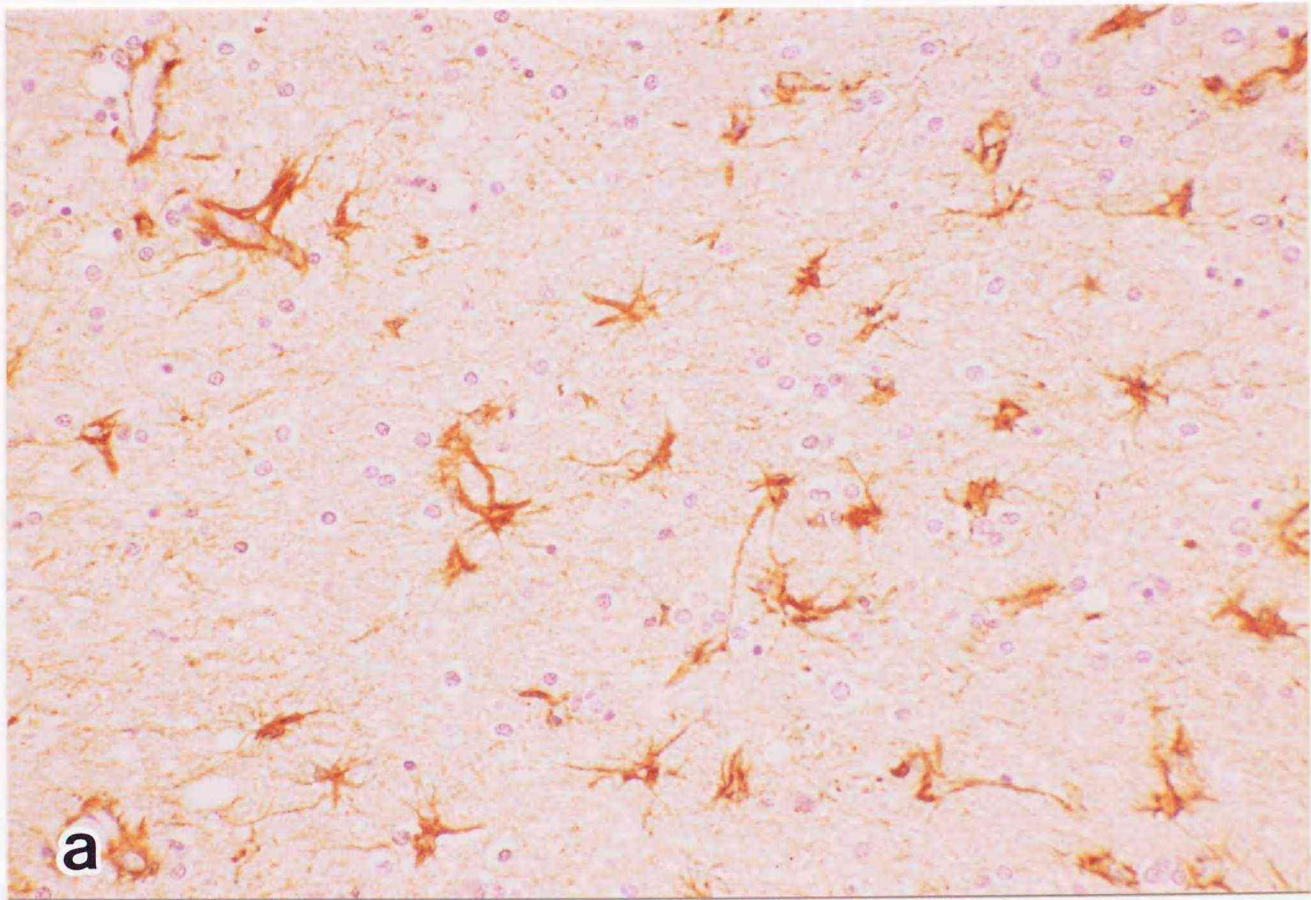
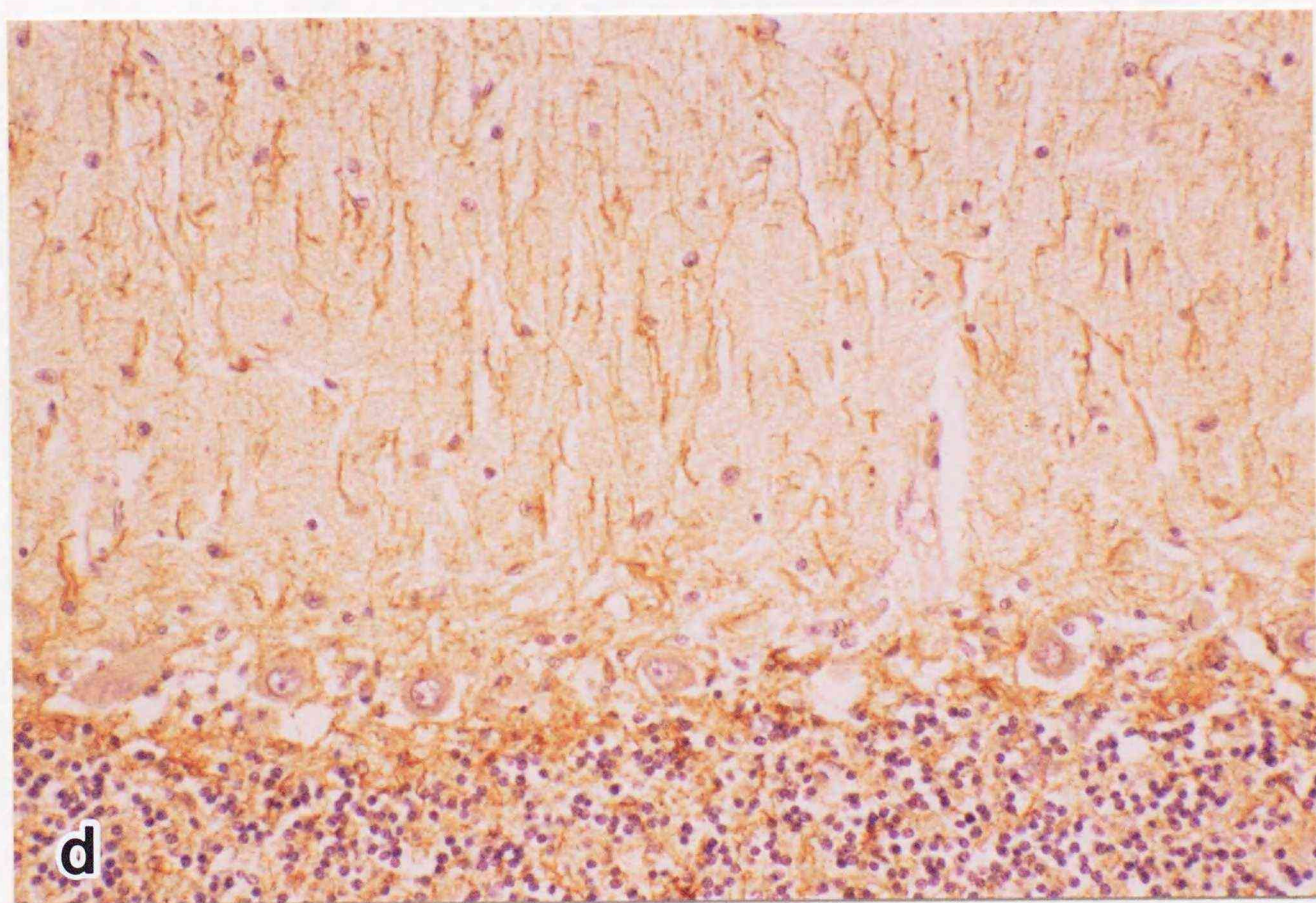
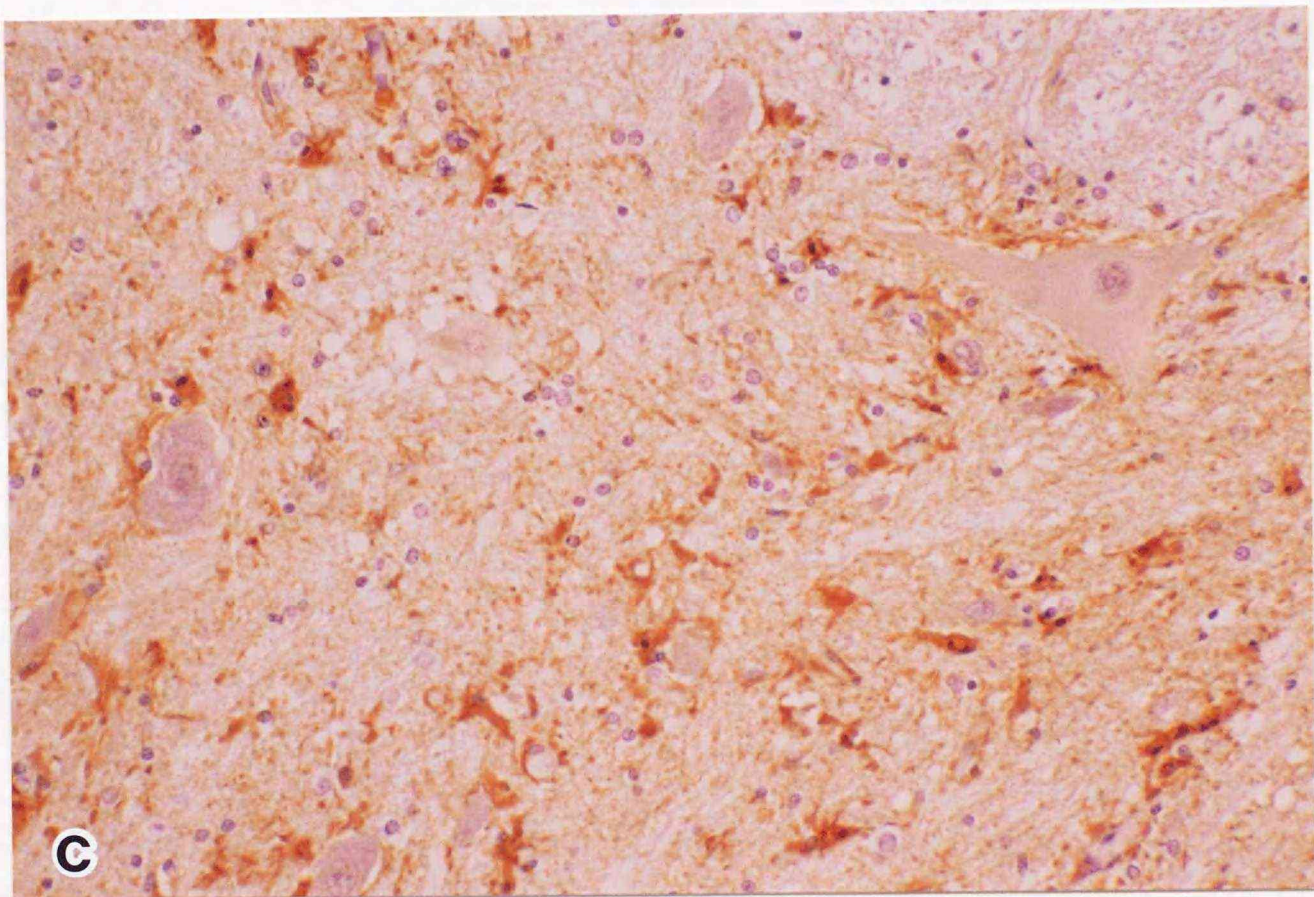
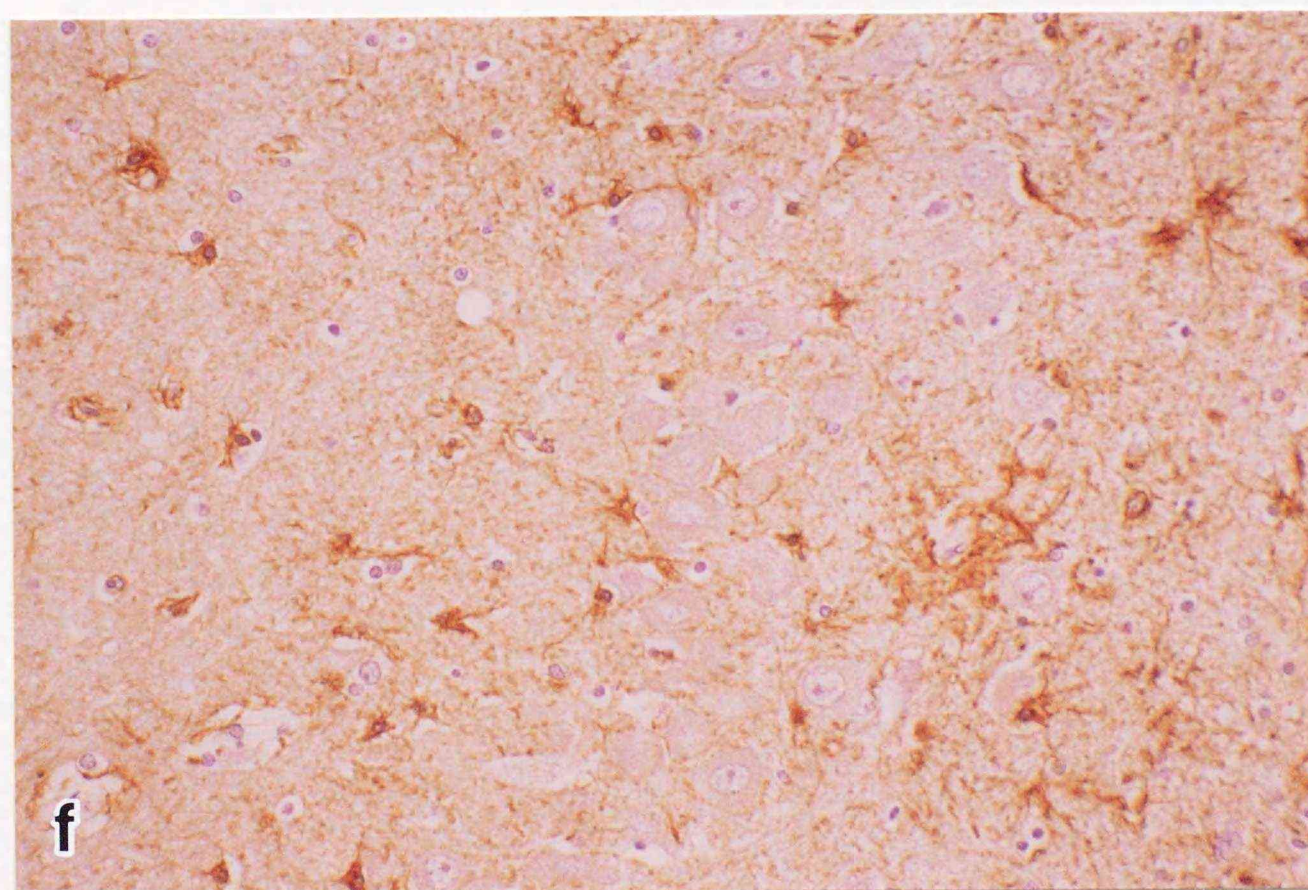
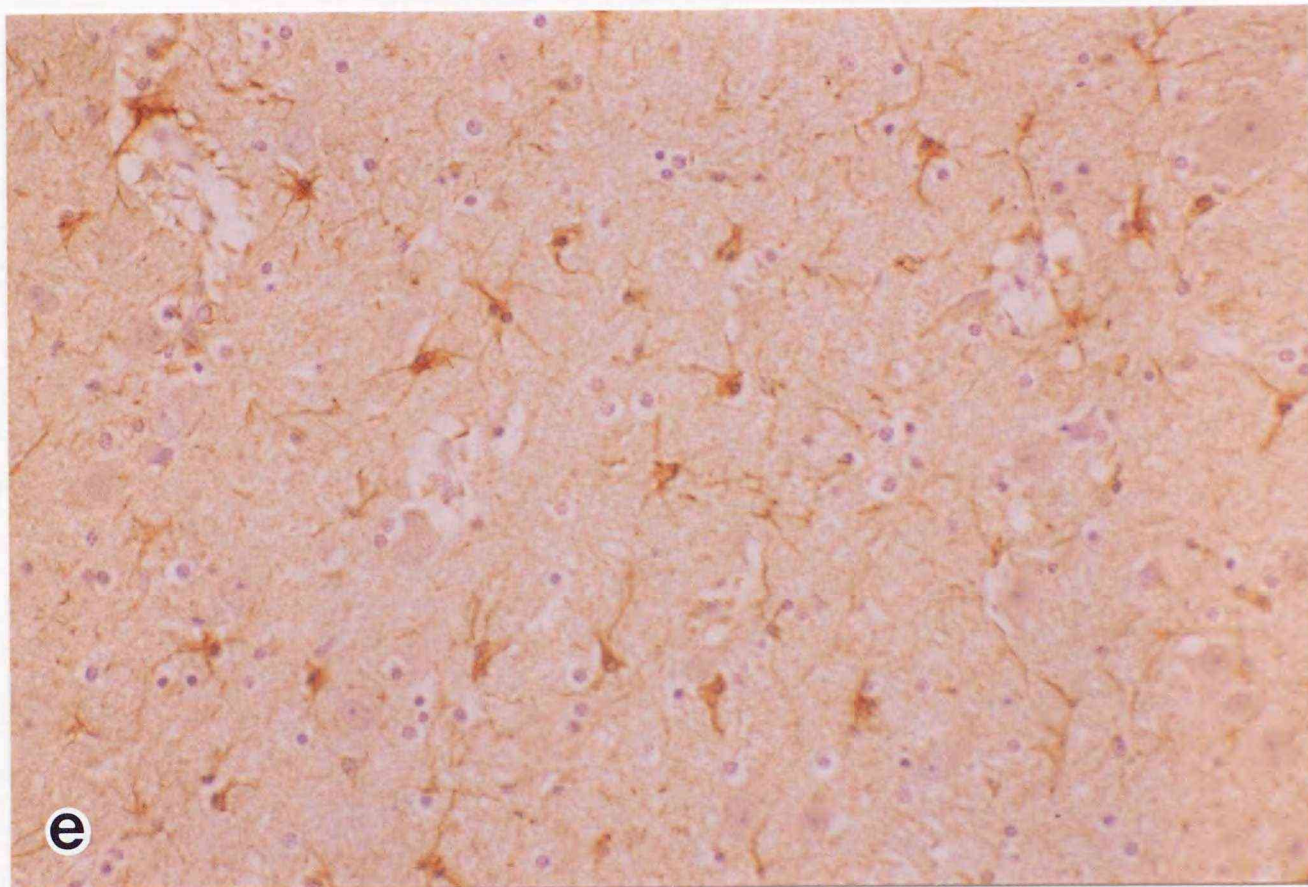
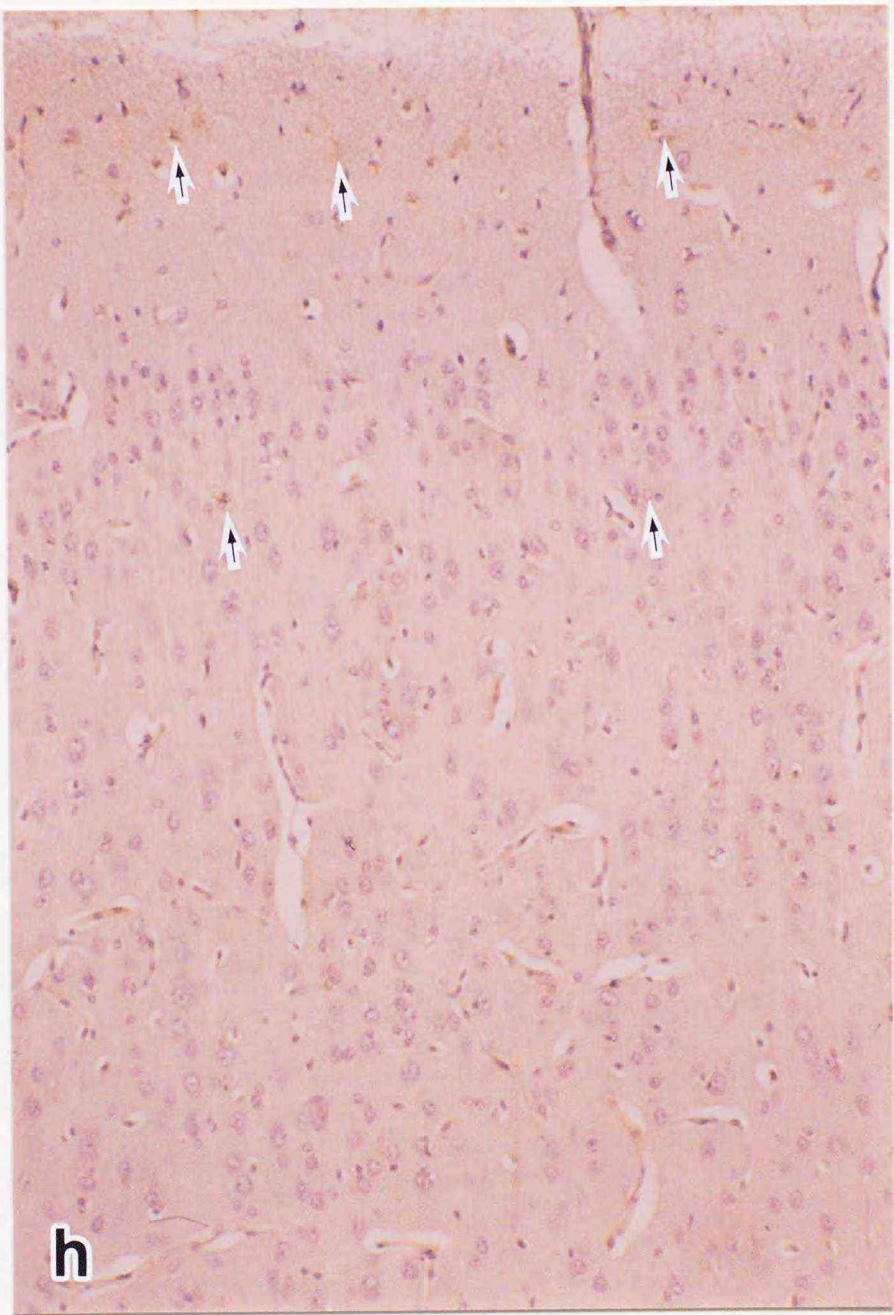
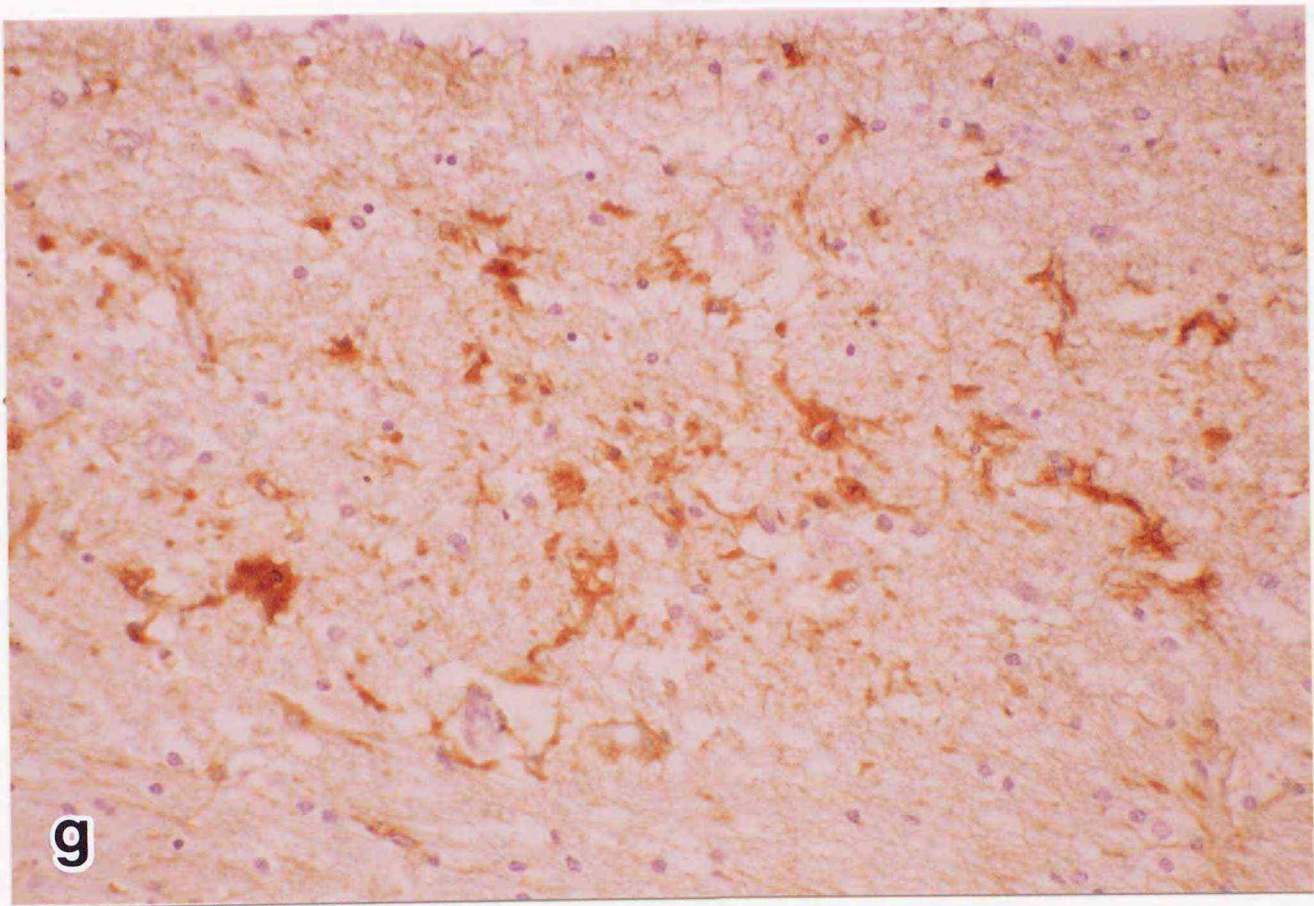


Fig. 16. GFAP immunohistochemistry of the central nervous system of the dogs. Note the moderate to severe astrocytic gliosis in the aged dogs: subcortical white matter of the cerebrum (a, Dog No. 21, x150); cortico-medullary junction of the cerebrum (b, Dog No. 23, x110); spinal cord (c, Dog No. 21, x150); cerebellum (d, Dog No. 24, x150); thalamus (e, Dog No. 20, x150); hippocampus (f, Dog No. 16, x150); subependymal area of the cerebrum (g, Dog No. 16, x150). Only occasional GFAP-positive fibers (arrows) are demonstrated in the cerebral cortex of a 6-month-old dog (h, Dog No. 5, x110).









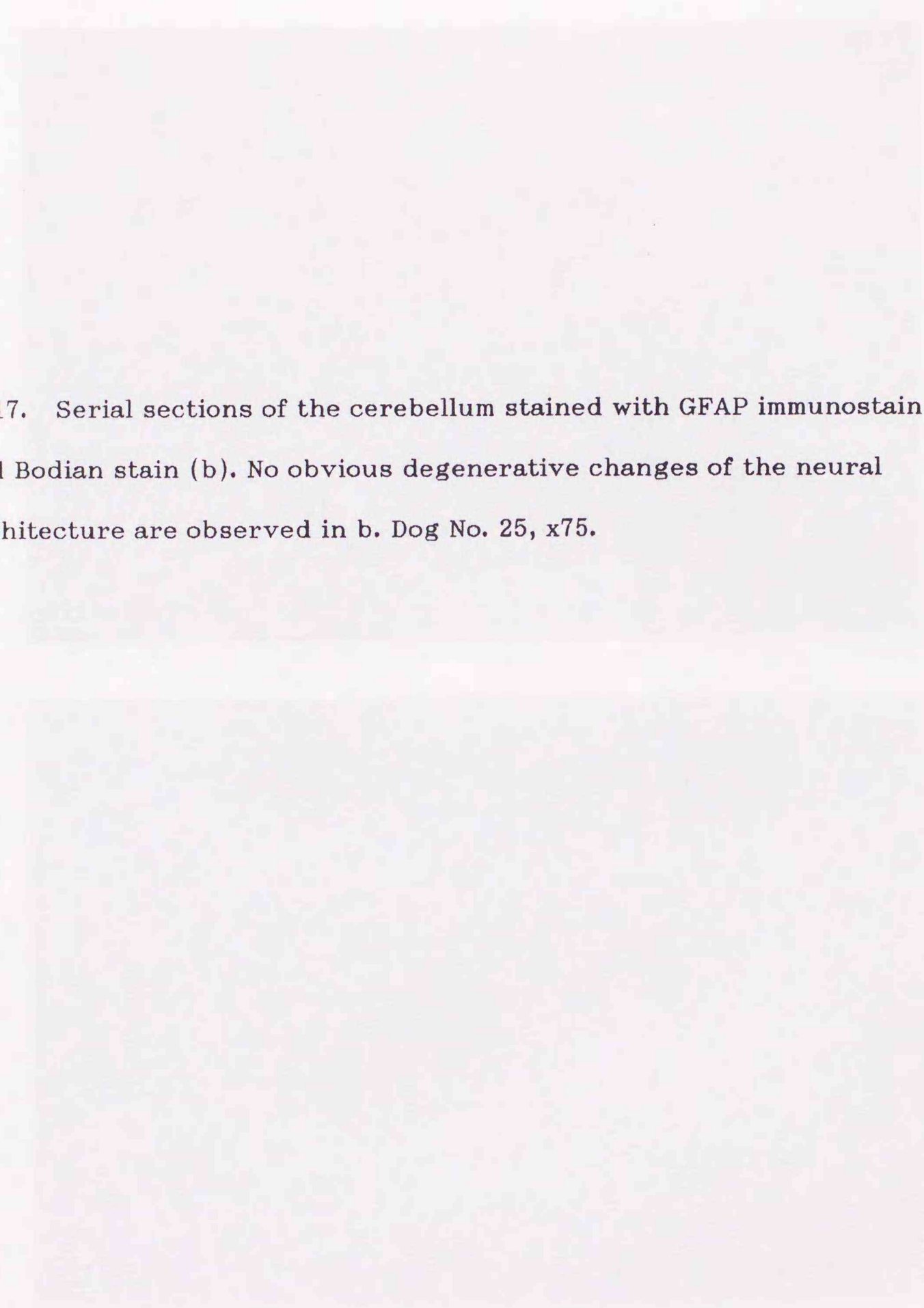


Fig. 17. Serial sections of the cerebellum stained with GFAP immunostain (a) and Bodian stain (b). No obvious degenerative changes of the neural architecture are observed in b. Dog No. 25, x75.

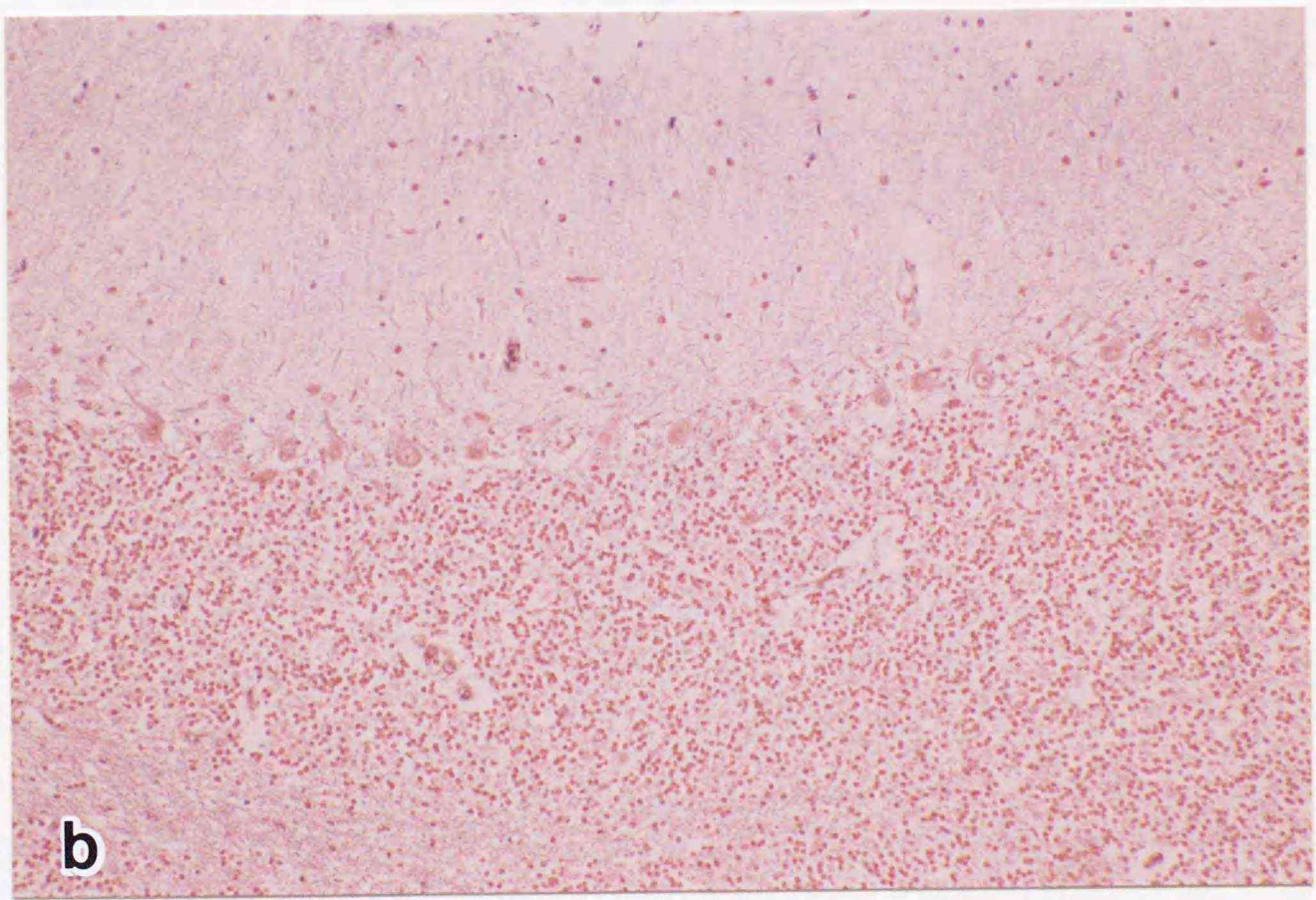
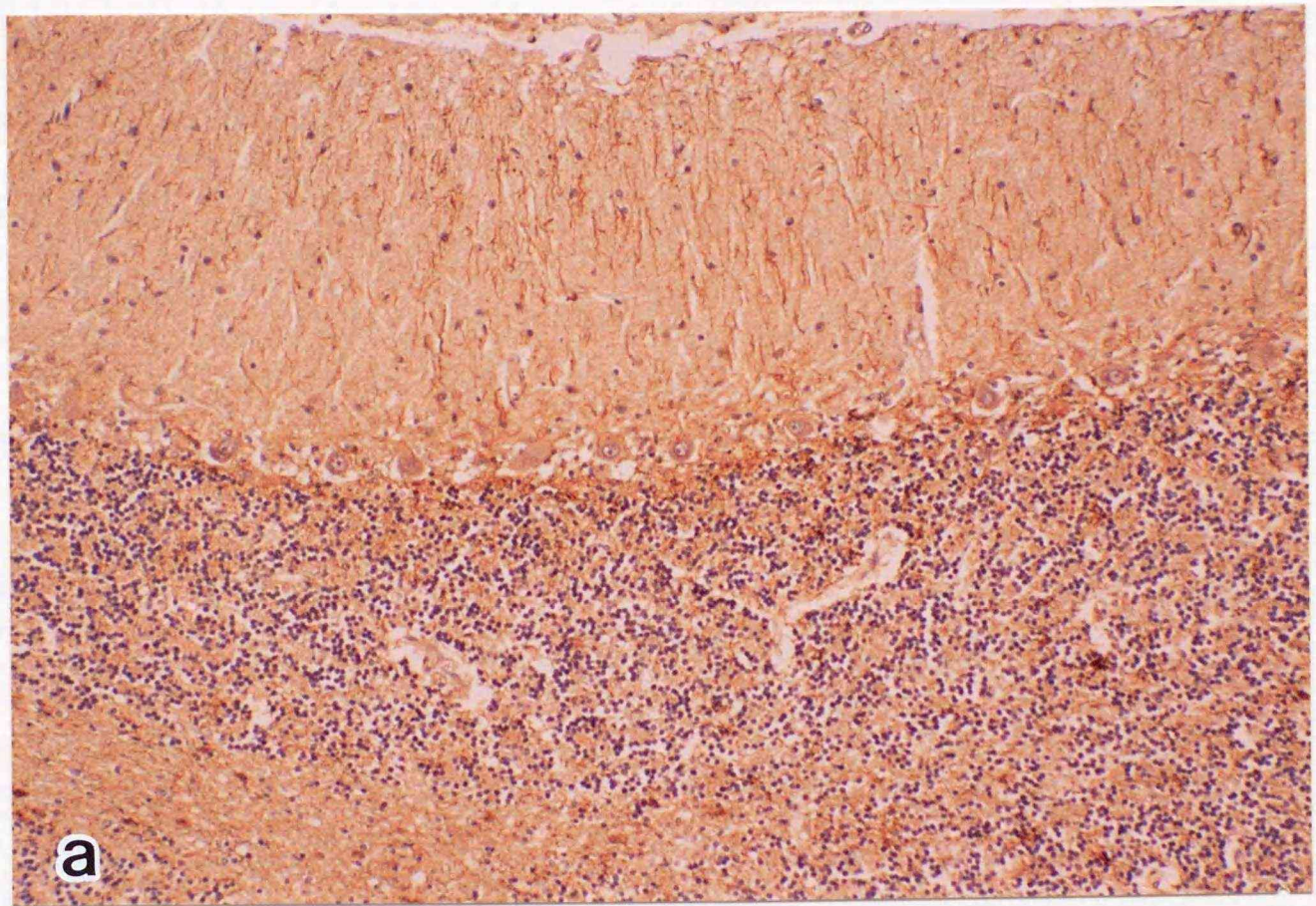
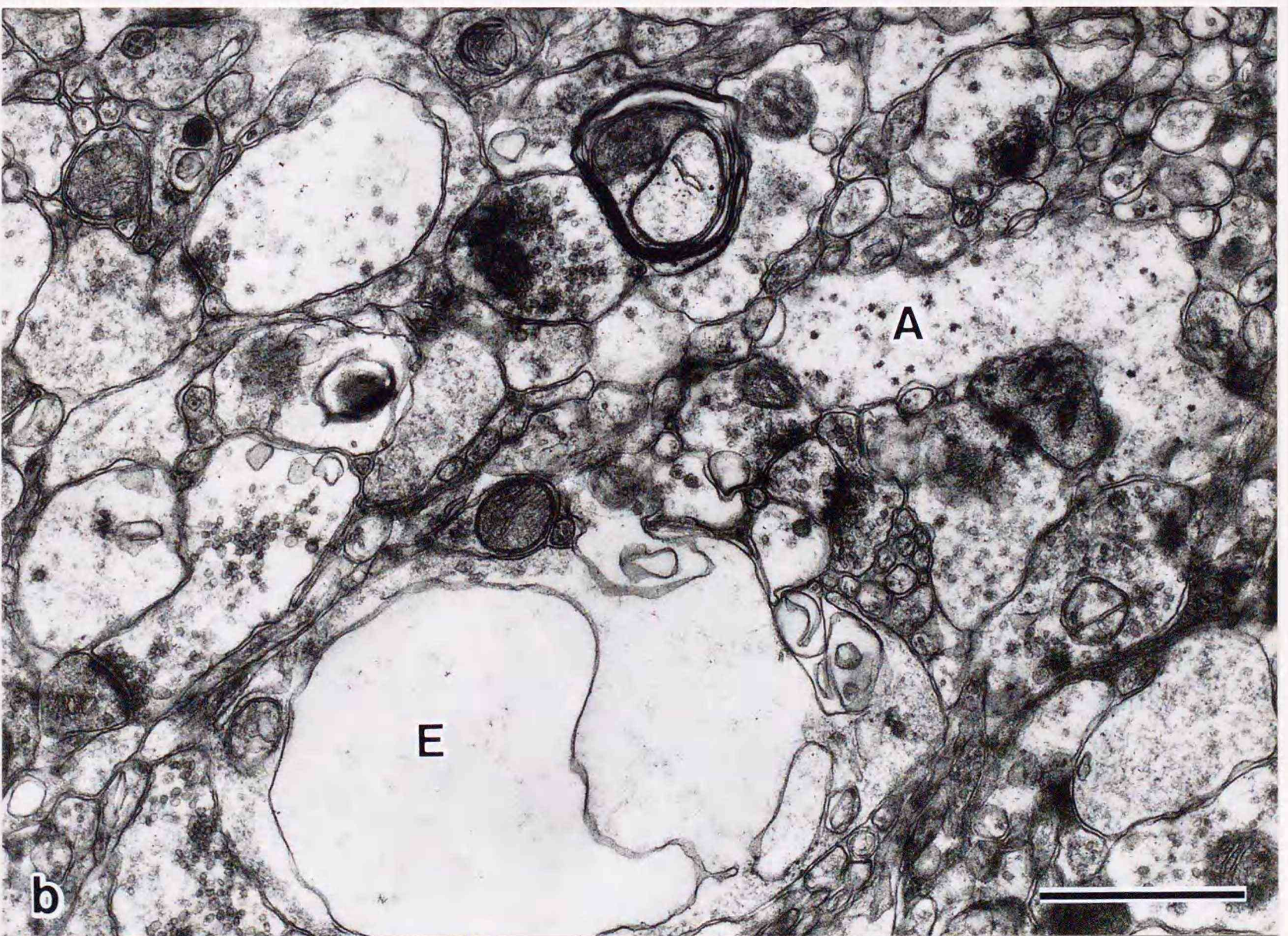
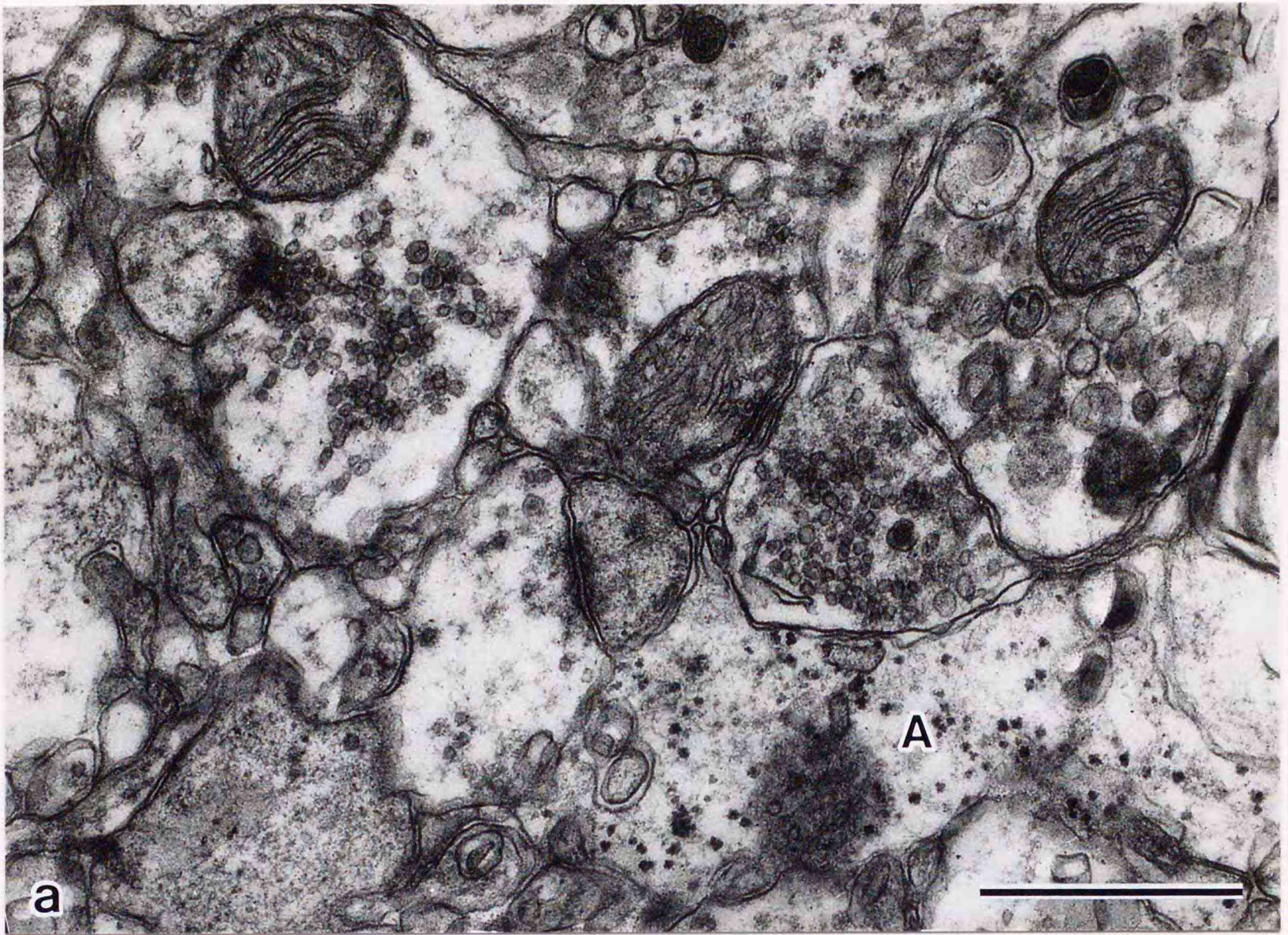
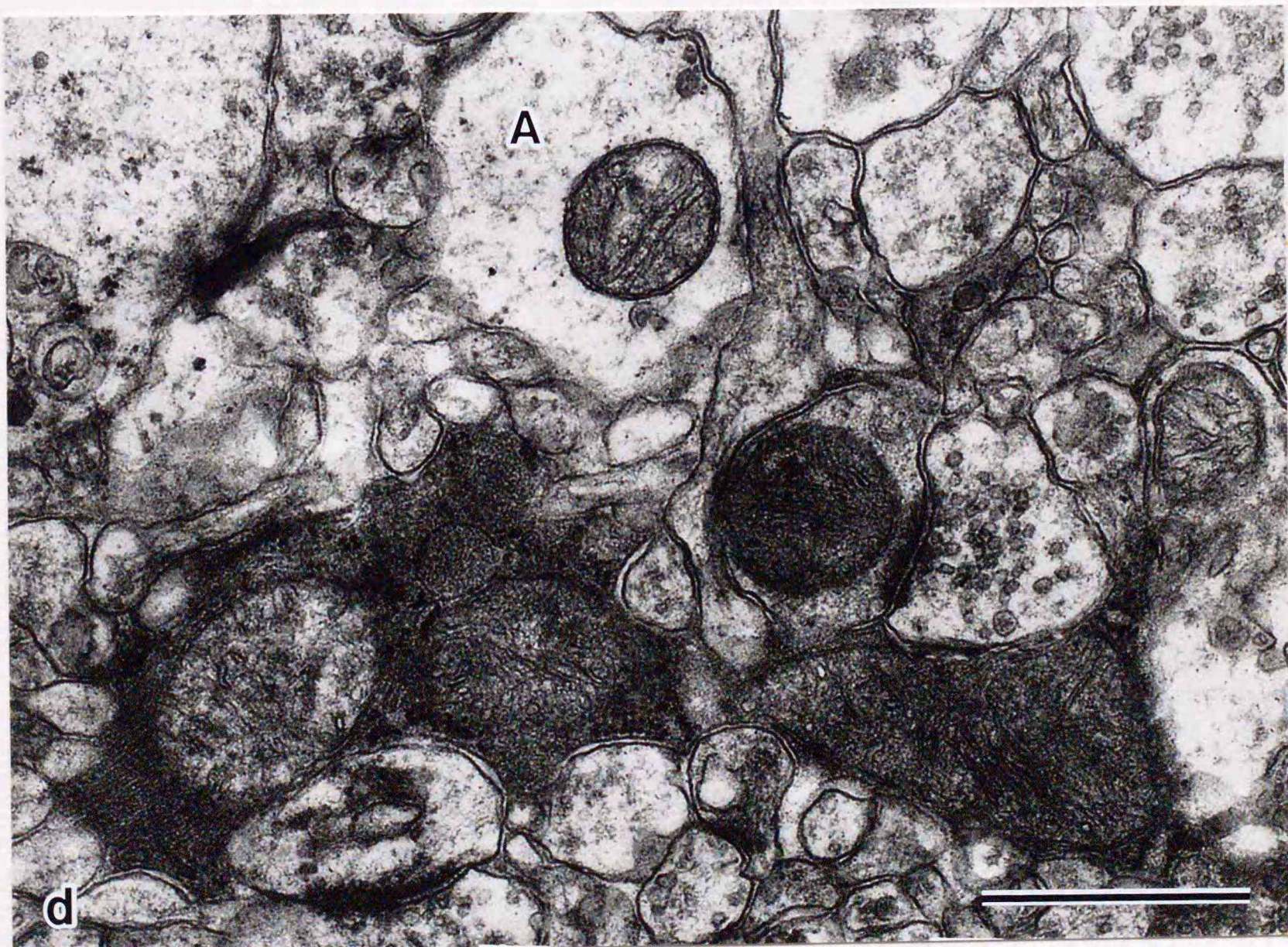
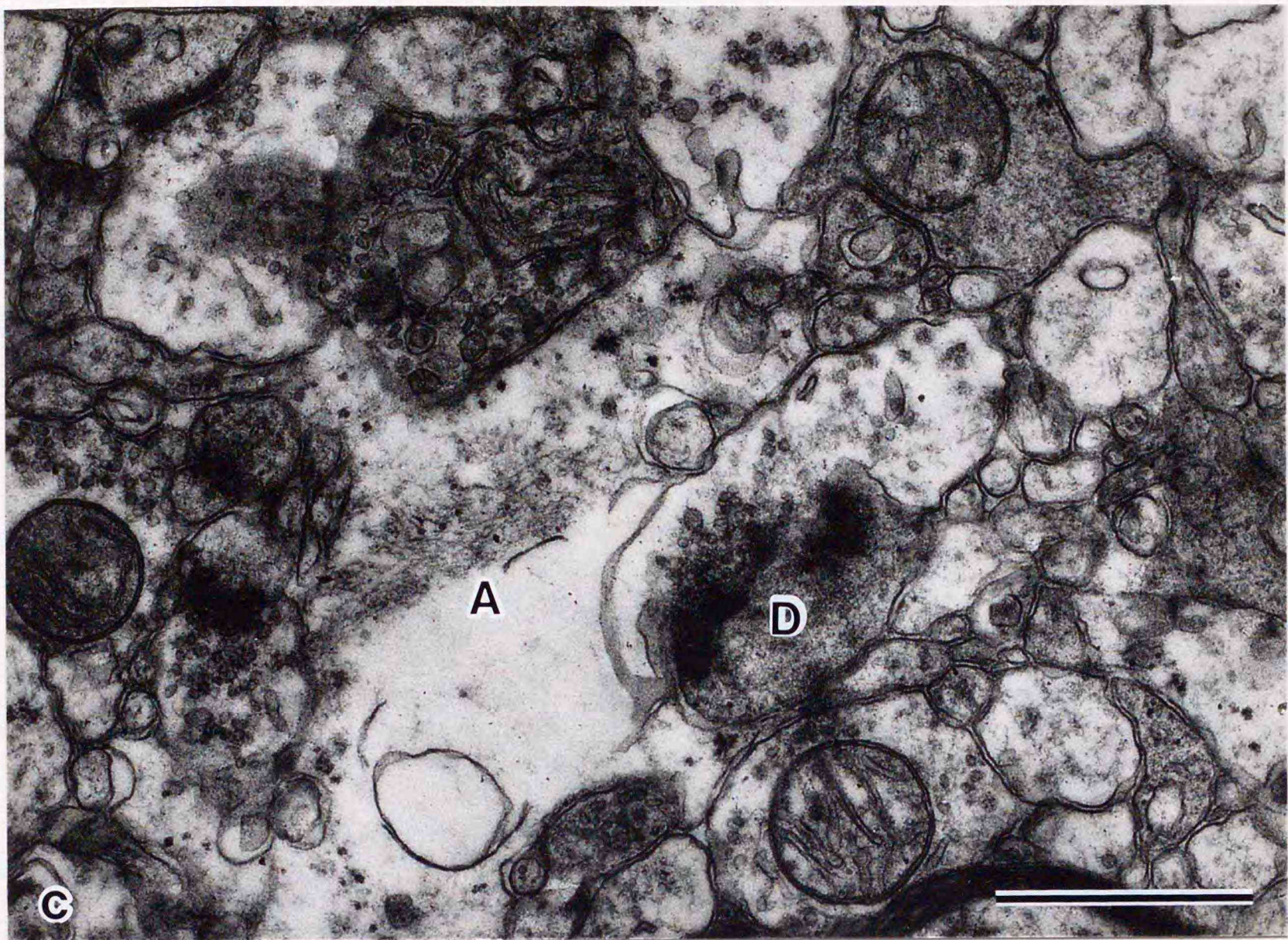


Fig. 18. Transmission electron micrographs of the cerebral cortex from the aged dog (Dog No. 17), showing a variety of degenerative profiles of neural components: swollen synapse-like vesicles with dense contents (a, x21,000); a dilated endoplasmic reticulum-like structure (E) (b, x15,000); heterogeneous dense bodies (D) (c, x22,000); dark axon terminals (d, x24,000). Note the increased number of glycogen granules in the astrocytic processes (A). Bar = 1 μ m.





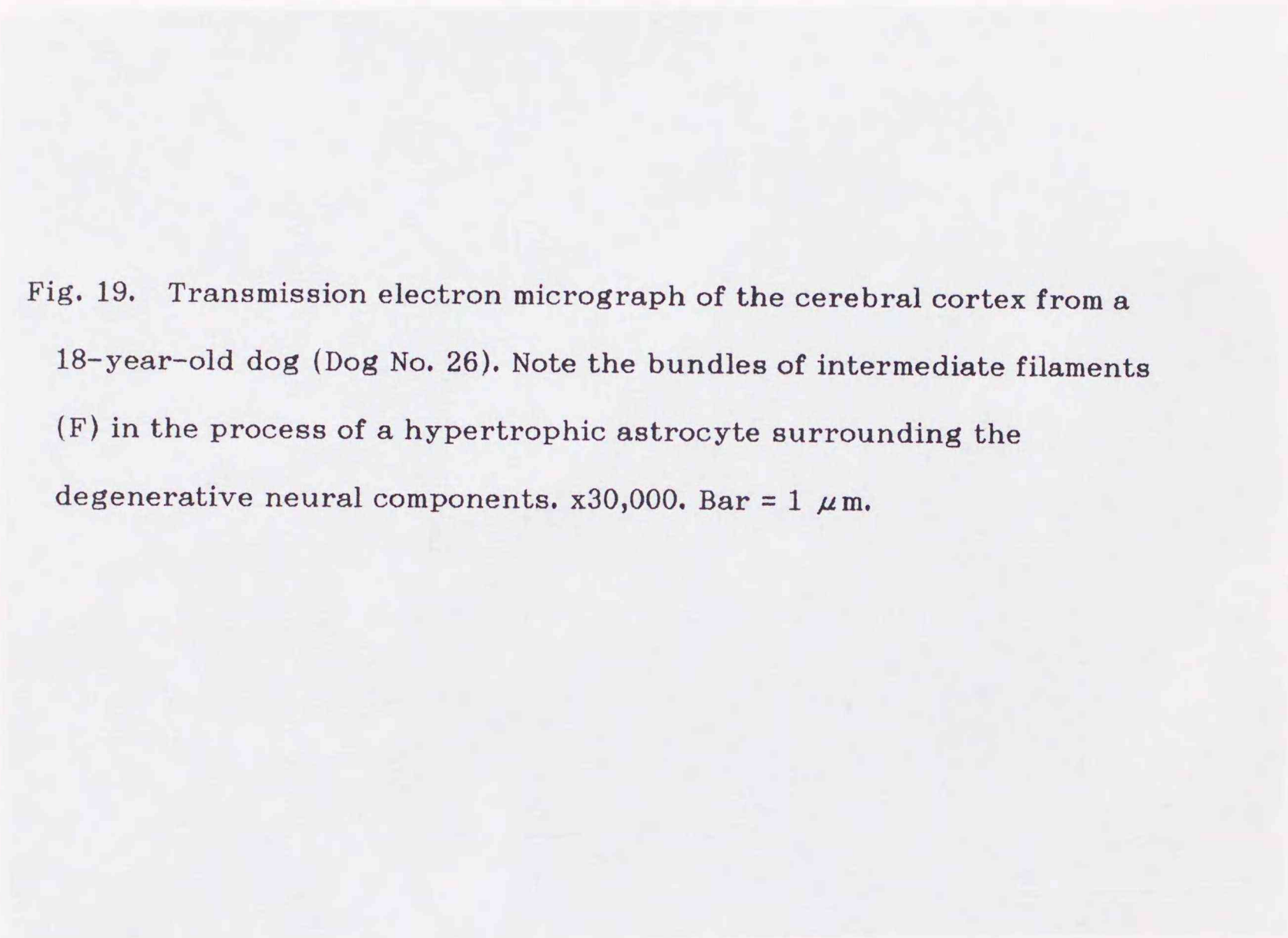
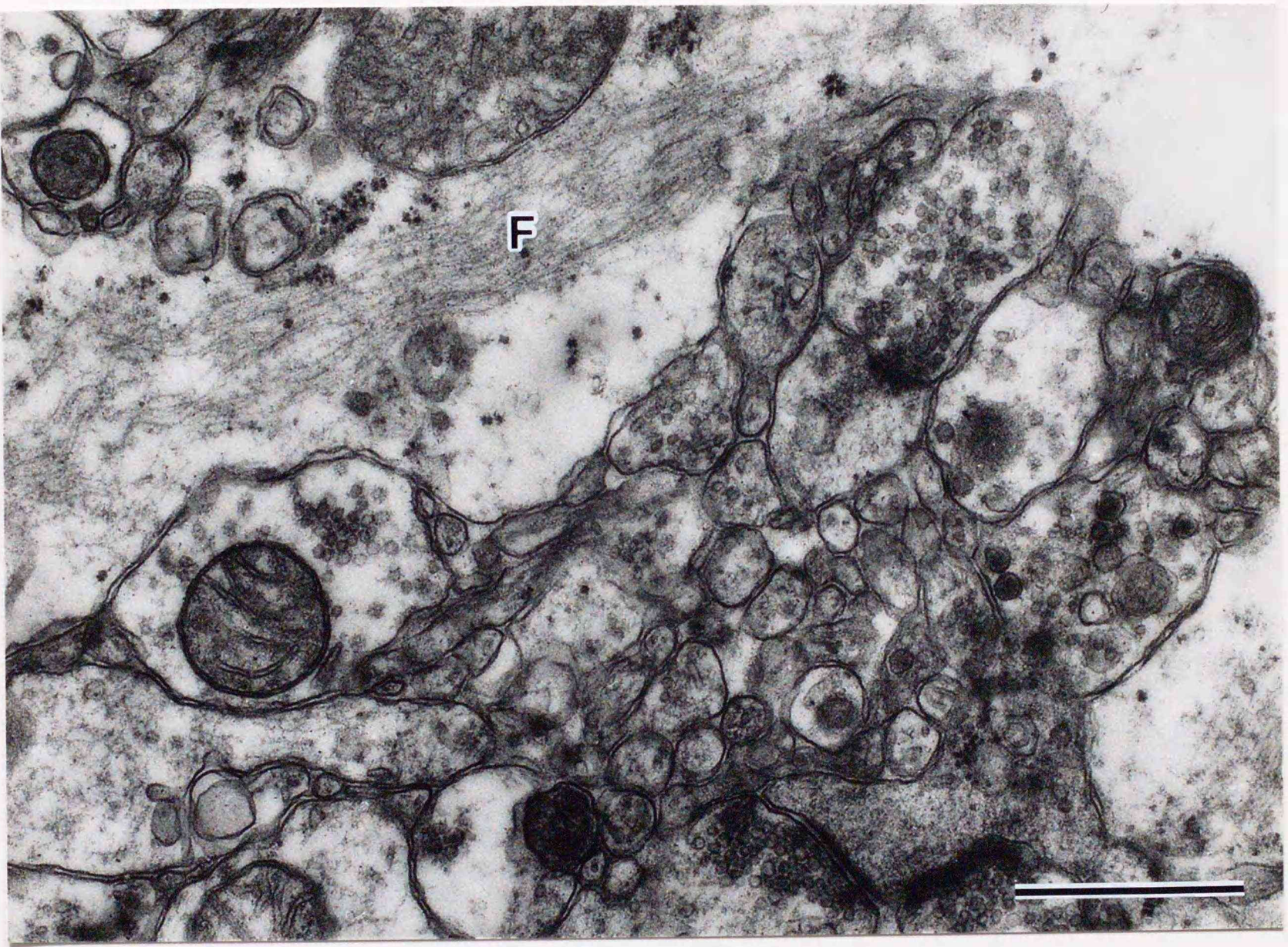


Fig. 19. Transmission electron micrograph of the cerebral cortex from a 18-year-old dog (Dog No. 26). Note the bundles of intermediate filaments (F) in the process of a hypertrophic astrocyte surrounding the degenerative neural components. x30,000. Bar = 1 μ m.



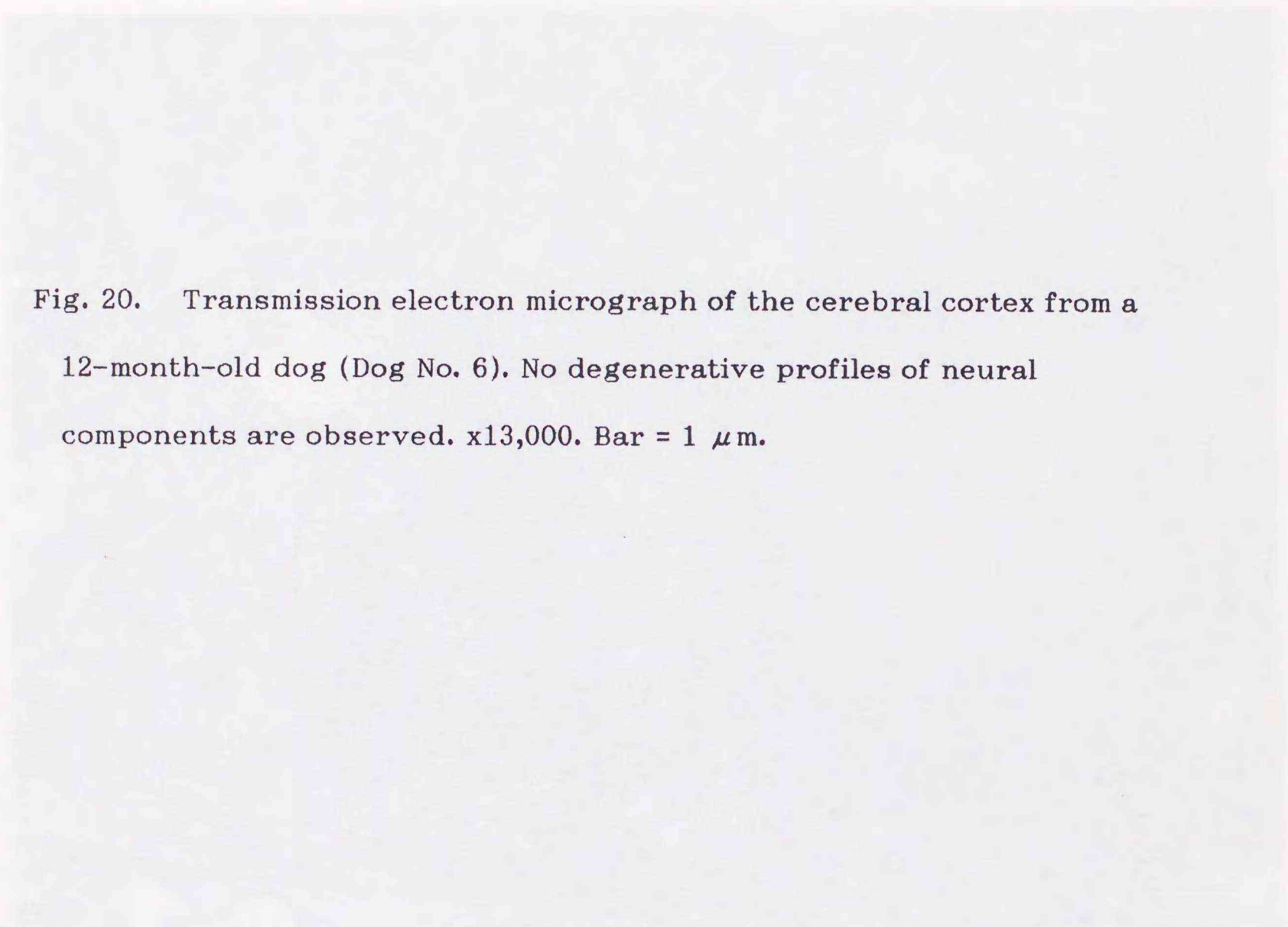
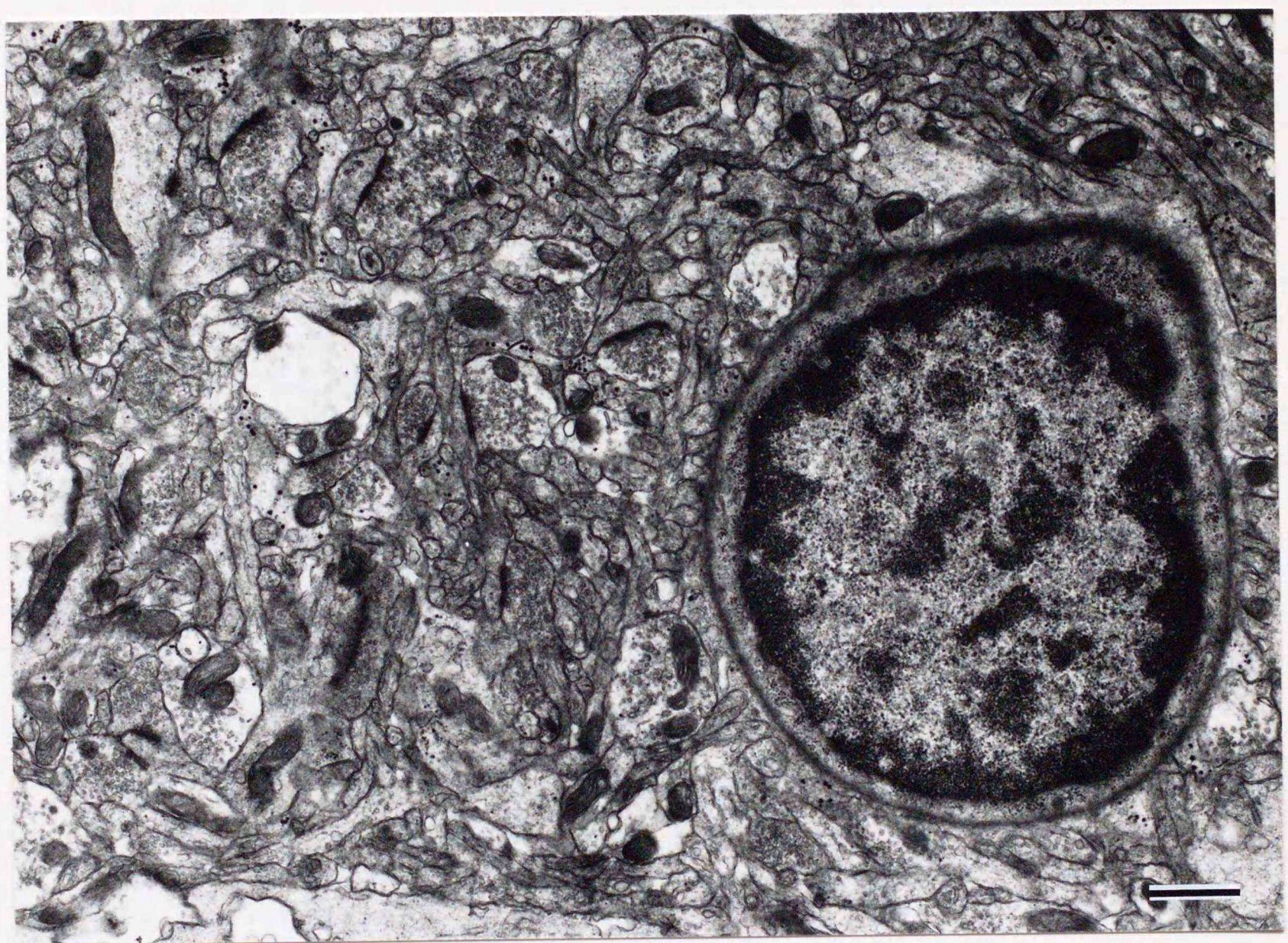


Fig. 20. Transmission electron micrograph of the cerebral cortex from a 12-month-old dog (Dog No. 6). No degenerative profiles of neural components are observed. x13,000. Bar = 1 μ m.



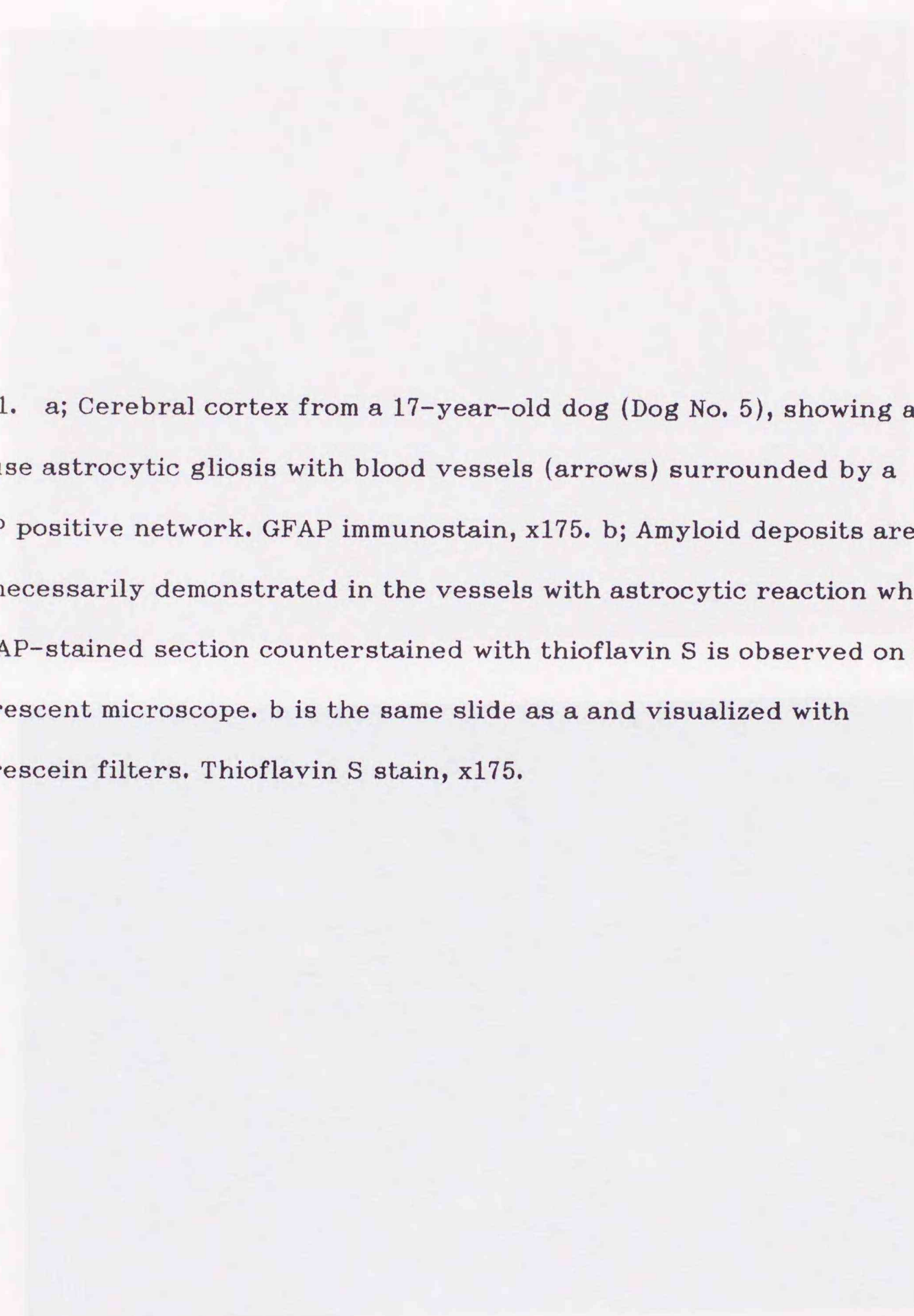


Fig. 21. a; Cerebral cortex from a 17-year-old dog (Dog No. 5), showing a diffuse astrocytic gliosis with blood vessels (arrows) surrounded by a GFAP positive network. GFAP immunostain, x175. b; Amyloid deposits are not necessarily demonstrated in the vessels with astrocytic reaction when a GFAP-stained section counterstained with thioflavin S is observed on a fluorescent microscope. b is the same slide as a and visualized with fluorescein filters. Thioflavin S stain, x175.

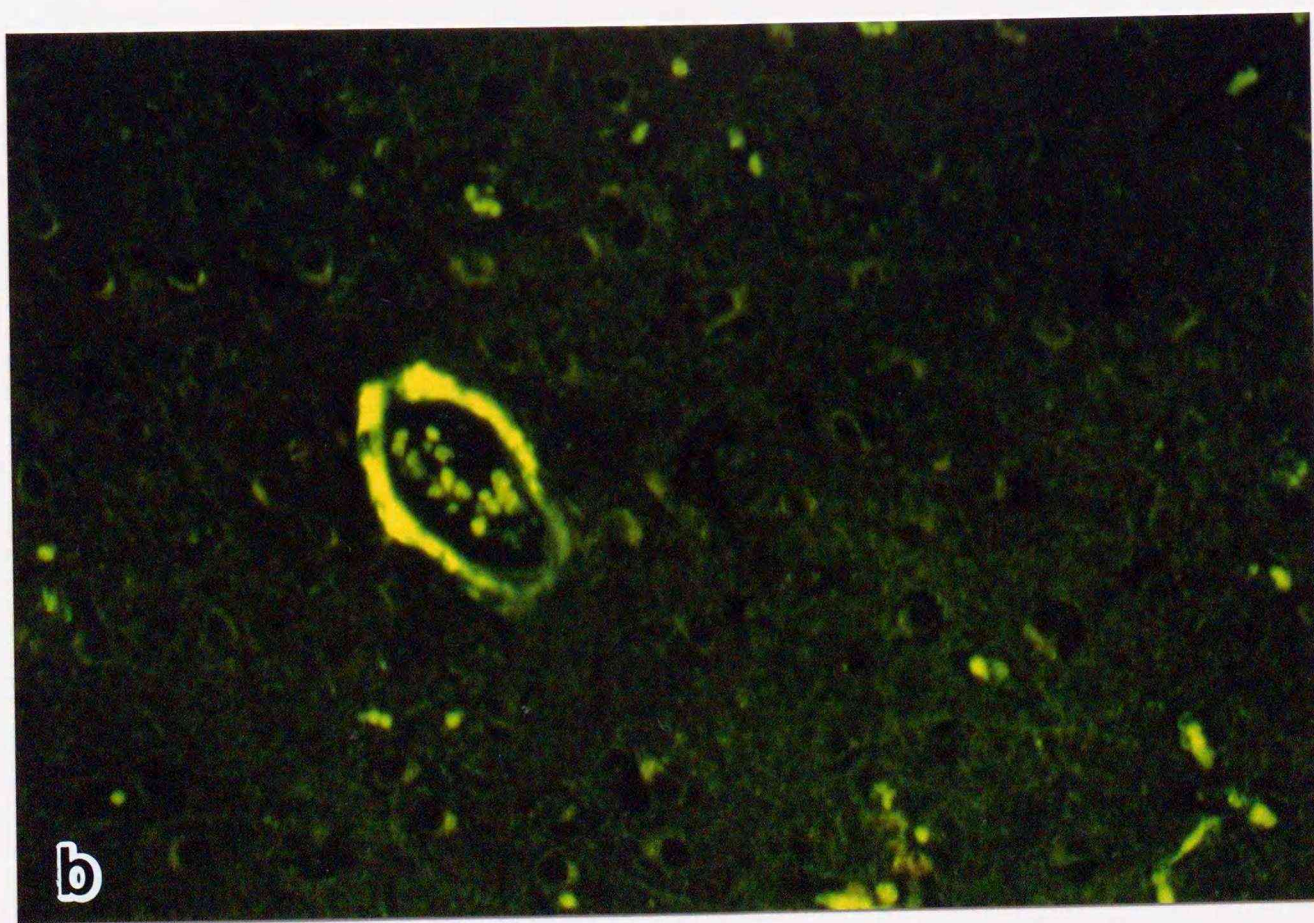
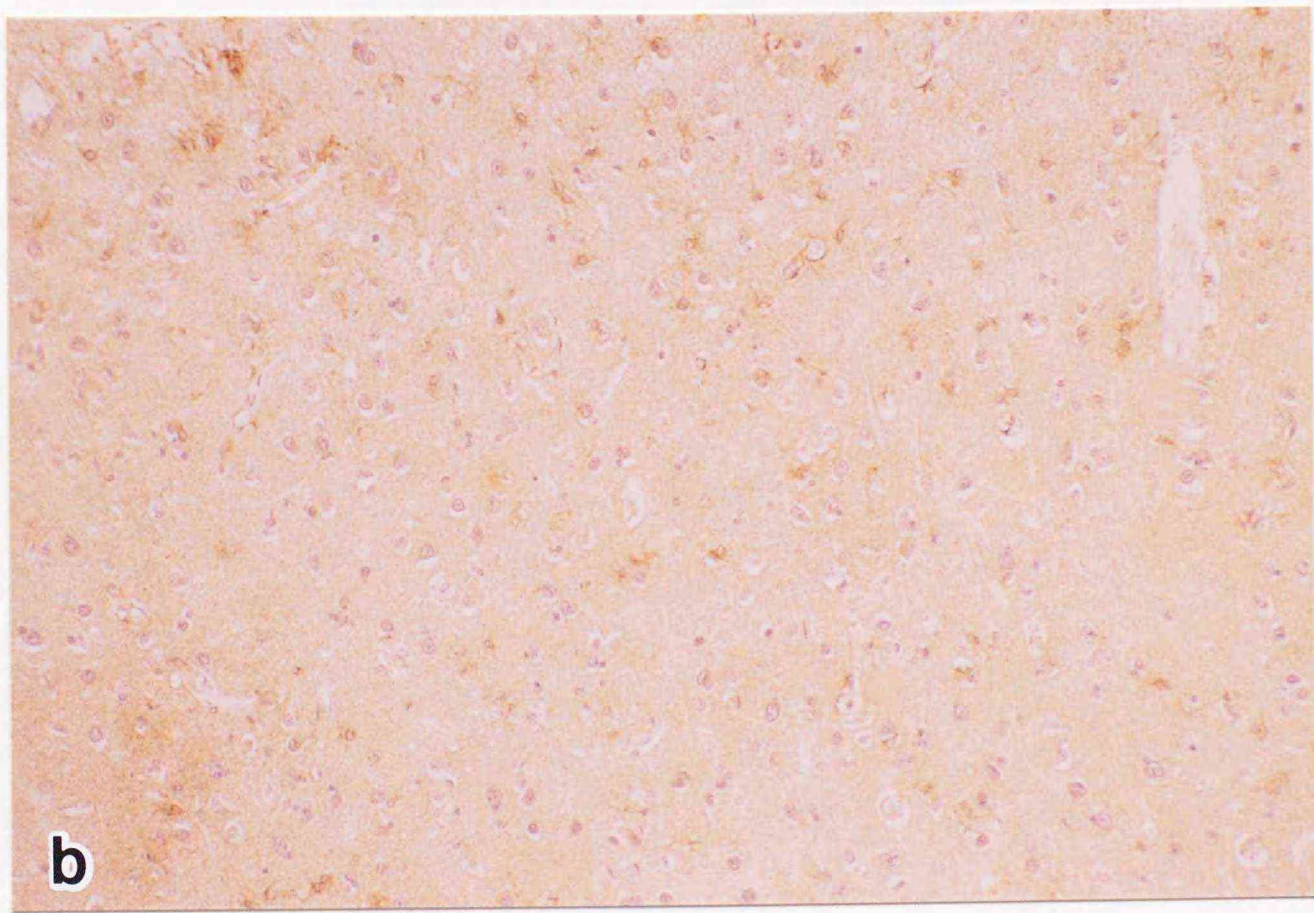
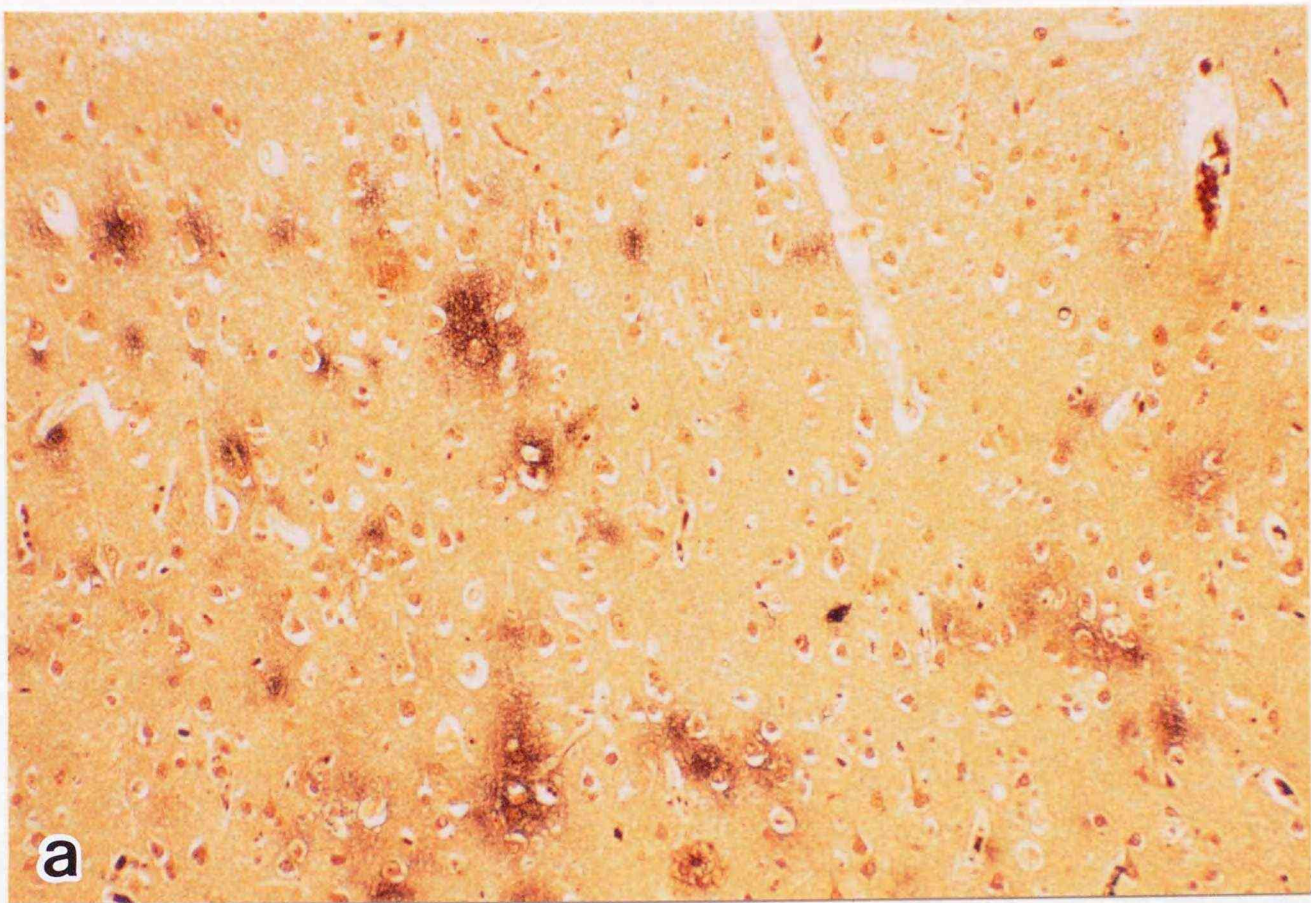
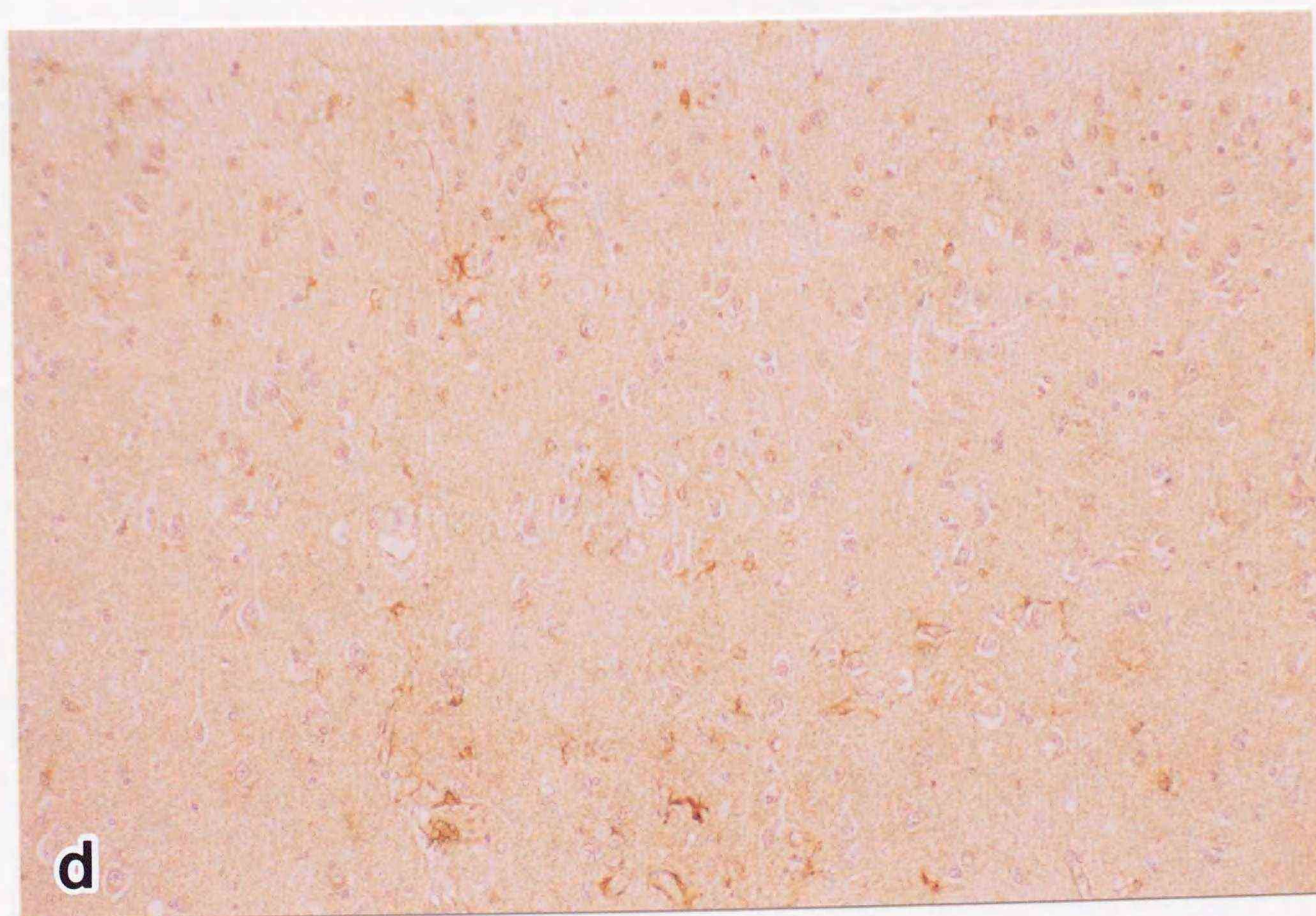
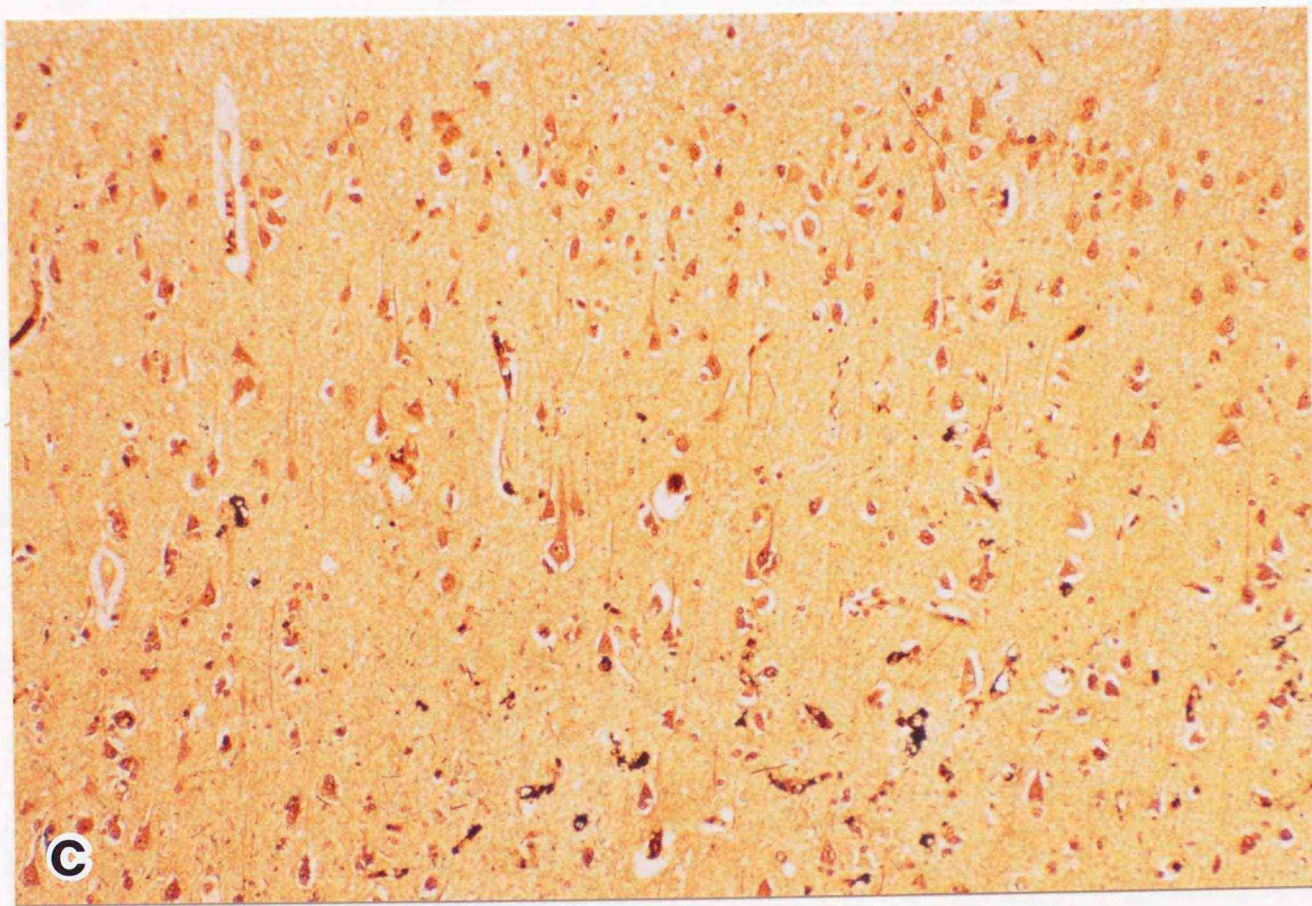




Fig. 22. Serial sections from the cingulate cortex (a, b) and parietal cortex (c, d) of a 17-year-old dog (Dog No. 5). Note no obvious difference in the pattern and intensity of astrocytic gliosis between the area with senile plaques (SP) (a, b) and the area without SP (c, d). a and c; modified Bielschowsky stain, x120. b and d; GFAP immunostain, x120.





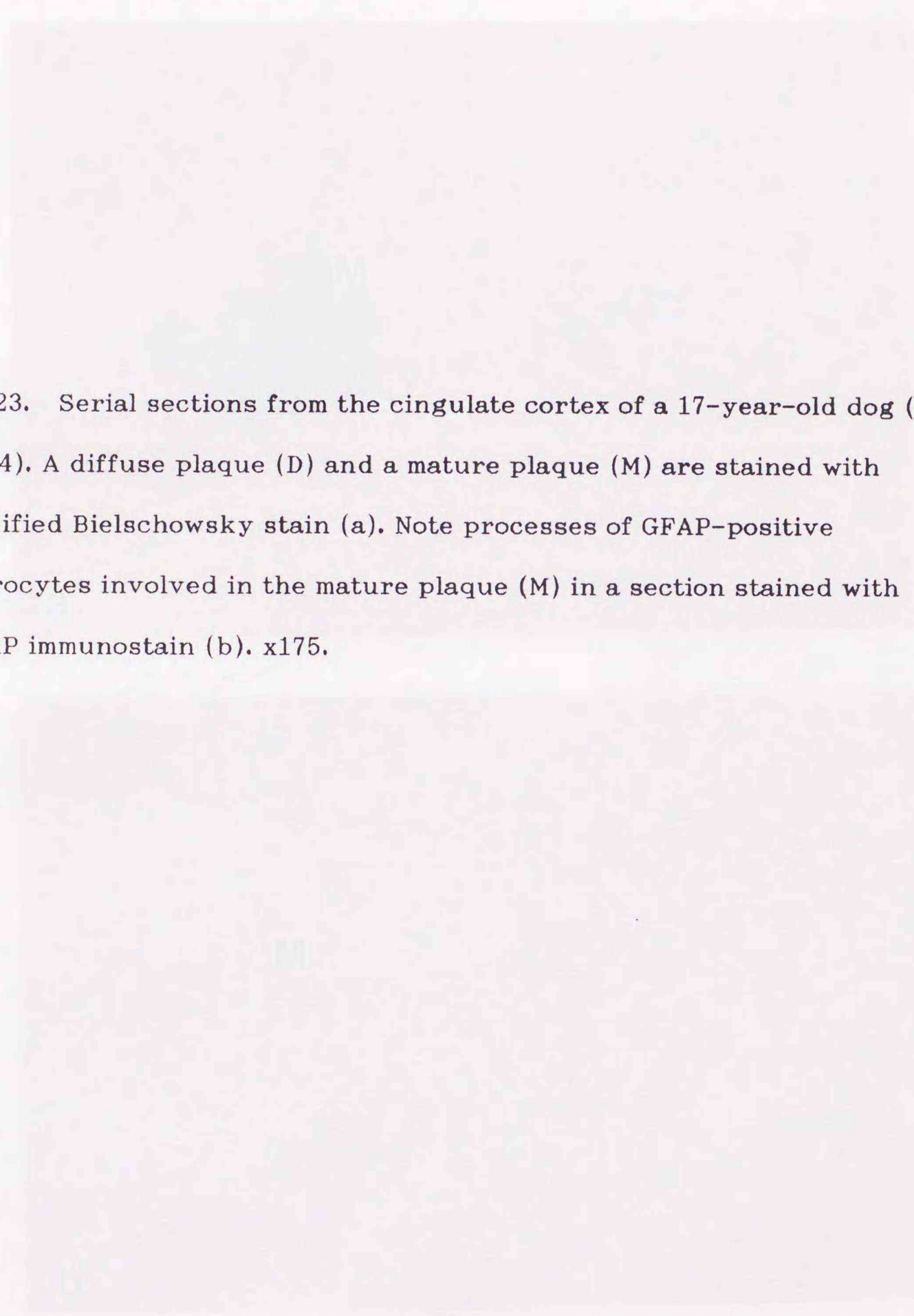
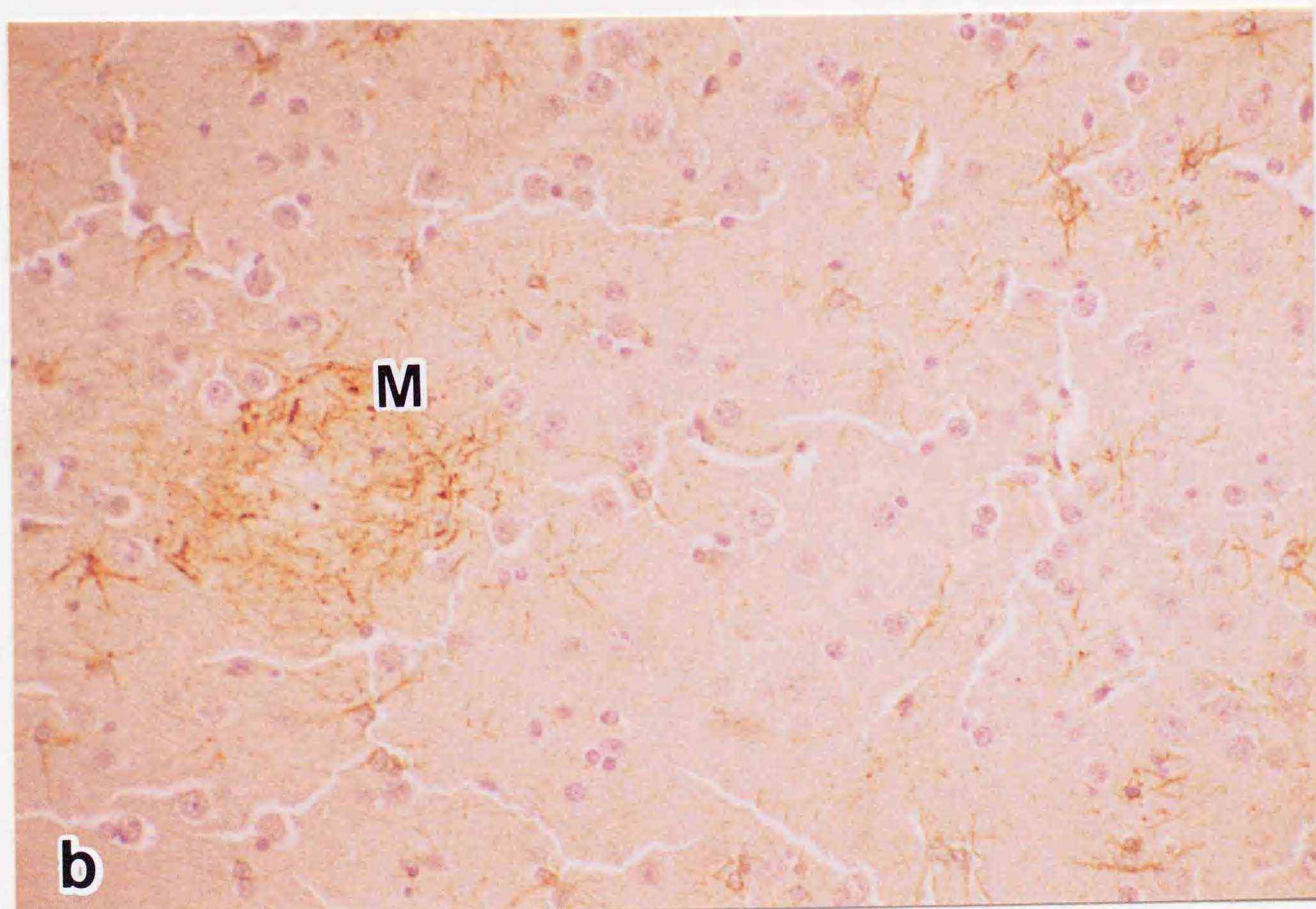
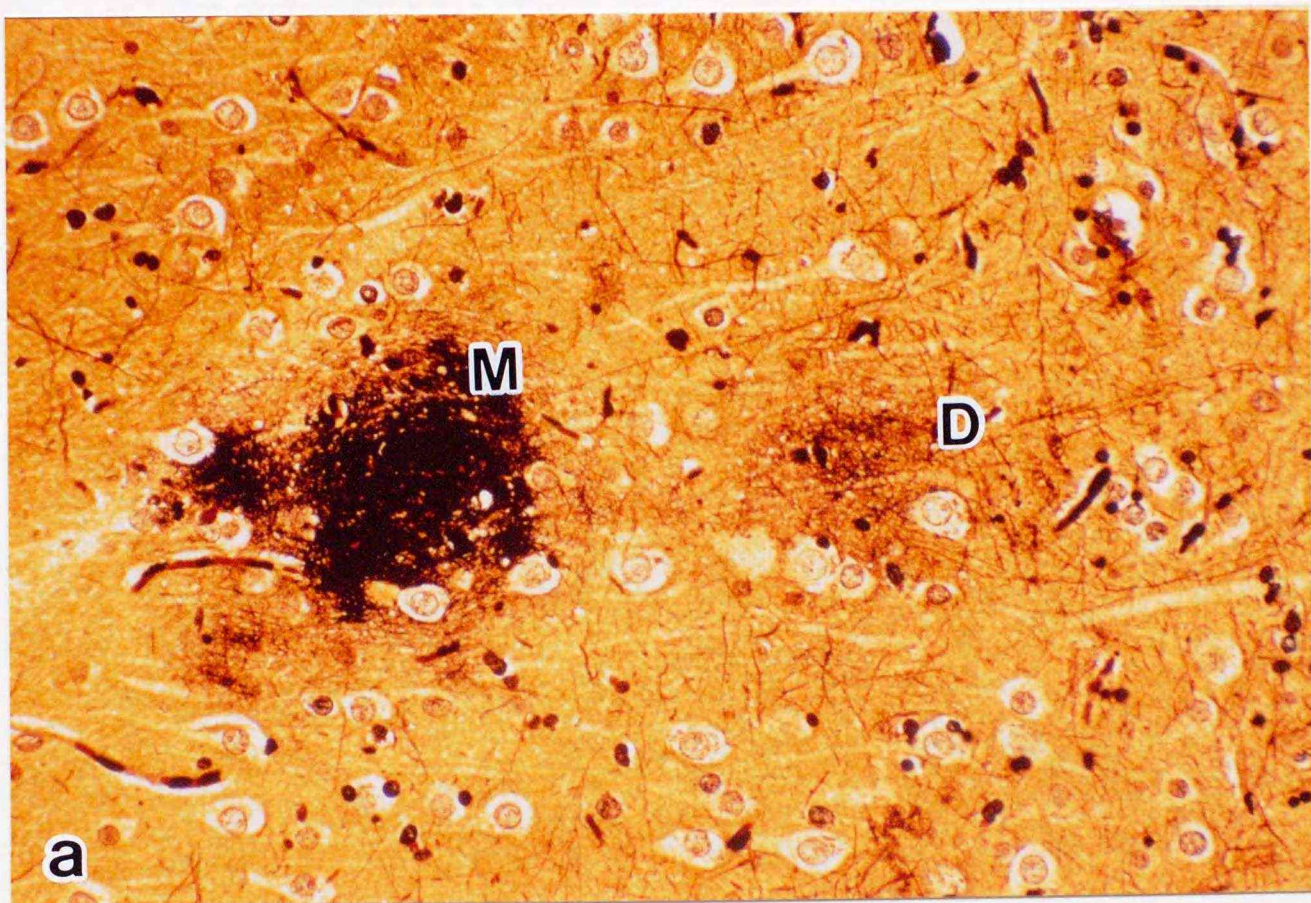


Fig. 23. Serial sections from the cingulate cortex of a 17-year-old dog (Dog No. 4). A diffuse plaque (D) and a mature plaque (M) are stained with modified Bielschowsky stain (a). Note processes of GFAP-positive astrocytes involved in the mature plaque (M) in a section stained with GFAP immunostain (b). x175.



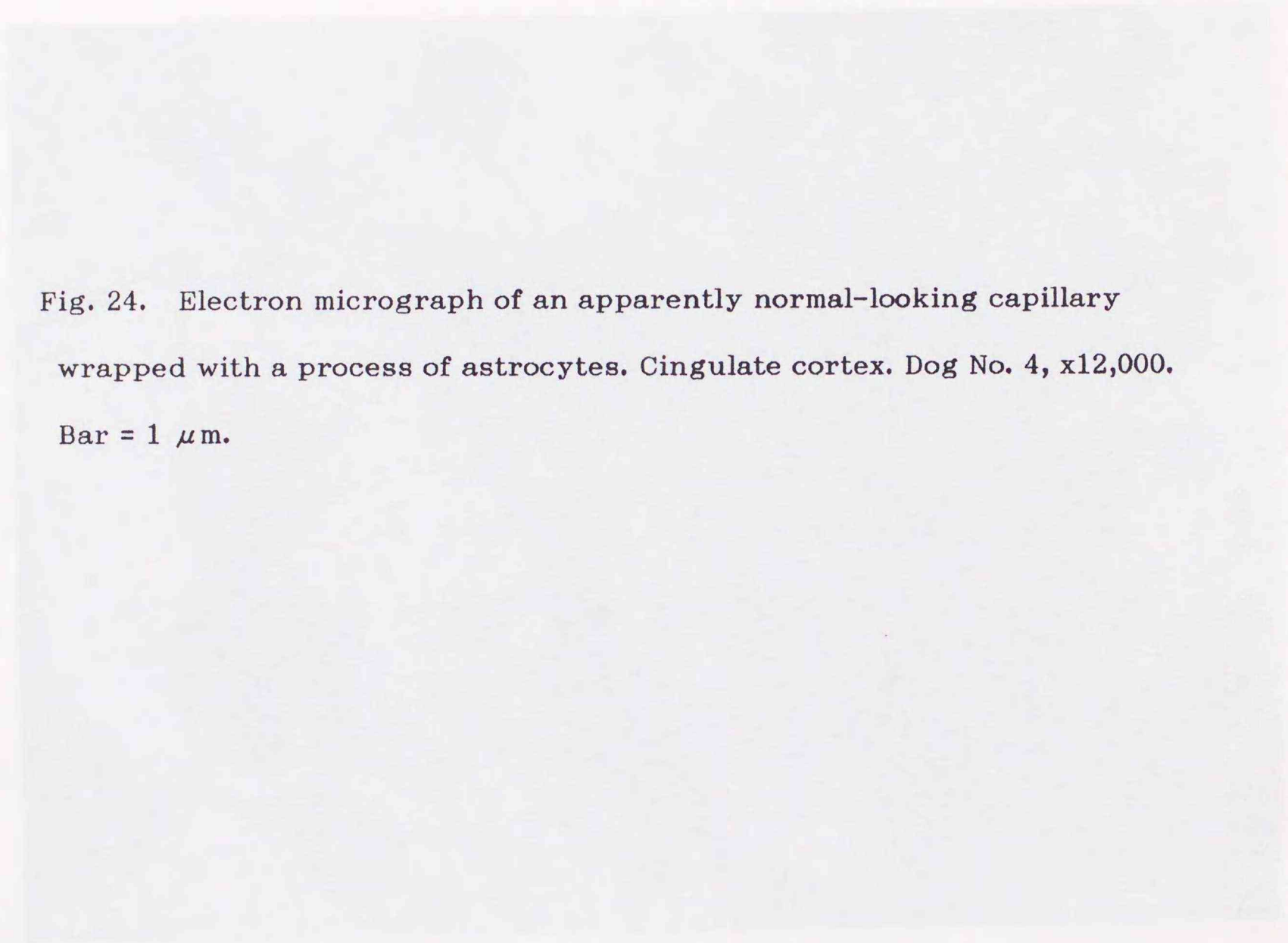
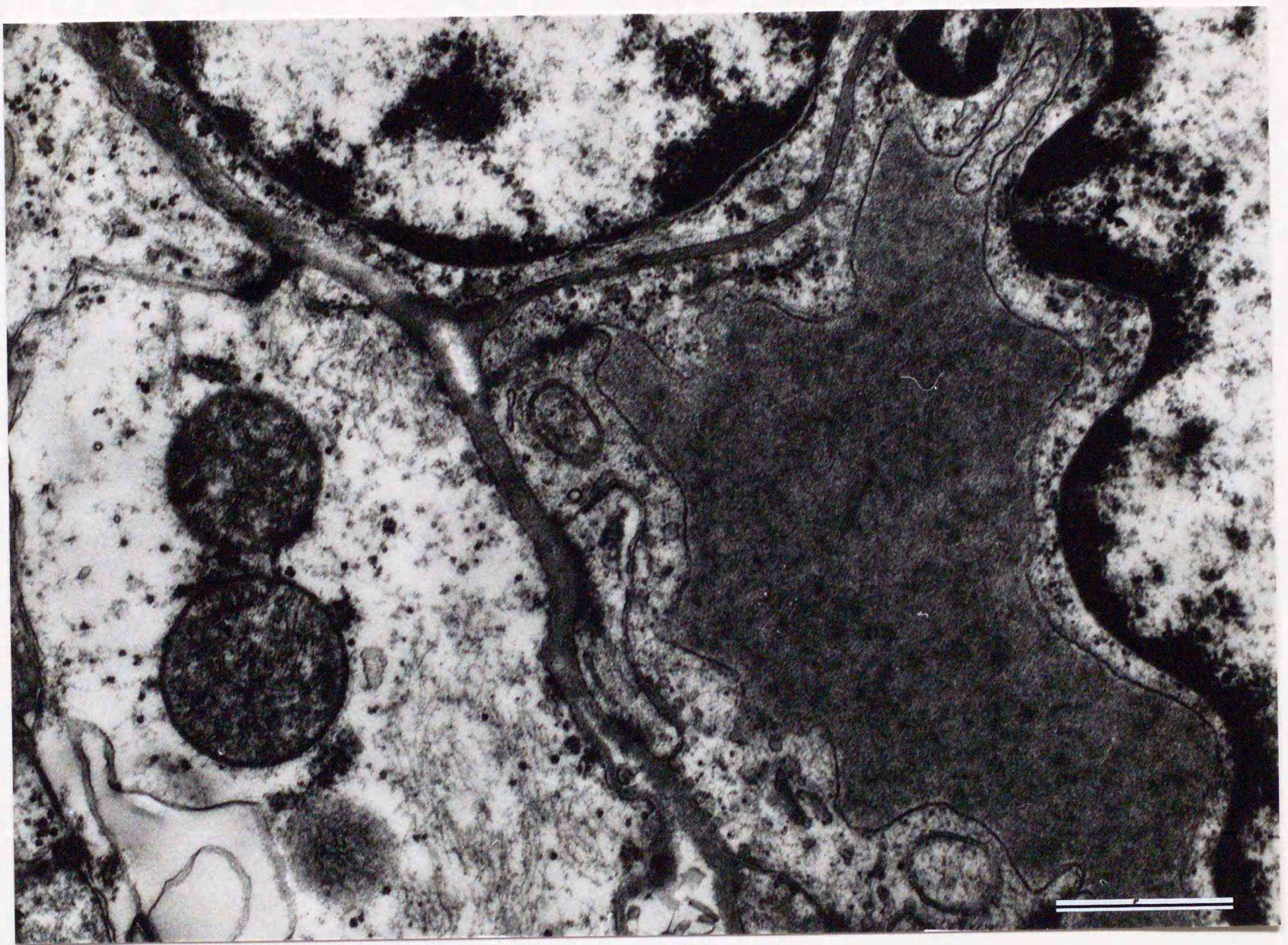
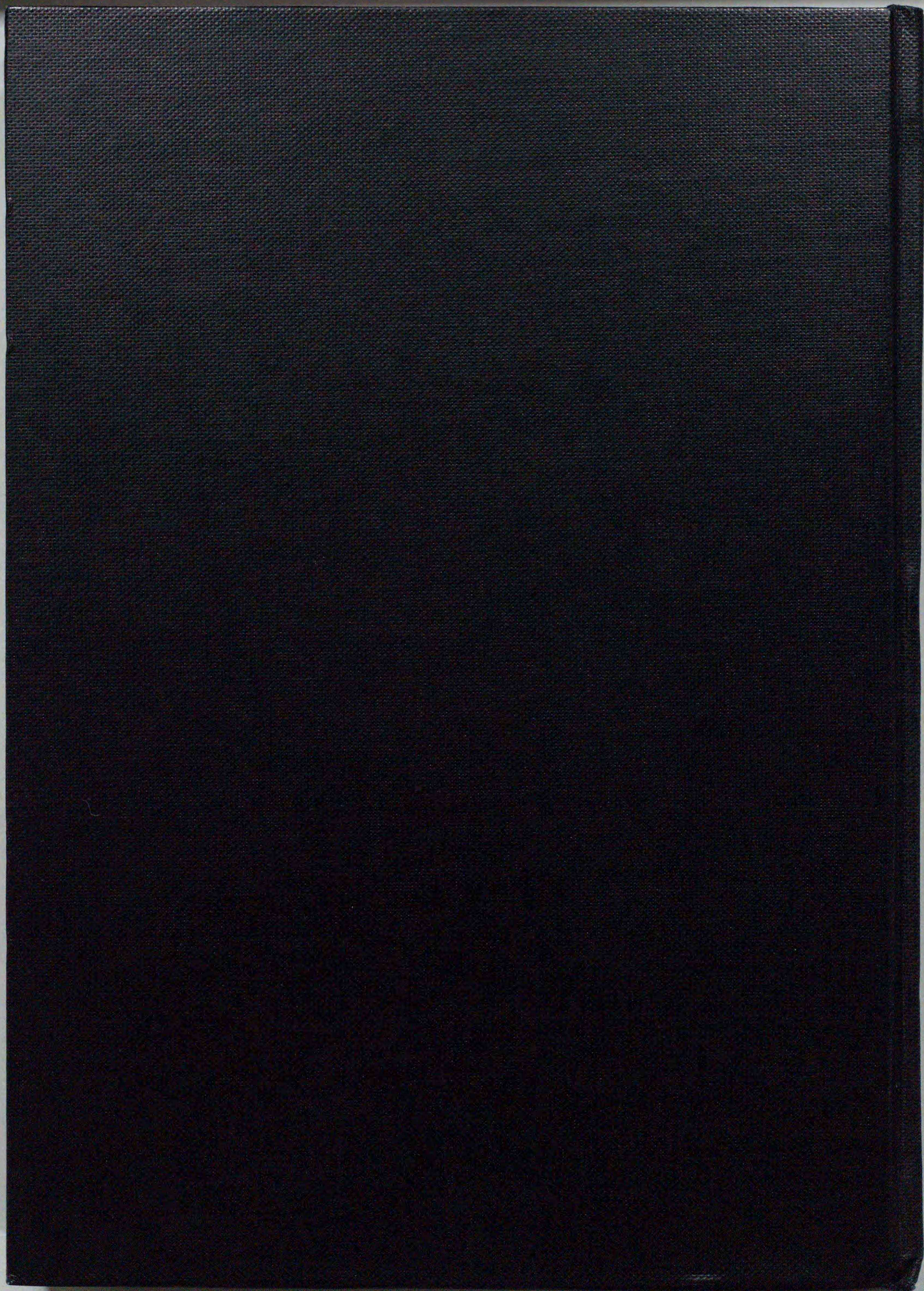
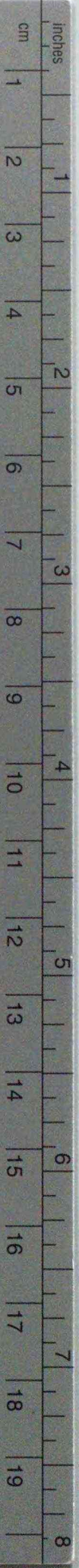


Fig. 24. Electron micrograph of an apparently normal-looking capillary wrapped with a process of astrocytes. Cingulate cortex. Dog No. 4, x12,000.
Bar = 1 μ m.







Kodak Color Control Patches

© Kodak, 2007 TM: Kodak

Blue	Cyan	Green	Yellow	Red	Magenta	White	3/Color	Black

Kodak Gray Scale



© Kodak, 2007 TM: Kodak

A 1 2 3 4 5 6 **M** 8 9 10 11 12 13 14 15 **B** 17 18 19

