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Altered angioarchitecture in selected areas of brains with Alzheimer's disease*

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Summary. It was the aim of this study to determine, qualitatively and quantitatively, alterations in the blood vessels of brains removed postmortem from patients with Alzheimer's disease (AD), and to compare these findings with the appearance of cerebral blood vessels in a group of individuals without brain disorders. Celloidin sections of brain tissue from four cerebral areas, pre-frontal (Brodmann's area 9), basal forebrain, sensorimotor, and hippocampus, were subjected to an alkaline phosphatase reaction to facilitate the evaluation of the vascular distribution. The vascular density in five sections was determined by counting the number of vascular intersections with a microscopic test grid of 100 squares; ten fields per section were examined in this manner. Analysis of 16 AD and 6 control brains, showed that there was a striking and statistically significant reduction in the vascular net density specifically in the basal forebrain region and the hippocampus of AD brains. In addition, vessels in the AD brains exhibited extensive topographical changes, such as kinking and looping. These results indicate that modifications in vascular density are present in AD brains with a marked regional specificity.

Key words: Brain – Alzheimer's disease – Arterioles and capillaries – Alkaline phosphatase reaction – Vascular density

A number of reports are available indicating that vascular patterns in the human brain undergo changes with age and in certain age-related disorders, such as dementia of the Alzheimer type. These studies incorporated a variety of techniques for the demonstration of blood vessels, ranging from visualization of hemoglobin pigment [8, 13], X-radiation following injection with radiopaque contrast medium [10], silver impregnation [1, 15], enzymatic reaction [2], and, most recently, scanning electron microscopy [1, 16].

Among the results reported by these authors who examined a number of different cerebral areas, an altered arteriolar topography in the senile brain emerged as a common finding. Additional changes were noted affecting the hippocampal region in the Alzheimer's brain [2] and the innervation of the microvasculature in Alzheimer's disease (AD) [16].

It was the aim of this study to identify and document quantitatively, alterations in the vascular patterns of brains in individuals with AD and to compare these findings with the vascular profiles obtained in individuals without abnormalities in the brain, drawn from an autopsy population ranging from adolescence to old age.

This project was based on the supposition that the vascular bed in brains with AD is altered and, specifically, that the vasculature is affected differentially in selected cerebral areas. Moreover, it is postulated that these differences (a) between Alzheimer brains and the control group and (b) within specific cerebral areas, can be demonstrated quantitatively.

A recently published alkaline phosphatase technique selectively demonstrating the microvasculature in the central nervous system [3] was utilized to facilitate the evaluation of the cerebral vascular arrangement.

Materials and methods

Segments of tissue from 16 brains of individuals with dementia of the Alzheimer type were obtained from the St. Louis University Alzheimer's Disease Research Center Brain Bank. The diagnosis

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Table 1. Case histories

Age at death	Sex	Cause of death	Age at onset of symptoms	Dementia scale [4]
Alzheimer's Disease [16]				
85	Male	Pneumonia	79	23.5[a]
89	Female	Pneumonia & septicemia	82	23.0
84	Female	Septicemia	83	26.5
88	Female	Bronchitis	78	26.0
88	Female	CA breast	83	23.0
81	Male	Cerebral hemorrhage	71	24.0
82	Female	Respiratory insufficiency	?	26.0
83	Male	CA prostate	74	21.0
91	Female	Bronchitis	71	24.0
89	Female	Resp. insufficiency & sepsis	87	17.5
82	Male	Cardiac arrest	?	N.A.
76	Female	C.O.P.D.	73	21.5
89	Female	Progressive primary hepatomegaly	?	23.0
79	Female	Senile dementia	65	25.0
92	Female	Congestive heart failure	87	23.5
79	Female	Cardiopulmonary arrest	72	25.0
Controls [6]				
23	Male	Cardiomyopathy & pneumonia		
82	Female	M.I. & A.S.H.D.		
90	Female	H.C.V.D.		
63	Female	Fibrinous pericarditis		
73	Male	Emphysema		
34	Male	Stab wound to heart		

[a] 28 = Most severe incapacity
C.O.P.D.: Chronic obstructive pulmonary disease; M.I.: Myocardial infarction; A.S.H.D.: Atherosclerotic heart disease; H.C.V.D.: Hypertensive cardiovascular disease; N.A. = not applicable

of the disease was based on senile plaque and/or neurofibrillary tangle counts, according to established criteria [12]. Six brains without CNS abnormalities were acquired from the St. Louis Medical Examiner's office. All case histories including the dementia scale [4] are listed in Table 1.

Four cerebral areas were selected: (1) pre-frontal lobe (3 cm from the anterior pole, Brodmann's area 9), (2) basal forebrain area, which includes the nucleus basalis of Meynert, (extending superiorly from the anterior perforated substance to the corpus striatum, bounded laterally by the amygdala and medially by the hypothalamus), (3) motor-sensory cortex, i.e., the pre- and postcentral gyri, and (4) hippocampus. Coronally sliced tissues from these areas, 5 mm thick, were fixed within 12 h after death in a formalin-calcium chloride-sodium barbital fixative, at 4° C, pH 7.0–7.2, with the bodies stored in a cold room until autopsy. Following fixation, the tissue blocks were dehydrated in ethanol and embedded in Parlodion, according to the protocol outlined by Bell and Scarrow [3].

Sections, cut 80 μm thick, were reacted for the presence of alkaline phosphatase [3] in arterioles and capillaries and mounted on glass slides; with the aid of a grid (100 squares) placed in a 10× ocular of a light microscope, a vascular density index was obtained at a final magnification of 100× by identifying arterioles or capillaries which touched either the bottom or right boundary of each square within the grid area (1 mm^2) of ten consecutive fields in five sections of each selected cerebral segment. A square in which a vessel touched either of these boundaries, regardless of vascular branching, was assigned the number 1; these squares, totalling 5,00/region were then summed and divided by 50, yielding the vascular density index. The data were subjected to a factorial design analysis of variance following by the Student-Newman-Keuls test to determine specific significant differences.

In addition, the incidence of topographically altered blood vessels was assessed by counting such vessels within ten consecutive grid areas in five sections of each cerebral area. For this purpose, a blood vessel was considered abnormal if it displayed either tortuosity or severe kinking and/or looping. The obtained values were examined by a statistical analysis of variance. The slides were examined without awareness of the subjects' status by marking them only with accession numbers prior to counting.

Following the morphometric procedure, the Parlodion was dissolved and the blocks of brain tissue were embedded in paraffin; sections cut at 8 μm were stained routinely with hematoxylin and eosin.

Results

Examination of the normal microvascular bed reacted for alkaline phosphatase yielded an appearance resembling that which has been described in detail by others [1]. Differences in the vascular density affecting the four selected areas were noted in the normal cortex; pre-frontal and motor-sensory cortices appeared equally profusely vascularized in control brains, however, a mild decrease in vascular bed density in the normal basal forebrain and hippocampus was noticeable (Figs. 1a, 2a, 3a, 4a). This modification in vascular density was overshadowed by the striking and sta-

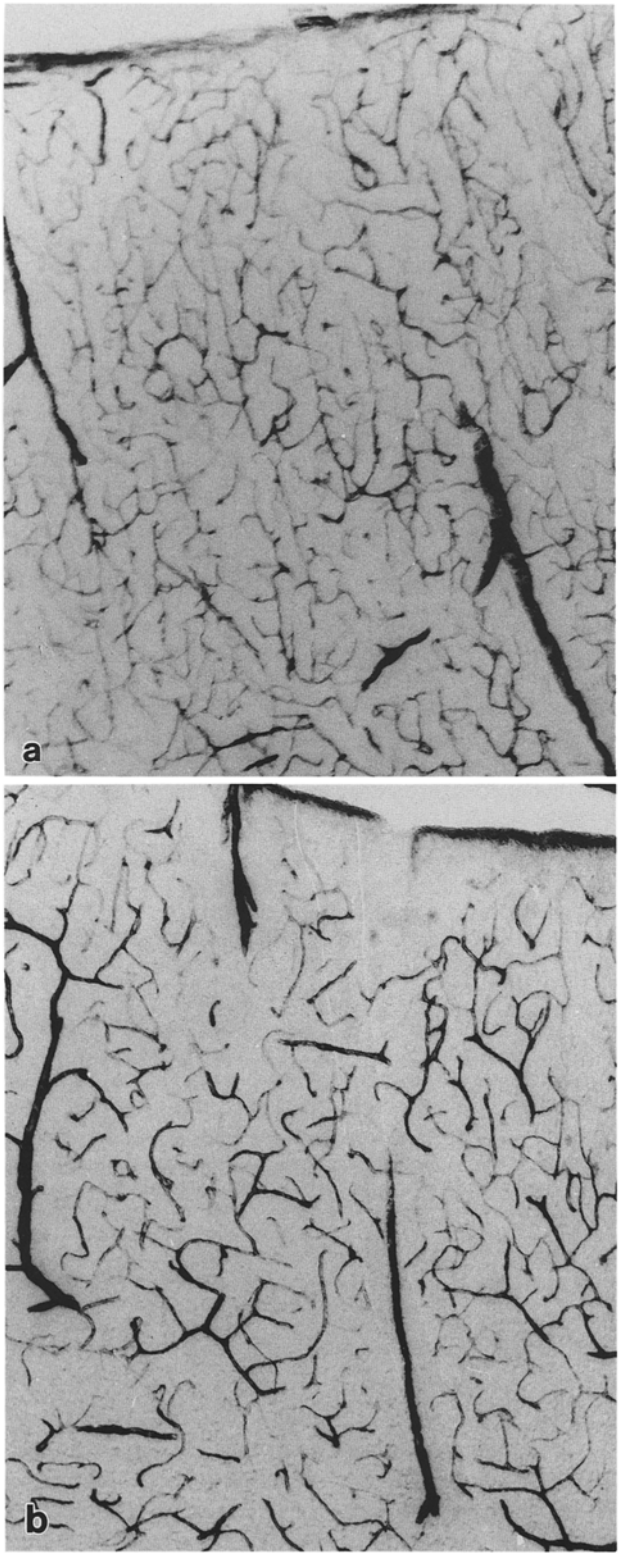

Fig. 1.a Prefrontal cortex, normal control, 63-year-old female. Arterioles and capillaries reacting positively for alkaline phosphatase. **b** Prefrontal cortex, Alzheimer's disease (AD), 83-year-old male, for comparison with **a**. A minimal decrease in vascular density is detectable by morphometry, **a, b** ×153

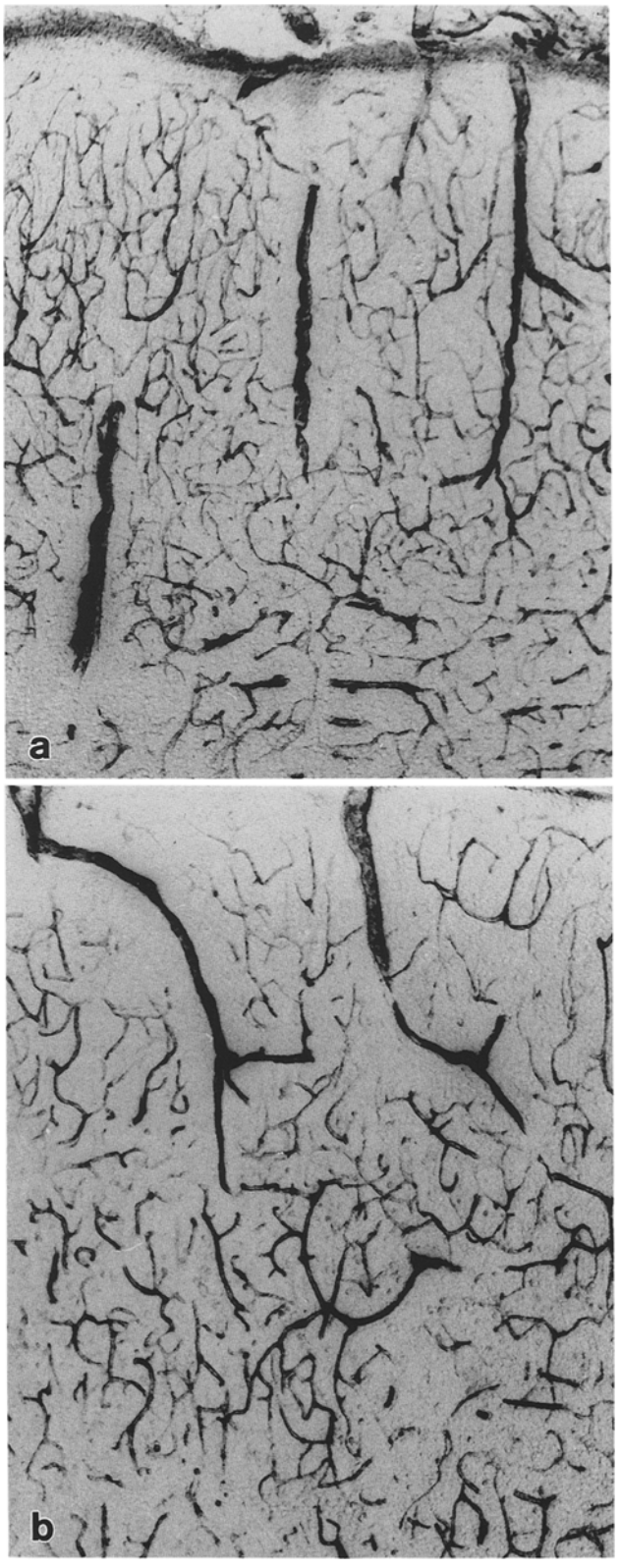

Fig. 2.a Sensorimotor cortex, control, 63-year-old female. Alkaline phosphatase. **b** For comparison with **a**; AD, 89-year-old female, note only minimal reduction of vascular density. Alkaline phosphatase. **a, b** ×156

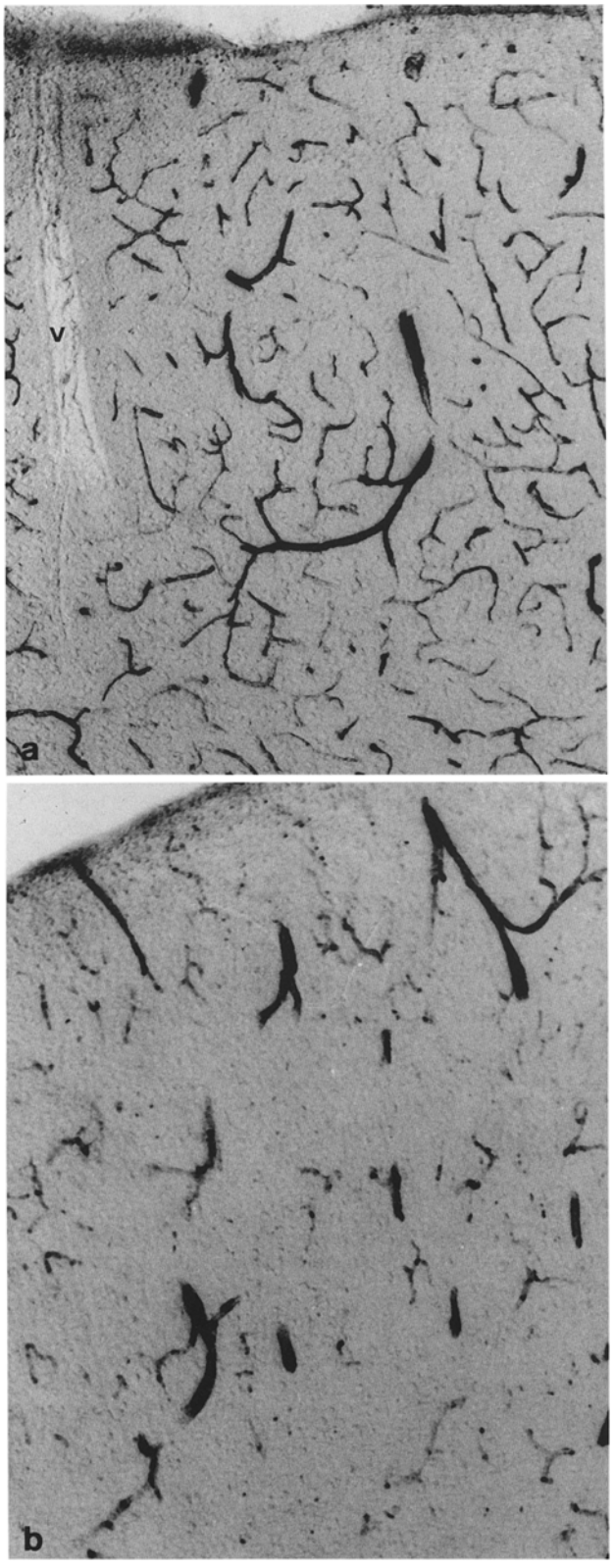

Fig. 3.a Basal forebrain, control, 63-year-old female. Note absence of alkaline phosphatase reaction products in venous endothelium (*v*). A slight reduction in vascularity, compared to that seen in prefrontal and sensorimotor cortices, can be noticed by morphometry. **b** Basal forebrain, AD, 83-year-old female. Note striking reduction in vascular density, compared to that seen in **a**. Alkaline phosphatase. **a, b** ×153

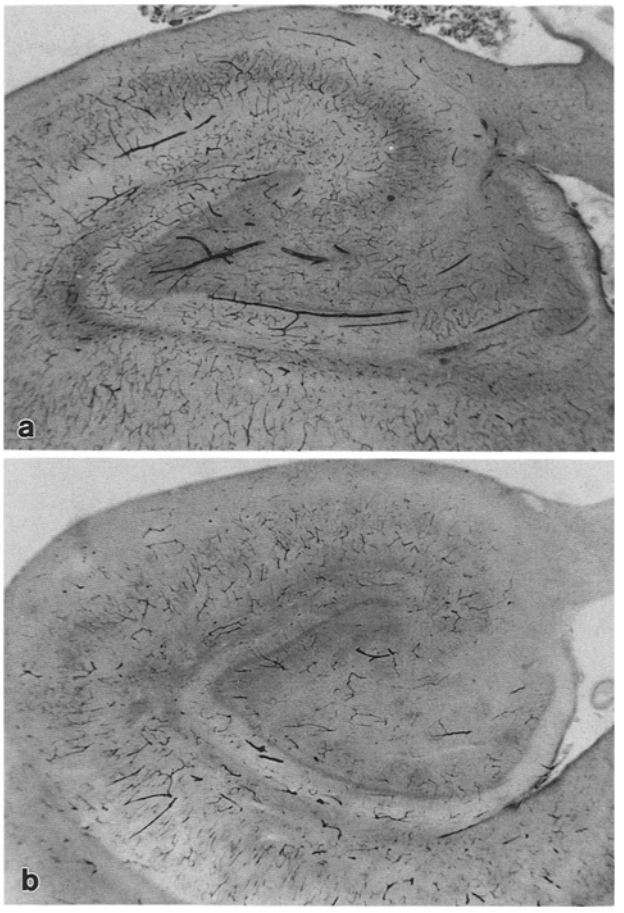

Fig. 4. a Hippocampus, control, 90-year-old female. Alkaline phosphatase. **b** Hippocampus, Alzheimer's disease, 89-year-old female. Note marked reduction in vascular density, contrasting with **a**. Alkaline phosphatase. **a, b** × 3

tistically significant reduction of the vascular bed evident in the basal forebrain and hippocampus of AD brains (Figs. 3b, 4b). This finding contrasts the moderate degree of diminished vascular density in prefrontal and sensorimotor cortices of individuals with AD (Fig. 1b, 2b). The morphometric data pertaining to all our cases are listed in Table 2.

To insure that the reduction in vascular density did not simply reflect an absence of enzymatic reaction, we applied a number of procedures to detect unstained vessels, such as polarizing light, closed-down apertures, and re-embedding of the celloidin blocks in paraffin, followed by H&E staining, and failed to see such vessels.

Topographical vascular changes included tortuosity or looping and kinking of blood vessels; tortuous vessels merely displayed an irregular contour, they never looped in their course. Such changes were seen only moderately in control tissue, however, they were observed frequently in AD brains (Figs. 5, 6). A numerical evaluation of such vessels revealed that par-

Table 2. Vascular density index

Cerebral area				Average	
Pre-frontal	Basal forebrain (BFB)	Motor/ sensory	Hippo-campus (HC)	All four areas	BFB & HC only
Alzheimer's disease (AD) [16]					
75.3	35.6	64.5	68.0	60.8	51.8
65.7	27.3	87.4	55.4	58.9	41.4
64.9	26.4	91.7	55.0	59.5	40.7
91.5	51.0	75.7	59.6	69.4	55.3
74.0	50.7	88.9	68.5	71.9	59.6
72.7	45.1	85.9	67.8	67.8	56.4
70.8	31.1	91.1	32.9	56.4	32.0
79.4	37.1	82.1	45.1	61.0	41.1
76.7	49.1	82.3	33.3	60.3	41.2
87.6	60.6	85.4	45.1	69.6	52.8
77.5	60.0	94.1	64.6	74.1	62.3
94.4	41.0	95.8	43.5	68.7	42.2
69.0	48.3	90.6	37.3	61.3	42.8
69.3	27.2	68.0	30.5	48.7	28.8
72.2	50.9	73.7	52.7	62.4	51.8
64.7	42.1	73.0	43.4	55.8	42.3
Average					
75.4	42.7*	83.1	50.2*	62.9	46.5*
Controls [6]					
94.2	76.9	97.4	73.5	85.5	75.2
92.6	86.9	95.3	84.1	89.7	85.5
95.6	92.3	92.6	85.0	91.3	88.6
91.0	89.6	90.4	78.4	87.3	84.0
96.4	88.9	93.9	82.0	90.3	85.4
97.5	86.2	96.4	90.2	92.6	88.2
Average					
94.6	86.8	94,3	82.2	89.8	85.1

* $P < 0.01$ (AD versus controls)

ticularly the number of blood vessels displaying kinking and/or looping, rather than a tortuosity, was significantly increased in all four areas of AD brains compared to the number of such vessels present in controls.

Tissue embedded in paraffin, subsequent to performing the enzyme reaction, and stained routinely with hematoxylin and eosin, showed no evidence of CNS disease in the control group; however, a moderate number of degenerating arterioles were seen in brains with AD (Fig. 7). These vessels, exhibiting a pronounced necrosis of the tunica media and, at times, an extensive calcification, appeared to be present largely in the basal forebrain region but were not seen consistently in all AD brains. Other neuropathological findings of note included the presence of cerebral lacunar infarcts in four cases, atherosclerotic vascular changes in nine cases, a congophilic angiopathy in two cases and hypertensive vascular disease in one case.

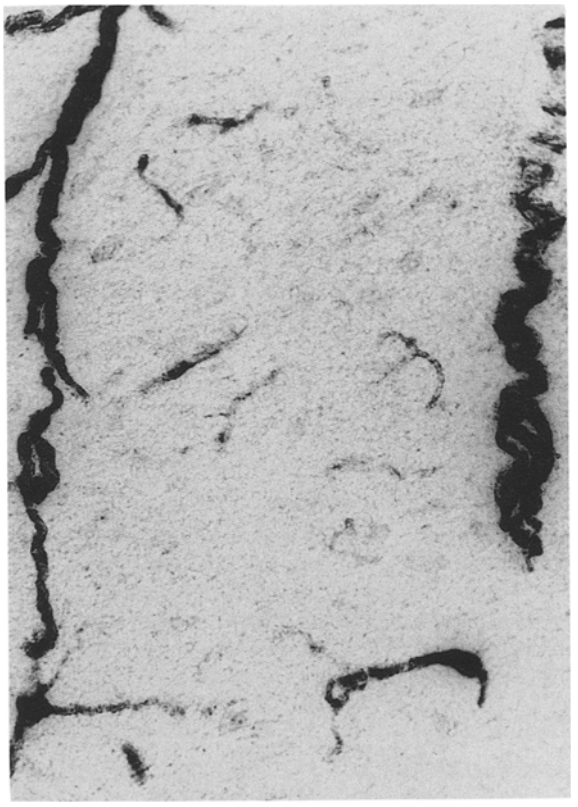

Fig. 5. Basal forebrain, AD, tortuous blood vessels. Alkaline phosphatase. × 360

Discussion

The findings of significance in this study can be summarized as follows: (1) the vascular density in AD brains is markedly reduced in contrast to the vascularity in normal brains; (2) the decreased density in AD brains is not demonstrable uniformly, but is limited specifically to two of the four areas which were examined, the basal forebrain and the hippocampus; (3) a pronounced increase in the number of abnormally contoured blood vessels, in the form of kinking and looping, was observed in all four areas of AD brains, and especially in the basal forebrain and the hippocampus.

It should be noted that the number of controls in our study is small and is not age-matched with the AD brains; as a result, conclusions regarding the statistical difference between the vascular density in AD and control brains should only be drawn with caution. Nevertheless, the calculations provide a comfortable assurance that the findings are not spurious, given the relatively tight fit of the control values to their mean and the lack of an age-related trend in values in the limited number of our six control brains.

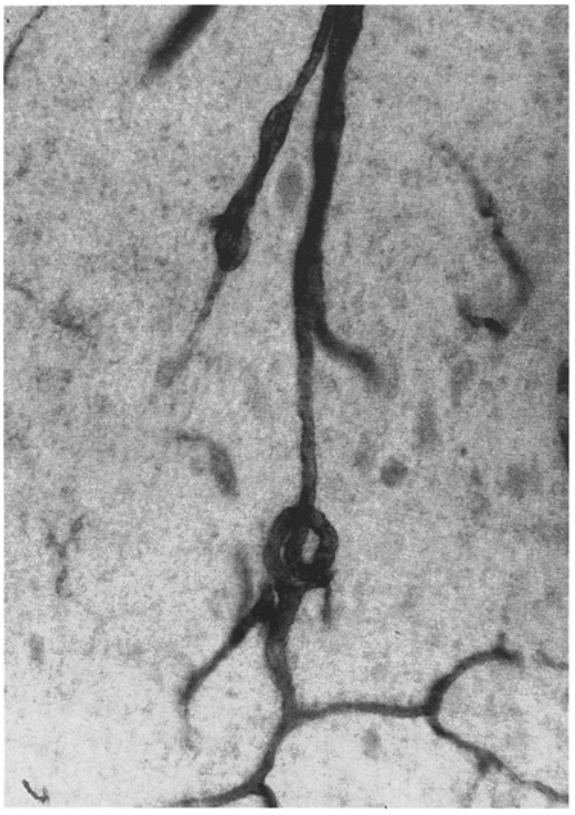

Fig. 6. Basal forebrain, AD, looped blood vessel. Alkaline phosphatase. × 360

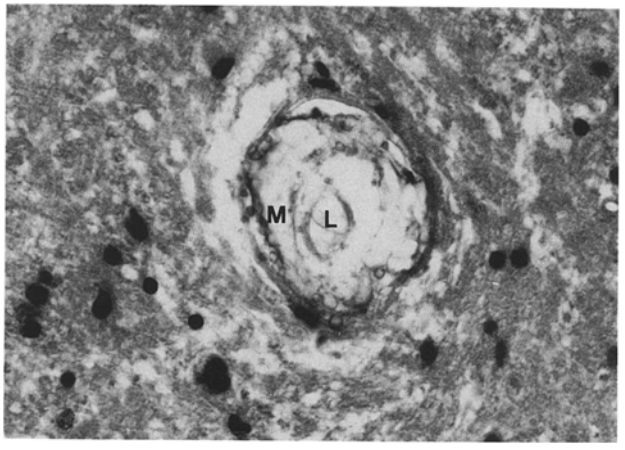

Fig. 7. Basal forebrain, AD, degenerating arteriole showing extensive medial (*M*) necrosis. *L*: Lumen. Paraffin, H&E. × 1,050

Isolated reports describing alterations of the vasculature in aging and demented brains have been available for over 100 years [15]. Initially, these contributions emphasized structural alterations, such as vascular coiling, loop formations, and tortuosity and kinking. More recently, Bell and Ball [2] reported decreased capillary and increased arteriolar densities in the aged hippocampus, while in the AD brains total hippocampal capillary density did not differ from that seen in the controls. However, in certain areas of the hippocampus, an increase in capillary density appeared to be related to the incidence of neuronal abnormalities, which led to the supposition that such an increase may provide access for a harmful factor to the cerebral cortex.

Our results differ from the above-mentioned findings, possibly because we express vascular density simply as the number of arterioles or capillaries present in a square millimeter of brain tissue and not as measurements of vascular lengths. Another study, however, using means of measurement similar to those reported by Bell and Ball [2], reached the conclusion that the blood supply in the aged cortex was not decreased [11], hence, this discrepancy fails to resolve the issue whether methodology can account for the differences in results. In our study a mildly lower vascular density was shown in the normal basal forebrain and hippocampus, regardless of age, in contrast to the profuse vascularity in the normal pre-frontal and motor/sensory areas. Most likely, this finding merely

Table 3. Number of abnormal vessels in cerebral tissue

	PF	BFB	M/S	HC	Total
Alzheimer's disease (AD) [16]					
Total no. of abnormal vessels	657	916	908	867	3348
No. per case	41.1	57.3	56.8	54.2	209.3
No. of tortuous vessels	392	474	505	505	1876
(%)	(59.7)	(51.7)	(60.1)	(53.5)	(56.0)
No. per case	24.5	29.6	31.6	31.6	117.3
No. of looped vessels	265	442	403	362	1472
(%)	(40.3)	(48.3)	(39.9)	(46.5)	(44.0)
No. per case	16.6	27.6	25.2	22.6	92
Controls [6]					
Total no. of abnormal vessels	213	167	218	230	828
No. per case	35.5	27.8	36.3	38.3	138
No. of tortuous vessels	183	128	185	187	683
(%)	(85.9)	(76.6)	(85.0)	(81.3)	(82.5)
No. per case	30.5	21.3	30.8	31.2	113.8
No. of looped vessels	30	39	33	43	145
(%)	(14.1)	(23.4)	(15.0)	(18.7)	(17.5)
No. per case	5	6.5	5.5	7.2	24.2
% increase of looped vessels/case in AD over controls	232*	324*	358*	214*	280*

* $P < 0.01$
PF: Pre-frontal; BFB: basal forebrain; M/S: motor/sensory; HC: hippocampus

reflects normal variations and extent of collateral blood supply, ranging from a most pronounced vascular density in the neocortex to the less abundant vascular permeation of archicortical limbic tissue.

A loss of cortical neurons in the normally aged individual is subject to controversy, at present. A recent report failed to find changes in the total number of neurons during aging [5]; moreover, it has been shown that cerebral senescence is not linked to impaired cerebral metabolism or cognitive ability, indeed "without superimposed disease, overall function can be maintained at high and effective levels" [7]. It is noteworthy that the profuse vascularity in the admittedly small number of normal aged brains which we examined, may point to a more reliable correlative factor for determining cerebral function, namely the vascular density within cerebral tissue. In support of this suggestion, most recently, the study by Prohovnik [14] showed that patients inhaling ^{133}Xenon in an early stage of AD are identifiable on the basis of a cerebral perfusion deficit in the temporoparietal cortex.

The result of our study that the reduction in vascular density is highly regionally specific in AD brains was not expected to the observed degree. As a consequence of a reported loss of neurons in the aging brain which does not affect all cerebral areas with equal severity [6], it is reasonable to assume an association between vascular supply and neuronal density and, hence, to expect a modest variation in the decrease of the vascular bed in AD brains; however, the marked, statistically significant decrease of the vascularity in two specific areas, the basal forebrain region and the hippocampus, exceeded this assumption. Our study, based on an absolute count of blood vessels, revealed a severe reduction in the number of vessels limited to two areas and suggests that especially the microvasculature in these specific areas is susceptible to an as yet unknown pathological factor of AD. This speculation is strengthened by data obtained by us from two individuals with non-Alzheimer's dementia. The vascular density index in these cases resembled that seen in the control group. While these findings are statistically insufficient, and hence, do not permit definitive conclusions, they intriguingly reinforce the hypothesis that regionally specific vascular loss is particularly pronounced in dementia of the Alzheimer type. It must be recognized, however, that the possibility of a congenitally deficient vasculature in AD individuals cannot be ruled out.

The finding that significant vascular architectural changes are present in the AD brain may be of considerable importance for an understanding of the pathogenesis in AD. In particular, the issue dealing with the origin of the beta-amyloid protein abnormally deposited in the wall of cerebral blood vessels and within cores of senile plaques, characteristically seen in AD brains, is controversial at present. A vascular derivation of an "amyloidogenic" protein has been postulated, i.e. chronic transudation through an abnormally permeable vascular wall into the cerebral parenchyma with subsequent deposition [9]; since it is reasonable to assume that the decreased vascular density, which we observed, temporally follows an abnormally permeable vascular bed, our results favor this supposition; however, the discovery that precursor proteins to amyloid are synthesized by neurons lends emphasis to a neuronal origin of such amyloid deposition [17]. The relationship, if any, between the presence of neuronal and vascular amyloid remains to be clarified.

The observation of abnormally contoured blood vessels in aging brains has been ascribed primarily to atrophy of the cortical parenchyma, with subsequent physical compression and rotation of blood vessels [15]. In our study, the separation of such vessels into two categories, tortuous vessels and those with a looped, kinked appearance, revealed that the former type was prevalent in both control (regardless of age) and AD groups of brains. For this reason, it is likely that these vessels are deformed as a consequence of tissue shrinkage due to artefactual processes postmortem.

Looping and kinking of vessels, however, was observed considerably more frequently in AD brains than in the controls. In contrast to tortuous vessels, the architecture of looped vessels is likely to exert more deleterious effects on tissue perfusion, since it is reasonable to assume that the hemodynamics in sharply looping and kinked vessels is disturbed. This alteration, present in all four regions which we examined, may represent an early stage of vascular reduction which is seen so prominently in the basal forebrain and hippocampus of AD brains, although it remains unclear why these two cerebral regions are particularly vulnerable in this regard.

In conclusion, this investigation showed that the AD process affects the cerebral vasculature and that this alteration exibits a marked regional specificity, underlining the involvement of the basal forebrain and hippocampus in this disorder.

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References

1. Akima M, Nonaka H, Kagesawa M, Tanaka K (1986) A study on the microvasculature of the cerebral cortex. Lab Invest 55:482–489
2. Bell MA, Ball MJ (1981) Morphometric comparison of hippocampal microvasculature in aging and demented people: diameters and densities. Acta Neuropathol (Berl) 53:299–318
3. Bell MA, Scarrow WG (1984) Staining for microvascular alkaline phosphatase in thick celloidin sections of nervous tissue: morphometric and pathologic applications. Microvasc Res 27:189–203
4. Blessed G, Tomlinson BE, Roth M (1968) The association between quantitative measures of dementia and of senile changes in the cerebral grey matter of elderly subjects. Br J Psychiatr 114:797–811
5. Haug H (1987) Brain sizes, surfaces, and neuronal sizes of the cortex cerebri: a stereological investigation of man and his variability and a comparison with some mammals (primates, whales, marsupials, insectivores, and one elephant). Am J Anat 180:126–142
6. Coleman PD, Flood DG (1987) Neuron numbers and dendritic extent in normal aging and Alzheimer's disease. Neurobiol Aging 8:521–545
7. Creasey H, Rapoport SI (1985) The aging human brain. Ann Neurol 17:2–10
8. Fang CH (1974) The studies of cerebral arterioles and capillaries in normal and pathologic states in man. In: Cervós-Navarro J (ed) Pathology of cerebral microcirculation. De Gruyter, Berlin, pp 431–441
9. Glenner GG (1985) On causative theories in Alzheimer's disease. Hum Pathol 16:433–435
10. Hassler O (1965) Vascular changes in senile brains. Acta Neuropathol (Berl) 5:40–53
11. Hunziker O, Abdel'Al S, Schulz U (1979) The aging human cerebral cortex: a stereological characterization of changes in the capillary net. J Gerontol 34:345–350
12. Khachaturian ZS (1985) Diagnosis of Alzheimer's disease. Arch Neurol 42:1097–1104
13. Pickworth FA (1934) A new method of study of the brain capillaries and its application to the regional localization of mental disorder. J Anat 69:62–71
14. Prohovnik I, Mayeux R, Sackeim HA, Smith G, Stern Y, Alderson PO (1988) Cerebral perfusion as a diagnostic marker of early Alzheimer's disease. Neurology 38:931–937
15. Ravens JR (1978) Vascular changes in the human senile brain. In: Cervós-Navarro J (ed) Advances in neurology, vol 20. Raven Press, New York, pp 487–501
16. Scheibel AB, Duong T, Tomiyasu U (1987) Denervation microangiopathy in senile dementia, Alzheimer type. Alzheimer Dis Assoc Disord 1:19–37
17. Selkoe DJ (1989) Aging, amyloid, and Alzheimer's disease. N Engl J Med 320:1484–1487

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