The Correlation of Vascular Capacity with the Parenchymal Lesions of Alzheimer's Disease

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ABSTRACT: Hippocampal capillary and arteriolar measurements showed a significant reduction in capacity with normal aging. Alzheimer's dementia was not associated with any further reduction; in fact regional variations suggested that the zones of Ammon's horn most severely affected by tangles and granulovacuoles retained the best vascular capacity.

The arterial system supplying the hippocampus, from the posterior cerebral artery to the series of small hippocampal arteries, was also assessed. Its capacity, as judged by arterial diameters, was similarly found to decrease significantly with age; the arterial diameters of Alzheimer cases were (insignificantly) greater than those of the normal old.

Calcarine capillary and arteriolar measurements also indicated a marked reduction of capacity with normal aging, and, as before, in Alzheimer's dementia there was no further significant change. All three phases of the study thus suggest that cerebrovascular capacity in Alzheimer's dementia is at least as good as in the normal old, if not better.

Senile (neuritic) plaques with amyloid cores and their relationship to the microvasculature were examined in the calcarine cortex. In both normal old and Alzheimer cases the plaques tended to congregate where capillaries were densest. Their correlation with capillary density was however better in the normal old, raising the question of whether their pathogenesis might differ in the two conditions.

RÉSUMÉ: Corrélation entre la capacité vasculaire et les lésions parenchymateuses dans la maladie d'Alzheimer. La mesure des capillaires et artérioles de l'hippocame a montré une réduction significative dans leur contenance au cours du vieillissement normal. La démence de la maladie d'Alzheimer n'était pas associée à une réduction additionnelle; en fait, des variations régionales suggèrent le fait que les zones de la corne d'Ammon qui manifestent l'atteinte la plus sévère par dégénérescence neurofibrillaire et granulo-vacuolaire conservent la meilleure capacité vasculaire.

Nous avons également évalué le système artériel irriguant l'hippocampe, de l'artère cérébrale postérieure à l'ensemble des petites artères de l'hippocampe. Il s'est avéré également que sa capacité, telle qu'évaluée par les diamètres artériels, décroissait de façon significative avec l'âge; les diamètres artériels des cas de maladie d'Alzheimer étaient plus grands que ceux des témoins âgés normaux (non significatif).

La mesure des capillaires et artérioles de la zone calcarine accusait aussi une réduction importante accompagnant le vieillissement normal, et, comme précédemment, il n'y avait pas d'autre changement significatif dans la démence d'Alzheimer. Ainsi, les trois phases de l'étude suggèrent que la capacité du système cérébrovasculaire est au moins aussi bonne, sinon meilleure, que chez le vieillard normal.

Nous avons examiné la relation entre les plaques séniles (névritiques) avec centre amyloïde et le réseau microvasculaire au niveau du cortex avoisinant la scissure calcarine. Chez les vieillards normaux ainsi que chez les cas de maladie d'Alzheimer il y avait tendance à ce que les plaques soient concentrées là où les capillaires étaient les plus denses. La corrélation entre les plaques et la densité des capillaires était cependant meilleure chez les vieillards normaux, ce qui soulève la question suivante: leur pathogénèse est-elle différente dans ces deux entités?

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Afferent vessels bring to every tissue the normal nutrients it needs to survive; they also provide an access route for infective agents or toxins that may affect the tissue's well-being, as well as for the components (or their precursors) of lesions that may form there. When the etiology of a given disease is unknown, it makes sense to examine the blood supply concerned: if its carrying capacity is compromised, a lack of some essential nutrient or the abnormal accumulation of some toxic metabolic waste product might be implicated; if the vascular capacity is increased, or if lesions can be shown to prefer sites close to vessels, suspicion falls on some blood-borne agent or nutrient initiating, contributing to or supporting the pathogenesis of the lesions.

The cerebral vessels of brains from patients with senile dementia of the Alzheimer type do not consistently display striking abnormalities either at autopsy or later when routine paraffin sections are inspected. Frequently, in fact, they are notably free from the severe atherosclerotic changes commonly seen elsewhere in the body after middle age.[1] Investigations into the etiology of this disease have therefore not focussed on the vascular supply of the affected parenchyma. Nonetheless, a search for more subtle relevant vascular changes is warranted.

Some vascular observations that may ultimately prove important have been made on Alzheimer brains. Amyloid angiopathy appears to affect brains of Alzheimer victims more frequently and more severely than those of the normal aged;[2] the effects of this proteinaceous infiltration of vessel walls are not clear, but marked reduction of the vascular lumen is not apparent. Electron microscopical studies of Alzheimer brain capillaries suggest that intraendothelial changes occur and that basal laminae may be thickened.[3] All of these pathological changes probably influence transport of substances across the vascular wall more than the carrying capacity of the system.

The present study was undertaken to answer the question of whether the vascular capacity of the brain in Alzheimer's dementia is increased, diminished, or unaffected relative to that of the normal aged brain. In three separate phases it examined the intraparenchymal microvessels of the hippocampus, the larger arteries supplying the hippocampus, and the intraparenchymal microvessels of the visual (calcarine) cortex. The third phase permitted some additional observations about the much-debated relationship of the vessels to senile (neuritic) plaques, which are common in this area in both normal aged and Alzheimer brains. This paper summarizes the results from all three phases; detailed findings from the first phase (hippocampal microvasculature) have been published elsewhere,[4] and complete accounts of the second and third are in preparation.

METHODS AND MATERIALS

The hippocampus was chosen for the examination of correlations between Alzheimer and vascular changes because, in the first place, this archicortex is the part of the brain most vulnerable to Alzheimer neuronal lesions: neurofibrillary tangles and granulovacuoles, which congregate there in significantly higher numbers than in any area of the normal aged brain.[5,6,7] Secondly, the hippocampus is known to be particularly susceptible to ischemic and hypoxic damage, and thirdly, its putative role in memory is exemplified by the loss of this function in Alzheimer's dementia, the most devastating and now most familiar effect of the disease on its victims. For a neocortical

comparison, the visual area was chosen. It has the most marked laminar variation in its capillary density,[8] allowing easy distinction and comparison of adjacent layers, and it accumulates significant numbers of senile (neuritic) plaques, particularly in Alzheimer's dementia, but also in normal aging.[9]

In all three phases of the study, comparisons were made between brains removed at autopsy from young (under 55), normal old, and Alzheimer subjects (Table 1). Patients from the first two groups were mentally normal, and their brains showed no neuropathological or severe cerebrovascular lesions. All in the third group had histories of dementia, and their brains showed typical Alzheimer lesion distribution.

The demonstration, for the first and third phases of the study, of intracortical arterioles and capillaries with alkaline phosphatase staining in 100μ celloidin sections, and the method of measuring the diameters and densities of these vessels, have been previously described.[10] From the hippocampus, six separate areas were compared: entorhinal cortex, presubiculum, subiculum, H1, H2 and endplate (5 slides per case; see Figure 1). From the visual cortex, three areas were sampled: upper lip, lower lip and sides of the calcarine fissure (3 slides per case). The senile plaques of occipital cortex were demonstrated by adding a Congo red counterstain to the alkaline phosphatase procedure; amyloid cores of plaques then showed bright green birefringence under polarized light (Figure 2). Only plaques with amyloid cores were considered.

The arteries supplying the hippocampus were demonstrated for the second phase of the study by injecting the posterior cerebral and anterior choroidal arteries on one side of the brain (the left for all except one young case) with warm (40-50°C) 70% Micropaque in 2.5% gelatin, coloured with yellow poster colour paint. Transorbital, lateral and Townes angiograms were obtained after formalin fixation of the brain. The varying patterns and lengths of the relevant arteries were ascertained by dissection under a Zeiss operating microscope. A short segment of each branching generation of each relevant artery was removed, embedded in paraffin, transversely sectioned, and stained with orcein to identify the internal elastic lamina, and with the van Geison stain combination to distinguish the muscular media from the collagenous adventitia (Figure 3). On photographs of these preparations, the circumferences of 1) the intimal (endothelial) surface, 2) the internal elastic lamina, 3) the outer edge of the media, and 4) the outer edge of the adventitia, were traced with the cursor of a Hewlett-Packard (9864A-9815A) digitizer-computer-calculator system. The areas enclosed by these outlines were also recorded.

RESULTS

Phase I — Hippocampal Microvasculature

Values for the diameters and densities of hippocampal arterioles and capillaries in the young, old and Alzheimer groups have been published previously in detail,[4] along with comments on the parameters that can be derived from these measurements such as capillary surface areas and volumes. Capillary densities illustrate the trends observed and are reproduced here in Table 2. There was a clear reduction of capillary densities associated with aging in all six hippocampal zones (17.7% down overall when the normal old were compared to the young). When the Alzheimer values were compared to the normal old however, no significant difference was seen overall, indicating

Table 1: Summary of Case Information for Three Phases of Study

		No. of brains	Age Range	Age Mean	Sex Male	Sex Female
Phase I	Young	5	21-51	38	5	0
Hippocampal microvasculature	Old	5	60-88	74	3	2
	Alzheimer	5	66-94	78	0	5
Phase II*	Young	5	32-51	43	5	0
Hippocampal arteries	Old	6	60-88	71	4	2
	Alzheimer	4	73-88	79	1	3
Phase III	Young	8	12-54	31	6	2
Calcarine microvasculature	Old	8	67-95	79	7	1
	Alzheimer	10	63-92	79	5	5

*Phases I and II ran concurrently and 7 (about ½) of these cases were used in both phases. Phase III was conducted later and none of its cases overlap. The total number of brains represented is 49.
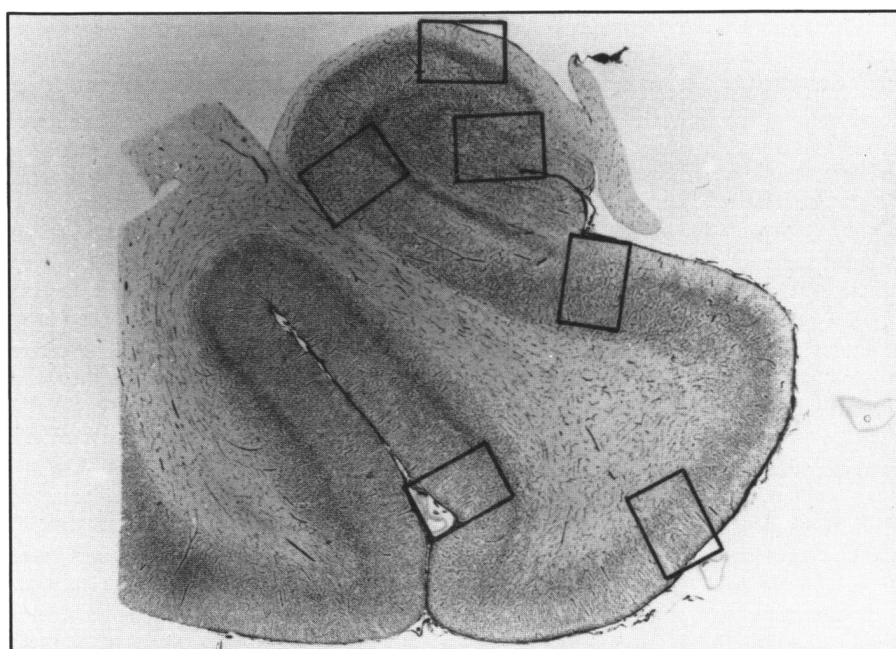

Figure 1 — Hippocampus. Low power light micrograph of 100µ celloidin section stained for vascular alkaline phosphatase. The six zones sampled (starting at bottom) were: entorhinal cortex, presubiculum, subiculum, H1, H2, endplate.

that while the predictable reduction with aging had occurred, no further compromise of hippocampal irrigation was associated with the presence of Alzheimer neuronal lesions. Indeed, in the three zones where numbers or increments of these lesions were greatest (subiculum, H1 and H2), there was a tendency (not usually significant) for the capillary densities to be higher than in the normal old (i.e. not as much reduced).

Phase II — Arteries supplying the hippocampus

The arteries sampled for this part of the study included all those believed to contribute to the intraparenchymal hippocampal vascular net in all the observed configurations of the circle of Willis and its posterior branches: anterior choroidal artery, posterior communicating artery, posterior cerebral artery, a variable number (0-3) of inferolateral arteries (which branch away from the posterior cerebral to supply the inferior temporal surface, giving off the hippocampal arteries en route), and a variable number (2-6) of hippocampal arteries (which arise either directly from the posterior cerebral or from its inferolateral branches, cross the subicular surface, and enter the hippocampal sulcus). The configuration of these arteries varied from case to case; Figure 4 shows a generalized pattern.

Details of the relevant measurements and calculations (including diameters, wall thicknesses, thickness/lumen ratios and a "distortion ratio") are being prepared for publication, but the diameters of the hippocampal arteries can be quoted here as an example of the changes observed. The diameter given is that of the internal elastic lamina, calculated from its measured circumference. Because it corrugates rather than increasing its thickness and shortening its circumference as the artery contracts, and because it will have the same circumference whether the artery is collapsed or rounded, this layer of the arterial wall provides the most dependable basis for comparisons (Figure 3). Distention of the artery under internal injection pressure would stretch the internal elastic lamina and yield unrealistic circumference values, but such pressure was dissipated by permitting some back-flow drainage from the freshly injected arteries before they were tied.

There was a significant decrease in diameter at the internal elastic lamina of the hippocampal arteries due to aging (Table 3). When the Alzheimer group was compared to the normal old, however, the mean laminar diameter of these arteries in the demented subjects' brains was slightly (but not significantly) larger, perhaps suggesting a greater carrying capacity for the

Figure 2 — 100µ celloidin section of calcarine cortex. Vessels are stained by alkaline phosphatase reaction; amyloid cores of plaques, stained with Congo red, show brilliant green birefringence in polarized light. Magnification (25 × objective) above shows good detail but whole thickness of section is not in focus (bar = 10µ). For measurements, a negative of the whole cortex was made with a 4 × objective, whose depth of focus is about 100µ. A small sample of the working magnification (300 ×) is seen below; all the vessels (arteriole and several capillaries) are in focus. Two plaque cores can be identified; other bright spots are birefringent artefacts.

Alzheimer group. This diameter in the Alzheimer group, being slightly less reduced than that of the old, was in fact not significantly different from that of the young.

Phase III — Calcarine microvasculature

Values that were measured or calculated for this phase of the study included arteriolar and capillary diameters and densities, packing densities and core radii of senile plaques, plaque-vessel distances, and percentages of arteriolar lengths affected by amyloid angiopathy. A detailed account is in preparation; capillary densities and a comparison of capillary and plaque distributions are given here as examples (Tables 4 and 5).

It has previously been established[8] that the greatest capillary density of this cortex is found in layer IV (corresponding approximately to the line of Gennari), followed in order of diminishing density by layers II-III, V-VI and I. Reductions of these densities (usually significant) were found with normal aging (15.5% down overall); slight further reductions were found with Alzheimer's dementia, but these were not significant. As in the first two phases of the study, no significant reduction of vascular capacity was found to be associated with the Alzheimer disease process.

In general, plaques were concentrated in the layers with most capillaries (IV and II-III), but their distribution differed slightly in the normal old and Alzheimer cases. In the old, the plaques showed a more marked tendency to cluster in layer IV amongst the most closely packed capillaries, whereas in the Alzheimer cortex they were more evenly distributed, the greatest density falling in layers II-III. As a result of this discrepancy, the correlation between plaque and capillary densities, at least in the layers where both were most plentiful, was closer for the normal old than for the Alzheimer group (Table 5).

Depending on the lamina observed, no more than 55% of the plaque cores (and usually much less) were seen to be in direct contact with a vessel wall. The normal old cases had a significantly higher percentage of these "touching" plaques.

DISCUSSION

None of the three cerebral vascular systems studied here revealed a significant difference in carrying capacity between normal old and Alzheimer cases; both were equally reduced compared to the normal young state. It seems unlikely therefore that Alzheimer neuronal lesions cause or result from inadequate irrigation of the tissue.

If any vascular tendency, albeit insignificant, is to be noted from the comparison of old and Alzheimer states, it would be the slightly greater capacity of the hippocampal vasculature associated with the presence of Alzheimer neuronal lesions. The hypothesis of some blood-borne agent initiating or sustaining the pathogenesis of these lesions is supported. It cannot of course be assumed that a greater potential vascular capacity automatically results in a greater blood flow; that question was not addressed in this study. Nor was the question of whether transport across the vessel walls, e.g., the blood-brain barrier, is altered. Nonetheless, in the light of suggested positive correlations between plaque and vessel distributions, it seems appropriate to speculate that observed ultrastructural changes in vascular wall structure result in an increased permeability rather than a restricted exchange.
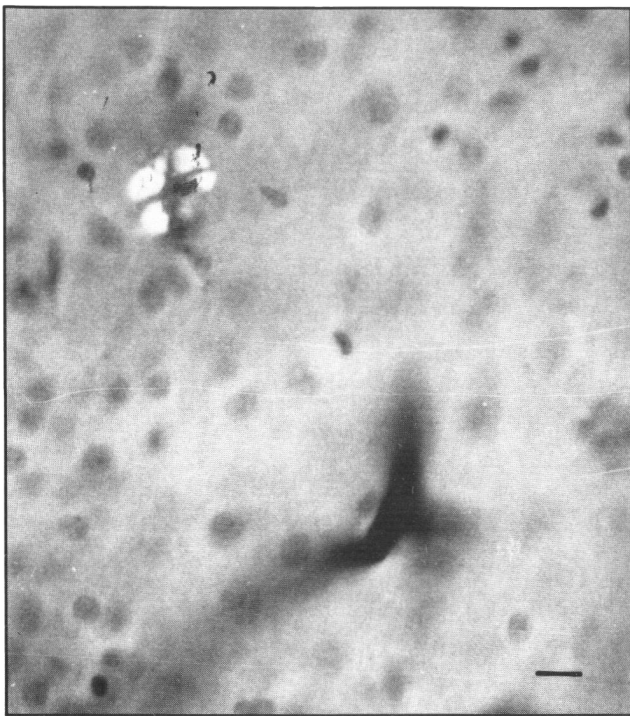
The clear preference of calcarine senile plaques for the most heavily vascularized laminae suggests a similar dependence on nutrients or blood-borne agents, but the data describing plaque distributions and percentages of plaques found in contact with vessels also suggest that this dependence is greater in normal aging than when the Alzheimer disease process is superimposed. It might be postulated that there are different pathogenetic mechanisms in these two states, or that there are two populations of amyloid-cored plaques with different dependencies on blood flow; normal aging would elicit one population, while the other would only be added, in a different distribution, with the advent of dementia.

Of course plaques might be found in areas with a high vascular density even though entirely independent of blood supply,
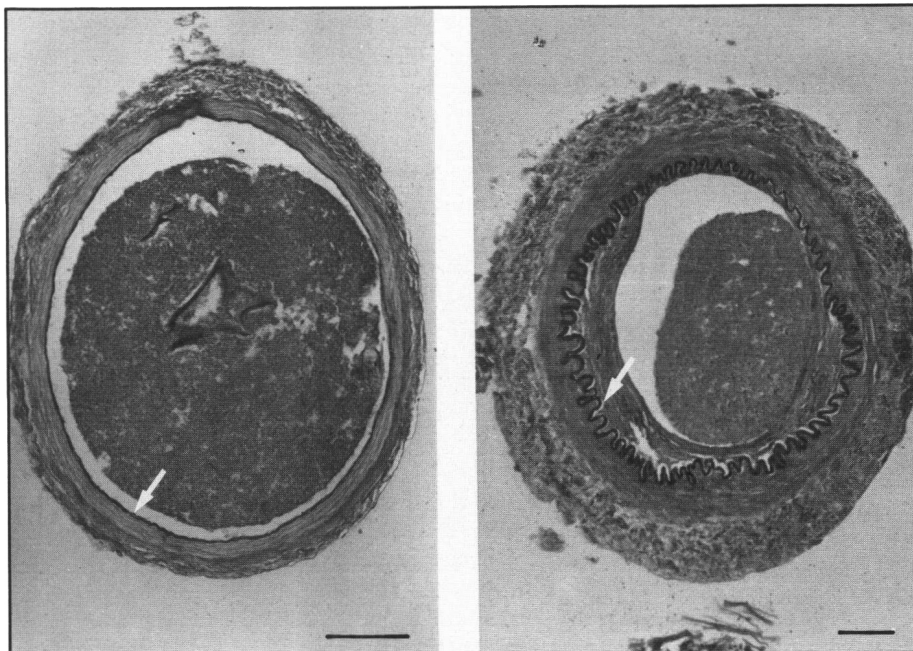

Figure 3 — Transverse paraffin sections of arteries, stained with orcein to demonstrate the internal elastic lamina (arrows). The artery on the left (PCA) has been fixed in a smoothly rounded state, the one on the right (PCoA) contracted (but not collapsed). The latter also shows a thickened intimal layer. (Bars = 100μ)

Table 2: Mean Densities (mm/mm³ ± 1 S.D.) of Hippocampal Capillaries

	Entorhinal	Presubiculum	Subiculum	H_1	H_2	Endplate	Overall mean (all six zones)
Normal young	146$^{(\pm 28)}$	111$^{(\pm 24)}$	134$^{(\pm 16)}$	131$^{(\pm 22)}$	119$^{(\pm 15)}$	102$^{(\pm 17)}$	124$^{(\pm 25)}$
	}15.1%↓*	}30.6%↓*	}17.2%↓*	}18.3%↓*	}15.1%↓*	}8.8%↓	}17.7%↓*
Normal old	124$^{(\pm 34)}$	77$^{(\pm 23)}$	111$^{(\pm 18)}$	107$^{(\pm 20)}$	101$^{(\pm 17)}$	93$^{(\pm 26)}$	102$^{(\pm 28)}$
	}10.5%↓	}13.0%↓	}8.1%↑	}11.2%↑*	}5.0%↑	}11.8%↓	}1.0%↓
Alzheimer	111$^{(\pm 21)}$	67$^{(\pm 21)}$	120$^{(\pm 21)}$	119$^{(\pm 21)}$	106$^{(\pm 14)}$	82$^{(\pm 18)}$	101$^{(\pm 28)}$

*The difference between the two bracketed values is significant (p<0.05 or better).

Table 3: Mean Diameters (μ ± 1 S.D.) of Hippocampal Arteries at Internal Elastic Lamina

Normal Young	328$^{\pm 88}$
	}17.1%↓*
Normal Old	272$^{\pm 63}$
	}7.7%↑
Alzheimer	293$^{\pm 76}$

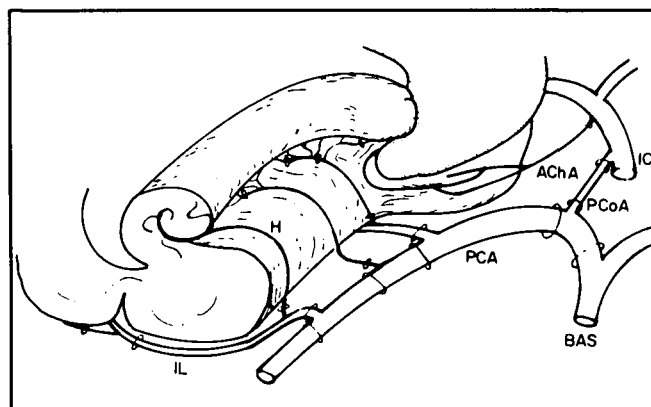*The difference between these two bracketed values is significant, p<0.02.

Figure 4 — Posteromedial view of hippocampus and its supplying arteries: IC internal carotid; AChA anterior choroidal; PCoA posterior communicating; BAS basilar; PCA posterior cerebral; IL inferolateral; H hippocampal. Generalized scheme; arterial patterns varied from case to case. Rings indicate typical sites for sampling arterial diameters.

Table 4: Mean Densities (mm/mm³ ± 1 S.D.) of Hippocampal Capillaries

Cortical Laminae	I		II-III		IV		V-VI		Overall	
Normal Young	$141^{\pm 64}$	$\}29.8\%\downarrow$*	$285^{\pm 60}$	$\}17.5\%\downarrow$*	$368^{\pm 65}$	$\}13.9\%\downarrow$*	$209^{\pm 45}$	$\}5.3\%\downarrow$	$251^{\pm 103}$	$\}15.5\%\downarrow$*
Normal Old	$99^{\pm 57}$		$235^{\pm 60}$		$317^{\pm 80}$		$198^{\pm 68}$		$212^{\pm 103}$	
Alzheimer	$97^{\pm 40}$	$\}2.0\%\downarrow$	$235^{\pm 43}$	$\}0$	$301^{\pm 51}$	$\}5.0\%\downarrow$	$193^{\pm 45}$	$\}2.5\%\downarrow$	$206^{\pm 88}$	$\}2.8\%\downarrow$

*Difference between bracketed figures is significant (p<0.02 or better).

Table 5: Ranking Orders of Capillary and Plaque Densities in Calcarine Cortex (1 = greatest density)

Cortical laminae	I	II-III	IV	V-VI
Capillaries (all groups)	4	2	1	3
Plaques, normal old	3	2	1	4
Plaques, Alzheimer	4	1	2	3

and associated instead with some other feature like synaptic density that coincidentally requires an increased capillary density. Even if this were true, the discrepancy between normal old and Alzheimer plaque distributions would still suggest a difference in their pathogenesis in these two states, only this time in relation to the synaptic network. Alternatively, perhaps plaques consistently represent degenerating synaptic contacts, a higher percentage of which might break down in layer IV in the aged and in layer II-III in Alzheimer's dementia — a pattern possibly dictated by some remote neuronal deterioration.

A dependence on vascular function is not equivalent to a direct origin from the vascular wall. Most plaque cores did not appear to be in contact with a vessel, confirming the assumption that they were parenchymal (neuritic?) in nature. The alkaline phosphatase stain displays only the arterioles and capillaries, but the background tissue colouration and manipulation of the light source renders most unstained vessels, including small venules, visible. Since every plaque core counted was microscopically inspected, it is probable that significant numbers of contacts with unstained venular walls would have been detected.

Only those plaques with amyloid cores (visualized with the Congo red stain) were considered. An evaluation of slides from 5 cases that had been stained with silver in addition to Congo red suggested that approximately 30% of the total number of plaques present were being detected. If plaques without amyloid cores represent a different population, nothing about their relationship to vessels can be inferred from the present study.

The progress of Alzheimer's dementia appears to be associated with at least an adequate blood supply. If future studies confirm the correlations observed here between lesion distributions and high microvascular capacities, blood-borne agents may be implicated in the genesis or maintenance of neurofibrillary tangles, granulovacuoles and senile plaques.

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