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Morphometric Comparison of Hippocampal Microvasculature in Ageing and Demented People: Diameters and Densities

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Summary. The diameters and densities of capillaries and arterioles in the hippocampal cortex of normal subjects and patients with Alzheimer's dementia were measured in thick celloidin sections stained for alkaline phosphatase. Microvascular diameters in general are affected more by age than by the presence of dementia of the Alzheimer type. The diameter of both capillaries and arterioles increases significantly with age. The density of capillaries decreases whereas that of the arterioles increases significantly. The capillary changes suggest that a reduced exchange potential accompanies ageing.

In brains of people with Alzheimer's disease the overall capillary diameters and densities do not differ from those of age-matched controls. Regional changes may, however, be important: those hippocampal zones showing the greatest severity of or increment in nerve cell lesions do correspond to those having the highest levels of or increase in capillary density and the greatest decrease in diameter, suggesting a direct association between neuronal susceptibility to Alzheimer changes and degree of regional blood supply. Capillary surface areas, volumes, and area/capillary volume ratios support the possibility of this relationship.

Neurofibrillary tangles and granulovacuolar degeneration do not correlate equally with the degree of capillary "irrigation"; tangles are more closely related to these morphological vascular parameters.

Key words: Dementia – Hippocampus – Alzheimer's disease – Ageing – Microvasculature

Introduction

Changes in the microvascular anatomy of the human brain have occasionally been observed in association with the ageing process. Hassler (1967) described "glomerular loops", "bundles", and "wickerworks" (after Cerletti 1910/1911). Fang (1976) also saw coiling and looping of the small vessels, with knob-like formations and sinusoidal enlargements of the venules, as well as areas of apparent decreased vascularity. Ravens (1978) confirmed earlier observations of tortuous changes in ageing small vessels. Beskow et al. (1971) found that vascular "loops", "bundles", and "wickerworks" do not occur in greater numbers in patients with senile dementia than in the normal aged population. A few non-specific pathologic changes in small cerebral vessels that are associated with ageing and dementing illnesses have also been mentioned by McMenemy (1971), Jellinger (1977), and O'Brien (1977).

Despite such descriptive reports, very little information is available about *quantitative* changes in the cerebral microvasculature either attributable to ageing, or associated with the pathology of senile dementia of the Alzheimer type. In the human neocortex Hunziker et al. (1979), using an image analyzer, found that capillary diameter, density, and volume increased in the 65–74-year-old group, while surface area decreased; values for still older subjects resembled the young. Bär (1978, 1980) reported that ageing in the rat produced little change in capillary diameters, an increase in their density and surface area, and a decrease in their volume.

The neurofibrillary tangle of Alzheimer (1907), granulovacuolar degeneration of Simchowicz (1910/1911), and neuronal fall-out all occur in the normal aged person's hippocampus, but are many times more severe when the memory disturbance of senile dementia of the Alzheimer type (SDAT) is superimposed. The severity of such changes in the human hippocampus in ageing and Alzheimer's disease has been quantified in our laboratory (Ball 1976, 1977; Ball and Lo 1977).

Regional variations in the hippocampal density of tangles and granulovacuoles, and also of the rod-like

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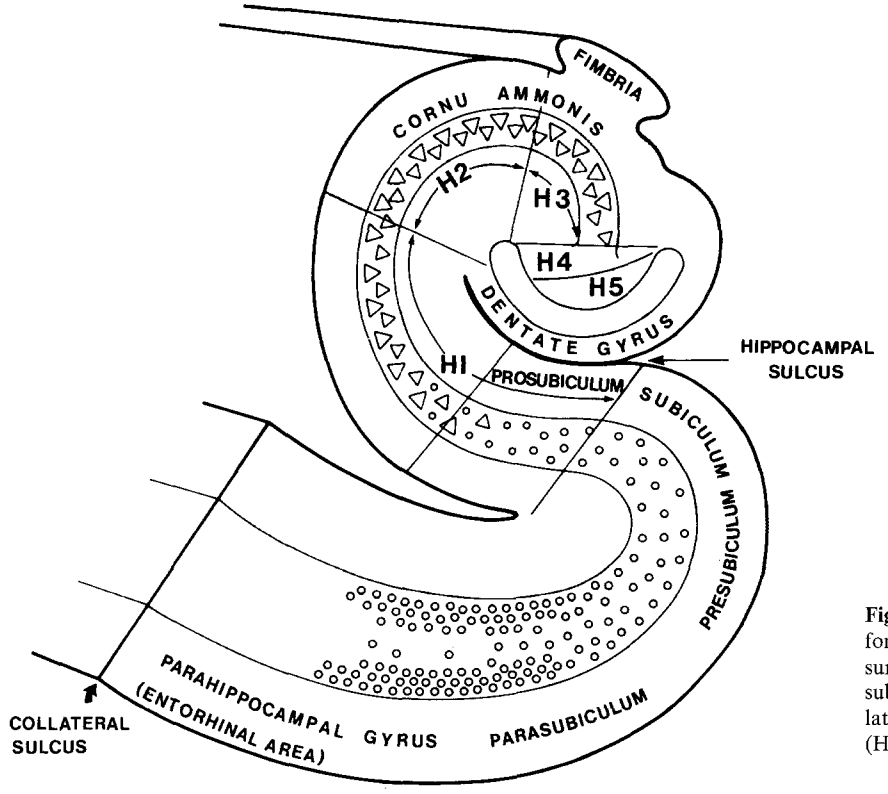

Fig. 1. Micro-anatomy of the hippocampal formation, showing the six cortical zones surveyed: entorhinal area; presubiculum; subiculum with medial H_1 (or prosubiculum); lateral portion of H_1; H_2; endplate (H_3, H_4, and H_5)

bodies of Hirano (Hirano et al. 1968), have also been noted (Ball 1978a, b). The microscopic anatomy of the human hippocampal pyramidal cortex can be divided into six "zones" (Fig. 1): the entorhinal area (including the parasubiculum and the parahippocampal gyrus); the presubiculum; the subiculum together with the medial portion of Rose's H_1 field (prosubiculum); the lateral portion of H_1 (the Sommer sector, which corresponds to Lorente de Nò's CA_1); H_2; and the endplate (H_3, H_4, and H_5). The relative topographic severity of the neuronal lesions of neurofibrillary tangles and granulovacuolar degeneration, as well as of the rod-like bodies of Hirano can thus be assigned a (representative) rank order for ageing and dementia (Ball 1978a, b). Comparison of these rank orders has shown that of the six zones, those three most severely affected by neurofibrillary tangles are: entorhinal cortex > subiculum > H_1. For granulovacuoles, the order is: subiculum > H_1 > H_2. For Hirano bodies, the order is: H_1 > subiculum > H_2. The increased predilection of the H_1 zone and adjacent subicular area in both ageing and dementia is notable, and has confirmed earlier observations of such regional predispositions by Hirano and Zimmerman (1962), Woodard (1962), Jamada and Mehraein (1968), Corsellis (1970), Tomlinson and Kitchener (1972) and Hooper and Vogel (1976). This remarkable pattern of regional hippocampal susceptibility resembles the "selective vulnerability" of neurons in the H_1, subiculum and endplate in hypoxia, ischemia and epilepsy, as first described by Spielmeyer (1925) and Uchimura (1928a) and recently reiterated by Brierly (1976) and Corsellis (1976). Vogt and Vogt (1937) ascribed this phenomenon to regional variations in parenchymal "physicochemical" characteristics, but Uchimura (1928b) suspected some regional arrangement of arterioles. Neither the vascular nor the "pathoclisis" hypothesis has been proven or refuted effectively (Altschul 1938; De Reuck et al. 1979).

Previous studies of the hippocampal vascular bed have either described patterns of distribution in the human (Uchimura 1928b; Hens and Van den Bergh 1977), in the rabbit and monkey (Lorente de Nò 1927) and in the rat (Coyle 1978), or have reported capillary measurements for a given age group in the rabbit (Cobb 1929), in the rat (Craigie 1930), in the cat (Campbell 1939), or in the child and dog (Mao Tseng-jung 1959). To explore the possibility of some correlation between the regional vasculature and the distribution of neuronal lesions in the hippocampi of aged and demented subjects, we have examined the anatomy of the blood vessels of the hippocampus in human post-mortem material. The study includes analyses of: (a) the angioarchitecture of the major arterial branches of the circle of Willis supplying the hippocampal formation; (b) the diameters of the microvasculature within the

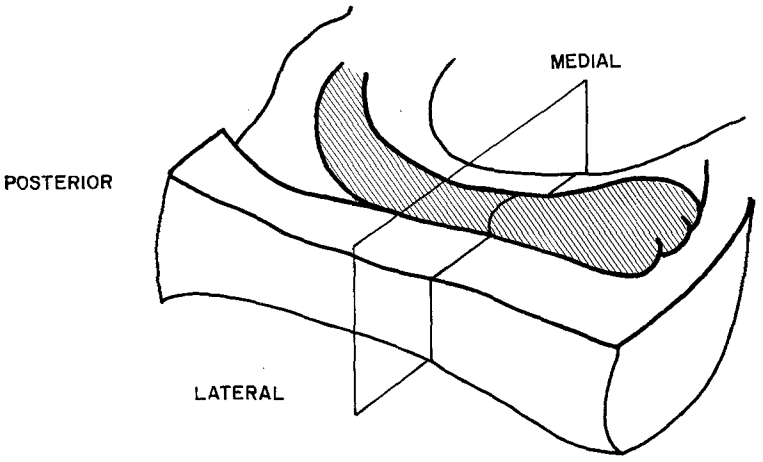

Fig. 2. Right hippocampus (shaded). The plane and approximate position of the surveyed sections are indicated

hippocampal parenchyma; and (c) the density of this microvasculature. This paper reports our observations on microvascular diameters and densities. The angioarchitecture of major arteries will be reported separately.

Material and Methods

Fifteen brains (eight men and seven women) were obtained at autopsy within 22 h of death (post-mortem interval does not appear to affect most microvascular parameters; Hunziker and Schweizer 1977). Ten of the brains were from patients dying without clinical or pathological involvement of the nervous system. These normal (control) brains were divided into two groups: five from "young" subjects (21–51 years, mean 38), and five "old" (60–88 years, mean 74). The remaining five brains (from subjects 66–94 years, mean 78) were from demented patients with clinically documented and pathologically confirmed dementia of the Alzheimer type. The age of onset of the dementing illness was known for three patients: 60, 63, and 71 years. For the remaining two, it was almost certainly after age 65. All five brains in this third group showed large numbers of senile plaques and neurofibrillary tangles in cerebral cortex, extensive granulovacuolar degeneration in the hippocampus, and no significant evidence of atherosclerotic or multi-infarct pathology. The mean age of the five old brains, 74 years, was not significantly different from that of the Alzheimer group, 78 years (Student's t-test, $P > 0.1$). The normal "old" group was therefore a suitable control for the demented cases. Both of these groups had average ages significantly greater than that of the five normal "young" brains, at 38 years (old > young, $P < 0.01$; Alzheimer > young, $P < 0.001$). Microscopic sections were prepared from the right hippocampus of each brain, except in three of the normal cases where only the left hippocampus was available. There is no significant lateralizing quantitative difference between the neuronal lesions of ageing or of Alzheimer's disease in left compared to right mesial temporal lobe (Ball 1977). Similarly, hippocampal neuron densities do not vary significantly from left to right (Dam 1979). Five transverse (coronal) celloidin sections, 120 µm thick and 500 µm apart, prepared from the mid-portion of each hippocampus (Fig. 2), were stained for the activity of non-specific alkaline phosphatase according to a modified Gomori technique (M. Bell, subm. for publ.). This method demonstrates the endothelium of human intraparenchymal arterioles and capillaries, leaving the post-capillary and larger venules unstained (Fig. 3). These celloidin sections provide a thicker sample of the microvascular bed than the thin frozen section technique. The pyramidal cortex was divided into those same six zones previously analyzed for neuronal changes (Ball 1978a, b); Fig. 1. The sample size, represented by a photograph from each zone, was 0.251 mm^3. A total of 30 such samples (six zones in five celloidin sections) was obtained from each hippocampus (Fig. 3a). Each rectangle delineates the area covered by one photograph (magnification of the negative

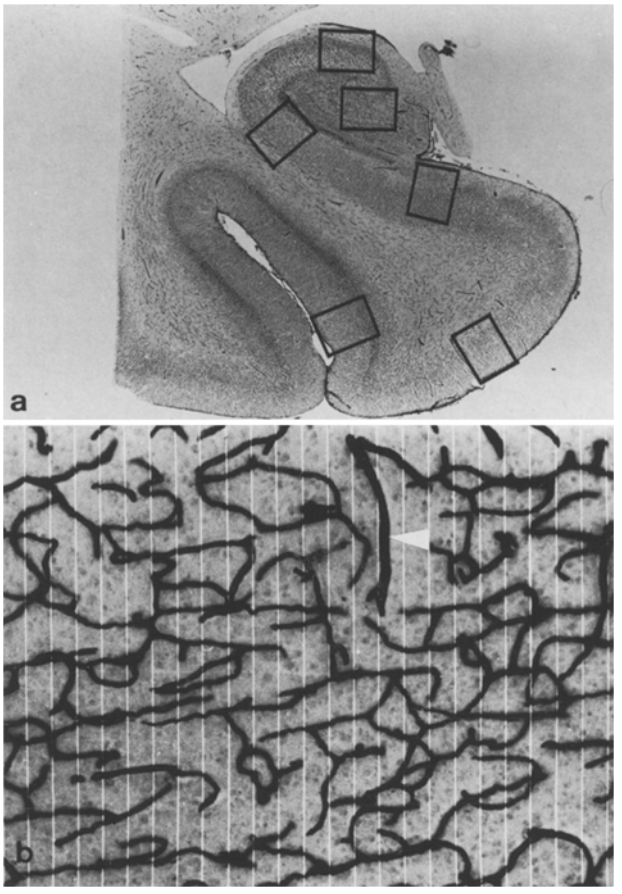

Fig. 3a, b. Celloidin section of hippocampus (120 µm) stained for the alkaline phosphatase activity of arterioles and capillaries. **a** Rectangles show the position and size of the samples from the six zones. **b** Example of photograph representing $^1/_4$ of one sample; vessel diameters measured at each intersection with grid line (working magnification 300×, grid lines 1 cm apart). Arrow on arteriole

Table 1. Two-way analysis of variance test for interobserver differences

Observer	Mean diameters in μm			
	Photo no. 1	Photo no. 2	Photo no. 3	Photo no. 4
1	5.3964	5.7340	6.5984	7.7805
2	5.2841	5.9727	7.0693	7.5031
3	5.1297	5.7764	6.5607	7.1422

Photo variance (columns): $F[3,11] = 73.2769$, highly significant, $P < 0.01$ (1-tailed)

Observer variance (rows): $F[2,11] = 2.5667$, not significant, $P > 0.05$ (1-tailed)

was 16×). Each of these negatives was then enlarged to cover 4 (8″ × 10″) prints (total mag. 300×), on which a grid with lines 1 cm apart was superimposed (Fig. 3b).

Diameter Measurements

The widths of the longitudinal images of the vessels were measured with a particle-size analyzer (Zeiss TGZ-3) at every intersection of a vessel with any grid line. The diameter of the adjustable light circle projected through the photograph was made to fit the true width of the vessel's image. The outer edge of each black silhouette corresponds to the outer (abluminal) surface of the endothelial cells in capillaries and probably in arterioles as well. Any potential pathologic thickening of the vascular walls external to the endothelium is not stained with the alkaline phosphatase technique, and should therefore not have affected the diameter measurements obtained. The particle-size analyzer permits sizing of objects in 48 equal classes, ranging from 0.5–9.14 mm on the photographs, and corresponding to actual vascular diameters in our sections of from 1.7–30.4 μm. The vessels measured thus comprise all the capillaries and those arterioles up to 30 μm in diameter. Larger arterioles and the unstained venules have been ignored. In order to assess the capillary bed separately from the whole population of micro-vessels, a convenient diameter – 10.0 μm – was chosen, below which all were considered to be capillaries. The term "capillary" in this study thus refers to all stained vessels below 10 μm diameter. Many such may in fact be terminal arterioles and pre-capillaries, but since the upper limit of diameter encountered in studies reporting relatively large capillaries has been 10 μm (Hale and Reed 1963; Fronek and Zweifach 1977; Schmid-Schoenbein et al. 1977; Conradi et al. 1979), it is felt that our definition, while not excluding all segments of the arteriolar tree, probably encompasses all vessels that could reasonably be considered capillaries. Five different observers performed the morphometry. In repeated analyses of the same photographs by the same or different observers in randomized order, there was no significant difference ($P > 0.1$) between the mean diameters found by (a) the same observer repeating the measurements of the same pictures, or (b) one observer repeating the measurement of 20 pictures previously done by a combination of three other observers. A two-way analysis of variance (Anova) was also applied to mean diameter measurements made in random order on the same four photographs by three different observers (Table 1). The variance ratio amongst the photographs was highly significant (1-tailed $F = 73.2769$, $P < 0.01$), whereas the variance ratio amongst the observers was not significant (1-tailed $F = 2.5667$, $P > 0.05$); i.e., the variations inherent in the data were far greater than the variations amongst different observers' measurements of that data. A further precaution was taken to minimize inter-observer variations: (a) all 6 zones from any one section were measured by the same observer; and (b) the five sections from a given case were distributed among at least three of the five observers. The numbers of vessels having each of the diameters were expressed with a Hewlett-Packard computer calculator (9815A) and peripheral plotter (9862A) in the form of histograms. The means and standard deviations of diameters for each case were compared in pairs of sets by Student's *t*-test. The total number of vessel diameters measured in the three groups of patients was 266,763.

Density (Length) Measurements

The entire lengths of all vessel images present in each photograph were measured by three observers with a computerized digitizer (Hewlett-Packard 9815A and 9864A) employing a cursor to trace the image. The same four pictures were measured in random order on the digitizer by three independent observers. The internal variabilities for each of the three observers' readings were 3.35%, 2.21%, and 2.97%, giving an average internal observer variability of 2.84%. To determine inter-observer variations, a two way Anova was done. The variance ratio amongst the observers was significant (1-tailed $F = 8.37$, $P < 0.01$), but the variance ratio amongst the photographs was ten times greater (1-tailed $F = 84.66$, $P < 0.01$). Thus, the variations in the data were much greater than those amongst different observers' measurements of that data. To neutralize any minimal effects of this inter-observer difference, the pictures from the five sections in each case were always distributed in the same way (three sections to one observer, one section to each of the other two).

The arterioles were measured and recorded separately before the total length of all stained vessels was obtained. A true diameter of 10 μm (3 mm on the photographs) was again chosen above and including which all stained vessels were considered arterioles. By subtracting the arteriolar lengths from those of the total, values for capillary lengths were also obtained. Mean vascular lengths and their standard deviations were obtained, and compared in pairs of sets, by Student's *t*-test, using the computer calculator. The Spearman rank correlation test was applied to comparisons among zones. Lengths measured from the photographs were also converted to densities (mm of vessel/mm^3 of tissue) by a conversion factor derived as follows:

$$\text{Tissue sample volume} = \frac{a \times b \times n \times t}{m}$$

$$= \frac{245\,\text{mm} \times 192\,\text{mm} \times 4 \times 0.12\,\text{mm}}{300 \times 300} = 0.2509\,\text{mm}^3$$

where a = length of photo, b = width of photo, n = number of photos/sample area, t = thickness of section, m = photographic magnification.

So

$$\text{density} = \frac{l \times 10}{m \times 0.2509} = \frac{l \times 10}{300 \times 0.2509} = \frac{l}{7.527}\,\text{mm/mm}^3$$

where l = length (cm) of vessel read on photo, 10 = conversion to mm, m = photographic magnification.

Thus, the conversion factor (divisor) is 7.527.

If the capillary bed is regarded as a continuous cylinder, estimates may be calculated for the total capillary surface area/mm^3 of tissue (S_v) and the total capillary volume/mm^3 of tissue (V_v) (Hunziker et al. 1974; Myrhage and Hudlická 1976; Schmid-Schoenbein et al. 1977; Bär 1978). For a cylinder, surface area $= 2\pi r \cdot l$, and volume $= \pi r^2 \cdot l$. These calculations, a marked simplification of the natural capillary network, can only provide approximations of the true values; they are, however, further indicators of the exchange potential of the capillary bed, and offer additional bases for comparisons. We also calculated a ratio of the capillary (exchange) surface area to the capillary (blood) volume, S/V. This ratio, representing S_v/V_v, can actually be calculated simply as $2/r$ because of the formulae terms,

Table 2. Mean diameter in μm (±1 S.D.). Arterioles and capillaries

	Mean age (yr)	Entorhinal	Pre-subiculum	Subiculum	H_1	H_2	Endplate	Overall mean (all six zones)	No. of diameters measured (*N*)
Normal young ($N=5$)	38	5.54(±0.47)	5.33(±0.23)	5.12(±0.50)	5.31(±0.38)	5.50(±0.58)	5.65(±0.46)	5.41(±0.45)	100,546
Normal old ($N=5$)	74	6.02(±0.88)	6.00(±0.80)	5.92(±0.99)	5.87(±0.97)	6.22(±1.13)	6.38(±1.10)	6.07(±0.91)	81,124
Alzheimer ($N=5$)	78	5.99(±0.76)	5.88(±0.71)	6.03(±0.58)	5.99(±0.79)	5.94(±0.79)	6.58(±0.69)	6.07(±0.70)	85,093
									266,763

Table 3. Percentage change in mean diameters of arterioles and capillaries

	Entorhinal	Pre-subiculum	Subiculum	H_1	H_2	Endplate	Overall mean (all six zones)
Young vs. old $\left[\frac{\text{old} - \text{young}}{\text{young}} \times 100\right]$	8.7↑	12.5↑	15.7↑	10.6↑	13.2↑	12.9↑	12.2↑[a]
Old vs. Alzheimer $\left[\frac{\text{Alzheimer} - \text{old}}{\text{old}} \times 100\right]$	0.5↓	2.1↓	1.8↑	2.0↑	4.6↓	3.2↑	0

↑ Increase, ↓ decrease
[a] Change is significant ($P < 0.001$)

and is a simple inverse representation of the diameter, but it focuses special attention on the area available to a given volume of blood for the exchange of nutrients.

Results

A. Arterioles and Capillaries Combined

(1) Diameters. The mean diameters (μm) of all vessels measured (small arterioles and capillaries) for the young, the old and the Alzheimer cases are given in Tables 2 and 3.

Overall Mean Diameters. The most striking difference is between the young and old controls. The mean overall diameter for all vessels in the older brains, 6.07 (± 0.91) μm, is 12.2% (0.66 μm) greater than the young, at 5.41 (± 0.45) μm. This difference is highly significant; $P < 0.001$. In contrast, there is no difference between the old and the Alzheimer group at 6.07 (± 0.70) μm, $P > 0.1$. The mean diameter of the Alzheimer group is also significantly greater than the young; $P < 0.001$.

Zonal Mean Diameters. When the mean diameter in a given zone of a single case is compared with that of other zones in the same case, significant differences appear. However, the ranking order of magnitude of these means is not consistent from case to case. Also, for any one zone, the mean vascular diameter of all five cases of each group does not vary significantly ($P > 0.1$) from any of the other zonal means. While the overall means of the young and the old are significantly different, the means of each individual zone of these two groups are not ($P > 0.1$), probably because of the smaller number of *t*-test entries (ten rather than 60). In Table 3, for each of the six zones, the differences in vascular diameter between young and old have been expressed as a "percentage change of the young"; between old and Alzheimer values as a "percentage change of the old". Comparison of the young and old pairs (Table 3, 1st row) shows an increase in all means with age, and considerable variability in the degree of this increase from zone to zone, the greatest being in subiculum and H_2. Comparison of the old and Alzheimer pairs (Table 3, 2nd row) shows a similar

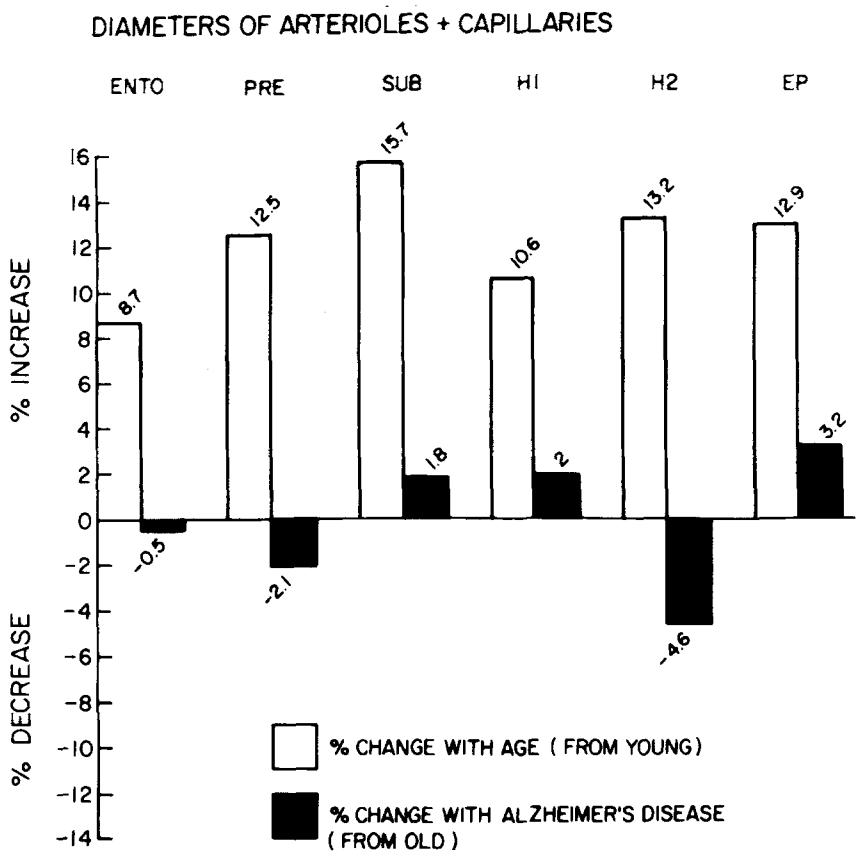

Fig. 4. Mean diameter changes of arterioles with capillaries, in the six hippocampal zones, associated with ageing (*white bars*) and Alzheimer's disease (*black bars*)

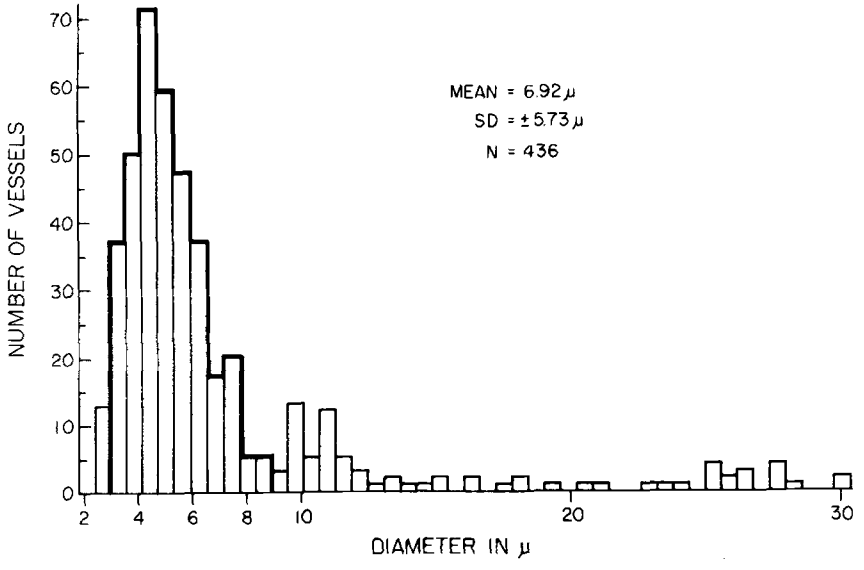

Fig. 5. Size distribution of vascular diameters in the presubiculum of one section from one old control. The 48 bars represent the classes measured with the particle-size analyzer. Most vessels typically fall in the 3–9 μm range (*dark outline*)

variability; the Alzheimer process is, however, accompanied by a reduced diameter in three zones (entorhinal, presubiculum and H_2) and increased diameter in the other three (subiculum, H_1 and endplate). The greatest change is the H_2 decrease (see also bar graph, Fig. 4).

Size Distribution. To illustrate the frequency with which the various diameters are encountered, Fig. 5 shows a histogram of the size distribution of all vessels measured in one zone of one section from one case. Each of the 48 size classes is represented by one bar; the corresponding true size in μm is indicated on the

Table 4. Mean vascular densities (mm/mm^3 ±1 S.D.) of arterioles and capillaries

	Mean age (yr)	Entorhinal	Presubiculum	Subiculum	H_1	H_2	Endplate	Overall mean (all six zones)
Normal young ($N=5$)	38	151 (±27)	116 (±25)	139 (±16)	136 (±22)	124 (±14)	107 (±17)	129 (±25)
Normal old ($N=5$)	74	129 (±36)	82 (±24)	118 (±19)	113 (±19)	105 (±18)	99 (±26)	108 (±28)
Alzheimer ($N=5$)	78	117 (±20)	72 (±21)	126 (±22)	127 (±24)	112 (±16)	89 (±19)	107 (±28)

Table 5. Rank order of vascular densities. Arterioles and capillaries[a]

Young:	Entorhinal	≧ subiculum	≧ H_1	> H_2	≧ presubiculum	≧ endplate
Old:	Entorhinal	≧ subiculum	≧ H_1	≧ H_2	≧ endplate	> presubiculum
Alzheimer:	H_1[a]	≧ subiculum[a]	≧ entorhinal	≧ H_2	> endplate	> presubiculum

≧ Greater than, but not significantly ($P > 0.1$). > Significantly greater than ($P < 0.05$ or better)

[a] This table also summarizes densities of capillaries alone, with the single exception that in the Alzheimer group the density of the subiculum is slightly higher (but not significantly, $P > 0.1$) than that of H_1

Table 6. Percentage change in mean densities of arterioles and capillaries

	Entorhinal	Presubiculum	Subiculum	H_1	H_2	Endplate	Overall mean (all six zones)
Young vs. old $\left[\frac{\text{young} - \text{old}}{\text{young}} \times 100\right]$	14.6↓[a]	29.3↓[a]	15.1↓[a]	16.9↓[a]	15.3↓[a]	7.5↓	16.3↓[a]
Old vs. Alzheimer $\left[\frac{\text{old} - \text{Alzheimer}}{\text{old}} \times 100\right]$	9.3↓	12.2↓	6.8↑	12.4↑[a]	6.7↑	10.1↓	0.9↓

↑ Increase, ↓ decrease

[a] Change is significant ($P < 0.05$ or better)

abscissa. In all six zones and all three groups, the greatest number of vessels falls between 3 and 9 μm (darker bars, Fig. 5), and the sparse distribution of vessels above 10 μm is variable and apparently random.

(2) Densities. Tables 4–6 give density (mm/mm^3) values for the three groups.

Overall Mean Densities. There is a clear reduction of microvascular density with ageing (Table 4), the overall mean for the young (129 ± 25 mm/mm^3) being significantly larger than the old (108 ± 28 mm/mm^3); $P < 0.001$.

Zonal Mean Densities. Table 5 shows for each group the ranking orders of the vascular densities found in the six zones, from highest to lowest, and indicates where significant differences were identified between consecutively ranked zones (in four of the 15 comparisons). Normal ageing was accompanied by a reduction of density in all six zones as well as in the overall mean. From Table 4 values, this reduction is significant ($P = 0.02$ or better) in every zone except endplate ($P > 0.1$). With Alzheimer's disease, the zonal effects are not as uniform. In three zones (entorhinal cortex, presubiculum, and endplate) density is decreased, although not significantly ($P > 0.1$). In the other three (H_1, subiculum, and H_2) it increases, significantly in H_1 ($P < 0.05$). Table 6 expresses the zonal percentage reduction in vascular density with ageing, and the percentage changes associated with Alzheimer's disease. The age changes are calculated as a percentage of the young values; the Alzheimer changes as a percentage of the old (see also bar graph, Fig. 6).

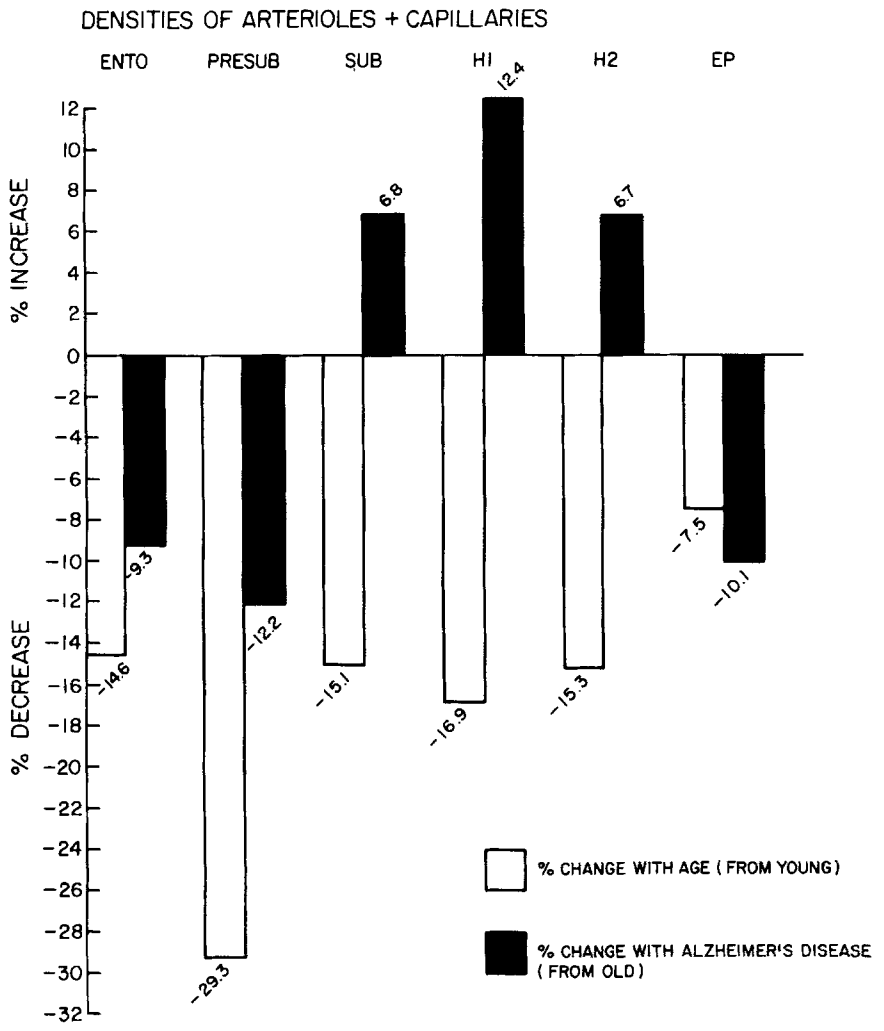

Fig. 6. Mean density changes of arterioles with capillaries, in the six hippocampal zones, associated with ageing (*white bars*) and Alzheimer's disease (*black bars*)

B. Capillaries Only

(1) Diameters. The same diameter calculations prepared for the combined population were repeated for the capillaries alone (Tables 7 and 8).

Overall Mean Diameters. Table 7 shows overall mean diameters of the normal old, 5.55 ($\pm$0.72) μm and of the Alzheimer, 5.41 ($\pm$0.49) μm, which do not differ significantly; $P > 0.1$. However, they are both significantly greater than the young, 5.08 ($\pm$ 0.40) μm; $P < 0.01$.

Zonal Mean Diameters. Capillary diameters in all six zones increase with age (Table 7). Unlike the variable direction of change in the combined vessel population, when capillaries are considered alone the mean diameter of the Alzheimer vessels in every one of the six zones is consistently smaller than in controls. When the means within each of the six zones are considered separately, neither the increase in capillary diameter with age (Table 8, 1st row), nor the decrease with Alzheimer's disease (Table 8, 2nd row) is statistically significant ($P > 0.1$, from data of Table 7). As with the combined arterioles and capillaries, however, the magnitude of these changes varies from zone to zone (Fig. 7). The greatest increases in capillary diameter with age occur in subiculum and H_2. The greatest decrease with Alzheimer's disease occurs in H_2.

Size Distribution. Differences among the three groups of patients in the distribution of their vascular diameters in the capillary range (under 10 μm) are demonstrated by three superimposed histograms (Fig. 8). Incorporating many more data than Fig. 5 these represent, for each group, the capillary vessels found in all six zones of one section from all five cases (i.e., 30 measured zones per group). The normal young cases have a larger proportion of their capillaries in the smallest (< 5 μm) size categories, consistent with the fact that their mean vascular diameters are significantly smaller than those of the aged and Alzheimer groups.

Table 7. Mean diameter in μm (±1 S.D.). Capillaries only

	Mean age (yr)	Entorhinal	Pre-subiculum	Subiculum	H_1	H_2	Endplate	Overall mean (all six zones)	No. of diameters measured (*N*)
Normal young ($N = 5$)	38	5.19 (±0.37)	4.95 (±0.26)	4.87 (±0.47)	5.03 (±0.36)	5.18 (±0.51)	5.23 (±0.44)	5.08 (±0.40)	96,875
Normal old ($N = 5$)	74	5.57 (±0.69)	5.32 (±0.46)	5.45 (±0.82)	5.41 (±0.74)	5.75 (±0.95)	5.79 (±0.86)	5.55 (±0.72)	75,827
Alzheimer ($N = 5$)	78	5.31 (±0.53)	5.24 (±0.62)	5.45 (±0.48)	5.38 (±0.59)	5.40 (±0.49)	5.71 (±0.26)	5.41 (±0.49)	78,446
									251,148

Table 8. Percentage change in mean diameters of capillaries only

	Entorhinal	Pre-subiculum	Subiculum	H_1	H_2	Endplate	Overall mean (all six zones)
Young vs. old $\left[\frac{\text{old} - \text{young}}{\text{young}} \times 100\right]$	7.4↑	7.3↑	11.9↑	7.5↑	11.0↑	10.6↑	9.3↑[a]
Old vs. Alzheimer $\left[\frac{\text{Alzheimer} - \text{old}}{\text{old}} \times 100\right]$	4.6↓	1.4↓	0.1↓	0.6↓	6.1↓	1.4↓	2.4↓

↑Increase, ↓decrease

[a] Change is significant ($P < 0.01$)

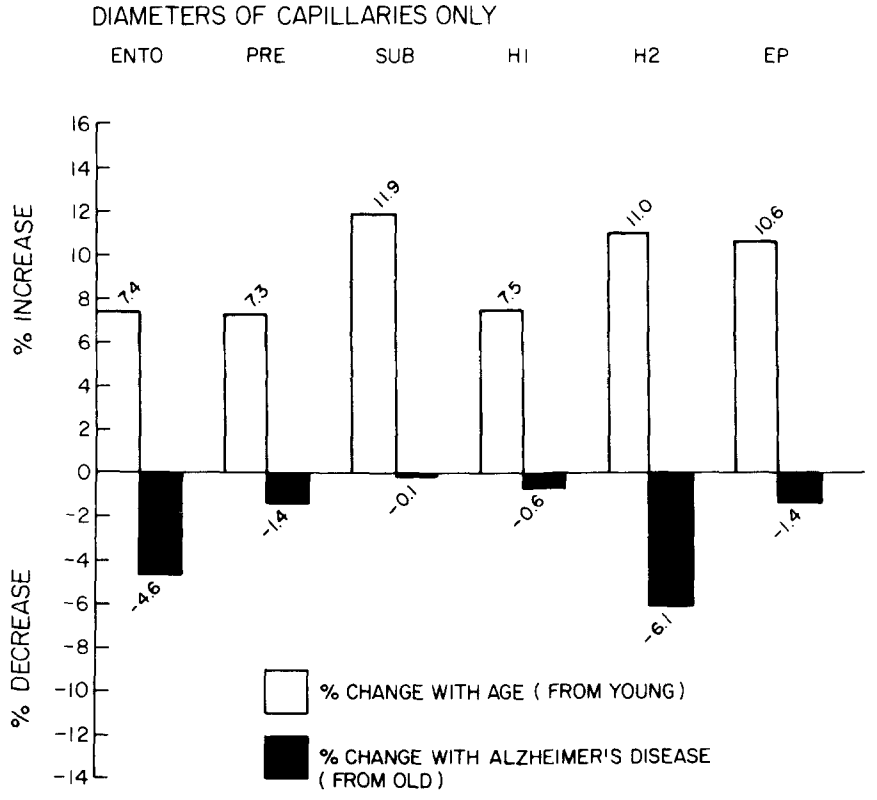

Fig. 7. Mean diameter changes of capillaries only, in the six hippocampal zones, associated with ageing (*white bars*) and Alzheimer's disease (*black bars*)

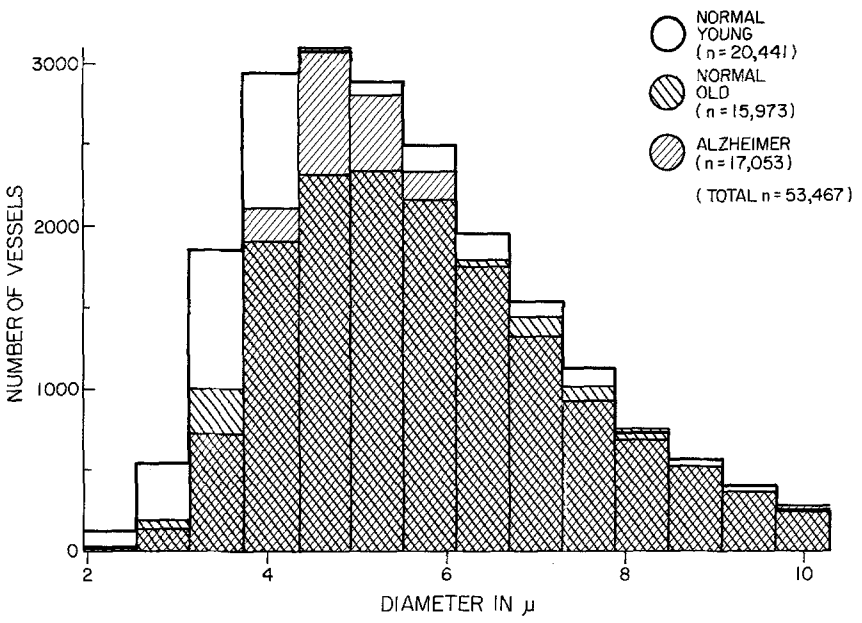

Fig. 8. Superimposed histograms of capillary diameter distributions from the three groups studied: each group histogram comprises values from five cases. (Mean diameters: young, 5.02 μm; old, 5.31 μm; Alzheimer, 5.26 μm)

Table 9. Mean vascular densities (mm/mm^3 ±1 S.D.) of capillaries

	Mean age (yr)	Entorhinal	Pre-subiculum	Subiculum	H_1	H_2	Endplate	Overall mean (all six zones)
Normal young ($N = 5$)	38	146 (±28)	111 (±24)	134 (±16)	131 (±22)	119 (±15)	102 (±17)	124 (±25)
Normal old ($N = 5$)	74	124 (±34)	77 (±23)	111 (±18)	107 (±20)	101 (±17)	93 (±26)	102 (±28)
Alzheimer ($N = 5$)	78	111 (±21)	67 (±21)	120 (±21)	119 (±21)	106 (±14)	82 (±18)	101 (±28)

(2) Densities. The lengths of capillaries alone were obtained by subtracting the measured lengths of arterioles (10 μm and above) from those of the total population. The same values presented above for the total population (Overall and Zonal Mean Densities) were also calculated for the capillaries alone (Tables 9 and 10).

Overall Mean Densities. As in the combined population, for the capillaries alone there is a significant reduction when the capillary density of the old (102 ± 28 mm/mm^3) is compared to that of the young (124 ± 25 mm/mm^3) ($P < 0.001$, Table 9).

Zonal Mean Densities. Table 5, showing ranking orders and significant differences in density of combined vessels of the six zones, also shows these characteristics for the capillaries alone. The high densities in entorhinal cortex and subiculum and low densities in presubiculum and endplate were suspected from our photographs (Fig. 3a). The reductions in capillary density found with ageing in five zones are significant ($P < 0.02$ or better, from Table 9 values); that in the endplate is not ($P > 0.1$). In Alzheimer's disease, three zones (entorhinal, presubiculum, and endplate) showed an insignificantly decreased capillary density relative to the old ($P > 0.1$). Capillary density increased in the other three zones (subiculum, H_1, and H_2), significantly in H_1 ($P < 0.05$). The percentage changes in capillary density (Table 10) with ageing are calculated as a percentage of young values; for the Alzheimer cases as a percentage of the old (see also bar graph, Fig. 9).

(3) Derived Parameters. Three additional parameters of the capillary network were derived from the diameter and density values given above: capillary surface area/unit tissue volume, S_v; capillary volume/unit tissue volume, V_v; and capillary surface area/unit capillary volume, S/V.

Table 11 shows that the calculated value for *capillary surface area/unit tissue volume (S_v)* – an esti-

Table 10. Percentage change in mean densities of capillaries

	Entorhinal	Pre-subiculum	Subiculum	H_1	H_2	Endplate	Overall mean (all six zones)
Young vs. old $\left[\frac{\text{young} - \text{old}}{\text{young}} \times 100\right]$	15.1↓[a]	30.6↓[a]	17.2↓[a]	18.3↓[a]	15.1↓[a]	8.8↓	17.7↓[a]
Old vs. Alzheimer $\left[\frac{\text{old} - \text{Alzheimer}}{\text{old}} \times 100\right]$	10.5↓	13.0↓	8.1↑	11.2↑[a]	5.0↑	11.8↓	1.0↓

↑ Increase, ↓ decrease
[a] Change is significant ($P < 0.05$ or better)

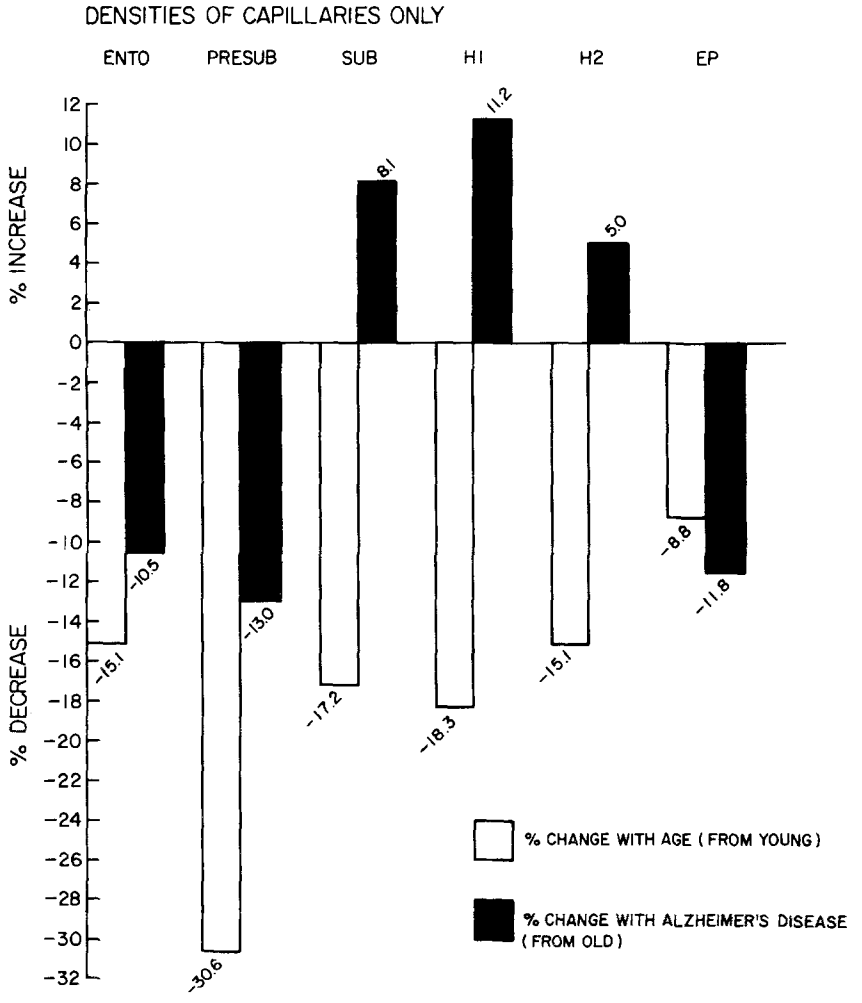

Fig. 9. Mean density changes of capillaries only, in the six hippocampal zones, associated with ageing (*white bars*) and Alzheimer's disease (*black bars*)

mation of the available capillary exchange area – drops (10.1 %) with age when the overall mean of all six zones in the old (1.78 mm^2/mm^3) is compared with the young (1.98 mm^2/mm^3). A small increase in surface area (2.3 %) is observed when the Alzheimer mean (1.82 mm^2/mm^3) is compared to the old. Surface area ranking orders vary among the six zones in the three groups of subjects: the largest occurs in entorhinal cortex for both young and old, but in the subiculum for the Alzheimer. The presubiculum and endplate consistently have the lowest surface area values in all three groups. The greatest decrease in area with ageing

Table 11. Derived parameters. capillaries only

	Entorhinal	Pre-subiculum	Subiculum	H_1	H_2	Endplate	Overall mean (all six zones)
Normal young							
S_v (mm^2/mm^3)	2.38	1.73	2.05	2.07	1.94	1.68	1.98
V_v (mm^3/mm^3)	0.0031	0.0021	0.0025	0.0026	0.0025	0.0022	0.0025
S/V (mm^2/mm^3)	768	824	820	796	776	764	792
Normal old							
S_v	2.17	1.29	1.90	1.82	1.83	1.69	1.78
Percent change young – old (%)	8.8↓	25.4↓	7.3↓	12.1↓	5.7↓	0.6↑	10.1↓
V_v	0.0030	0.0017	0.0026	0.0025	0.0026	0.0025	0.0025
Percent change young – old (%)	3.2↓	19.1↓	4.0↑	3.9↓	4.0↑	13.6↑	0
S/V	723	759	731	728	704	676	712
Percent change young – old (%)	5.9↓	7.9↓	10.9↓	8.5↓	9.3↓	11.5↓	10.1↓
Alzheimer							
S_v	1.95	1.19	2.15	2.14	1.90	1.59	1.82
Percent change old-Alzheimer (%)	10.1↓	7.8↓	13.2↑	17.6↑	3.8↑	5.9↓	2.3↑
V_v	0.0026	0.0016	0.0029	0.0029	0.0026	0.0023	0.0025
Percent change old – Alzheimer (%)	13.3↓	5.9↓	11.5↑	16.0↑	0	8.0↓	0
S/V	750	744	741	738	731	691	728
Percent change old – Alzheimer (%)	3.7↑	2.0↓	1.4↑	1.4↑	3.8↑	2.2↑	2.3↑

S_v = cap. surface area (mm^2)/tissue vol. (mm^3), V_v = cap. (blood) volume (mm^3)/tissue vol. (mm^3), S/V = cap. surface area (mm^2)/cap. (blood) vol. (mm^3). Percent change young – old $= \frac{\text{young} - \text{old}}{\text{young}} \times 100$, Percent change old – Alzheimer $= \frac{\text{old} - \text{Alzheimer}}{\text{old}} \times 100$

(25.4%) occurs in presubiculum. With Alzheimer's the greatest decrease (10.1%) is in entorhinal cortex, but three other zones actually increase their capillary surface areas: H_1 (17.6%), subiculum (13.2%) and H_2 (3.8%).

The calculated values for *capillary volume/unit tissue volume (V_v)* – an estimate of the blood volume available in the capillary bed – show that the overall mean for the six zones of all three groups is the same ($0.0025\ mm^3/mm^3$, Table 11). Zonal variations do occur, however, resembling but not duplicating those of the capillary surface area. In the young and old, the largest capillary volume is in the entorhinal; in the Alzheimer subjects, in the subiculum and H_1. In all three groups, the lowest values are in endplate and presubiculum. Ageing is associated with decreases in capillary volume in some zones (greatest in presubiculum, 19.1%), and increases in others (greatest in endplate, 13.6%). Alzheimer's disease is likewise accompanied by both decreases (greatest in entorhinal cortex, 13.3%) and increases (H_1, 16.0%; and subiculum, 11.5%); H_2 remains unchanged.

Since the overall mean capillary volume does not change in ageing or Alzheimer's dementia, the changes in the overall mean *capillary surface area/unit capillary volume (S/V)* ratio are exactly the same as those of the surface area: a 10.1% drop in normal ageing, and a 2.3% rise in Alzheimer's disease. Zonal variations in this ratio (which depends entirely on the capillary radius, $2/r$) do not closely resemble those of the capillary surface area or volume. In the young and old the presubiculum and subiculum have the highest ratios; in Alzheimer's disease, entorhinal cortex and presubiculum. The endplate has the lowest in all three groups. The ratio decreases in all zones in ageing (most in the endplate, 11.5%; least in entorhinal cortex, 5.9%). It varies in dementia, decreasing only in presubiculum (2.0%) and increasing most in H_2 (3.8%) and entorhinal cortex (3.7%).

C. Arterioles Only

The densities of arterioles, whose lengths were measured separately, are in Table 12. Percentage changes in

Table 12. Mean vascular densities (mm/mm^3 ±1 S.D.) of arterioles

	Mean age (yr)	Entorhinal	Pre-subiculum	Subiculum	H_1	H_2	Endplate	Overall mean (all six zones)
Normal young ($N = 5$)	38	5.31 (±2.79)	4.78 (±2.26)	4.25 (±2.13)	5.05 (±3.72)	4.52 (±3.06)	4.25 (±1.99)	4.65 (±2.79)
Normal old ($N = 5$)	74	5.58 (±3.99)	5.58 (±2.52)	6.64 (±4.12)	5.45 (±2.92)	4.38 (±2.39)	5.58 (±3.59)	5.58 (±3.32)
Alzheimer ($N = 5$)	78	6.78 (±3.19)	4.92 (±2.26)	6.51 (±3.45)	7.57 (±3.72)	6.38 (±3.72)	7.57 (±4.78)	6.64 (±3.72)

Table 13. Percentage change in mean densities of arterioles

	Entorhinal	Pre-subiculum	Subiculum	H_1	H_2	Endplate	Overall mean (all six zones)
Young vs. old $\left[\frac{\text{old} - \text{young}}{\text{young}} \times 100\right]$	5.1↑	16.7↑	56.2↑[a]	7.9↑	3.1↓	31.3↑	20.0↑[a]
Old vs. Alzheimer $\left[\frac{\text{old} - \text{Alzheimer}}{\text{old}} \times 100\right]$	21.5↑	11.8↓	2.0↓	38.9↑[a]	45.7↑[a]	35.7↑	19.0↑[a]

↑Increase, ↓ decrease

[a] Change is significant ($P < 0.05$ or better)

ageing and Alzheimer's dementia are in Table 13; the age changes are expressed as a percentage of the young, the Alzheimer changes as a percent of the old.

Overall Mean Densities. Arteriolar density increases significantly from 4.65 (±2.79) mm/mm^3 in the young to 5.58 (± 3.32) mm/mm^3 in the old ($P = 0.02$); Table 12. It increases still further with Alzheimer's dementia to 6.64 (± 3.72) mm/mm^3 ($P < 0.01$).

Zonal Mean Densities. The greatest arteriolar density is in entorhinal cortex in the young, subiculum in the old, and H_1 in the Alzheimer group ("ties" in Table 12 resolved by raw data). The lowest occurs in subiculum in the young, H_2 in the old and presubiculum in the Alzheimer group. Significant differences amongst the arteriolar densities of the six zones appeared rarely, and never between zones adjacent in ranking order. Five of the zones increase in arteriolar density during ageing (Tables 12 and 13); the greatest increase (56.2%), in subiculum, is significant ($P < 0.02$, from Table 12). H_2 is the only zone to undergo an (insignificant) decrease in arteriolar density with ageing (3.1%, $P > 0.1$). With Alzheimer's disease the arteriolar density increases in four zones, significantly in H_2 (45.7%) and H_1 (38.9%) ($P < 0.05$, Table 12). Density decreases in two zones. H_2 appears to be the zone whose arterioles are the most affected by Alzheimer's disease; its increase in density is the greatest, and is notable because H_2 is the only zone in which the arteriolar density might have been expected to decrease with normal ageing.

Arteriolar Contribution. Table 14 shows the arteriolar densities as percentages of the total vascular densities measured. The old have a significantly higher percentage of arterioles (5.2%) than the young (3.6%, $P < 0.01$). The percentage of arterioles in the Alzheimer group (6.2%) is higher than the old, though not significantly ($P > 0.1$). It is clear from Tables 12 and 9 that these rising arteriolar percentages are the result of rising arteriolar as well as falling capillary densities, The highest arteriolar percentages (as opposed to densities) occur in presubiculum and endplate in all three groups. Subiculum has the lowest arteriolar percentage in young and Alzheimer groups, H_2 the lowest in the old.

D. Other Observations

Largest Vessels. When the mean diameters of all six zones are ranked, in all three groups (young, old, and Alzheimer), whether for the combined vessels or for the capillaries alone, the largest mean diameter is always found in the endplate.

Age Correlations. Regression analysis shows no striking correlations between overall mean vascular diameters (for all six zones) of the ten normal subjects and their

Table 14. Arterioles as percentage of total measured vascular population

	Entorhinal	Pre-subiculum	Subiculum	H_1	H_2	Endplate	Overall mean (all six zones)	
Normal young	3.5	4.1	3.1	3.7	3.6	4.0	3.6	old > young ($P < 0.01$)
Normal old	4.3	6.8	5.6	4.8	4.2	5.7	5.2	old = Alzheimer ($P > 0.1$)
Alzheimer	5.8	6.8	5.2	6.0	5.7	8.5	6.2	

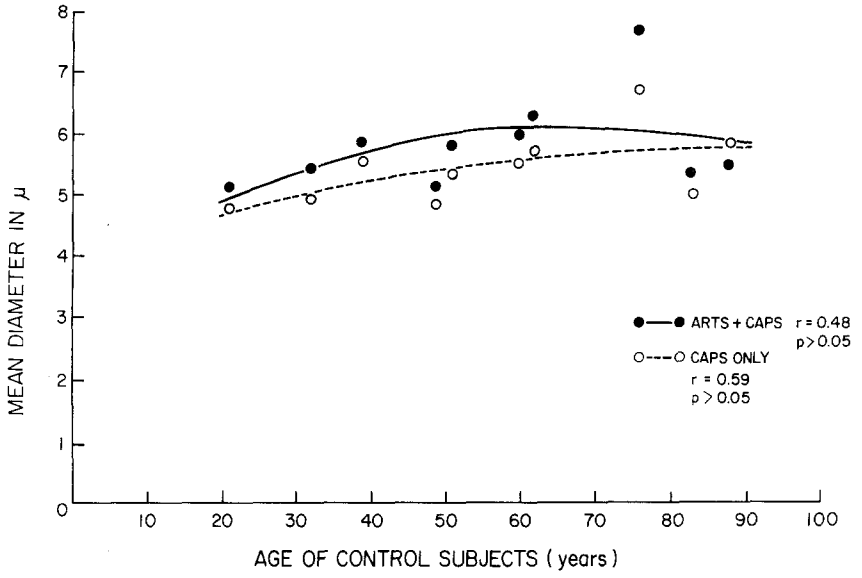

Fig. 10. Correlation of age with vascular diameters in ten normal subjects: *solid line*, arterioles and capillaries combined; *dotted line*, capillaries only

ages. The best fit is parabolic (Fig. 10); however, its correlation coefficient, r, is only 0.48 for the combined population ($P > 0.05$); r is 0.59 for capillaries alone, but still $P > 0.05$. Whether this trend would acquire statistical significance in a larger series should be determined. The microvascular densities of the ten normals are compared with their ages in Fig. 11. For the combined population as well as for capillaries alone, the best fitting regression is parabolic ($r = 0.64$ for combined, 0.65 for capillaries alone); both correlations are significant ($P < 0.05$).

High and Low Exchange Potentials. In those three capillary parameters for which a high value reflects high exchange potential (density, surface area, and volume) the entorhinal cortex and subiculum of all three groups of subjects always fall within the highest three of the six zones. They may have a good configuration for transmural exchanges. Conversely, endplate and presubiculum are the two last ranked zones in all subjects, suggesting poorer exchange potentials.

Comparison of Capillary Data with Other Studies. Values for mean capillary *diameters* in our study (5.08 μm for the young; 5.55 μm for the old) fall within the same range as those reported by others, using a variety of different species and histological techniques: 2.6–3.0 μm in rat hippocampus, with paraffin sections of injection preparations and light microscopy (Craigie 1930); 4.07 μm in ground squirrel brain, with low viscosity nitrocellulose and light microscopy (Drummond 1962); and 4.4–5.4 μm in rat cortex of different ages, in perfusion-fixed epoxy resin sections, with electronic image analysis (Bär 1978). Thin frozen sections, stained for alkaline phosphatase and measured by electronic image analysis, yielded diameters of 4.9 μm in rat cortex (Laursen and Diemer 1977); 5.41–5.61 μm in human temporal lobe (Hunziker and Schweizer 1977); and 5.74–6.49 μm in young and old human parietal cortex (Hunziker et al. 1978). A wide range of capillary *densities* for different animals and brain regions is reported: in the rat, 618 mm/mm^3 (hippocampus) to 1,155 mm/mm^3 (parietal cortex) from injected specimens and light microscopy (Craigie 1930), and 809 mm/mm^3 (young adult neocortex) to 939 mm/mm^3 (old) from 2 μm resin sections and image analysis (Bär 1978); in the rabbit, 400 mm/mm^3 (fascia dentata) to 555 mm/mm^3 (visual cortex) from injected specimens and light microscopy (Cobb 1929); in the cat, 440–780 mm/mm^3 (hippocampus) to 860–1,110 mm/mm^3 (parietal cortex) and 1,350 mm/mm^3

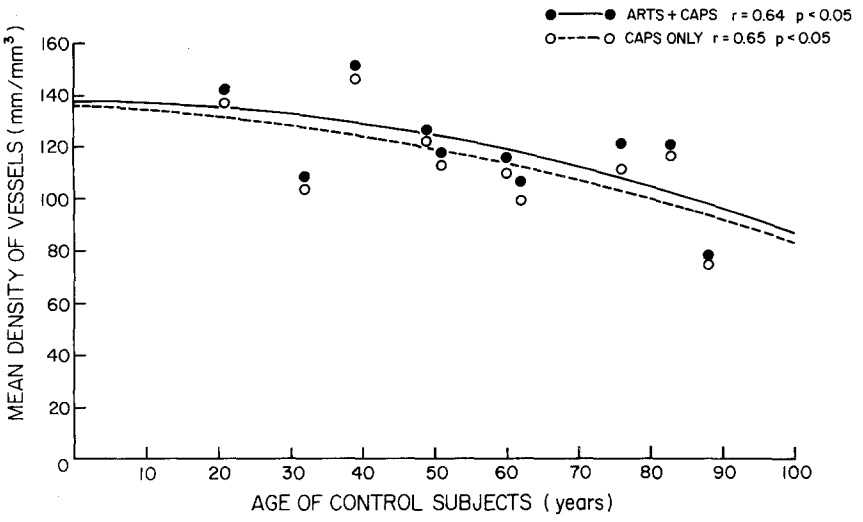

Fig. 11. Correlation of age with vascular densities in ten normal subjects: *solid line*, arterioles and capillaries combined; *dotted line*, capillaries only

(lateral geniculate) from vital injection preparations and light microscopy (Campbell 1939), and 369–420 mm/mm^3 (four neocortical areas) from thin alkaline phosphatase sections and image analysis (Hunziker et al. 1974); and in the dog, 523 mm/mm^3 (hippocampus) to 623–837 mm/mm^3 (neocortex) (Mao Tseng-jung 1959). Fewer measurements on human material have been reported: 425 mm/mm^3 (hippocampus) to 602–868 mm/mm^3 (neocortical) in children 1–2.5 years (Mao Tseng-jung 1959); 140 mm/mm^3 (average of four neocortical areas) in two aged humans (Hunziker and Schweizer 1977); and 189 mm/mm^3 to 261 mm/mm^3 in precentral gyrus of 34 subjects aged 19–94 (Hunziker et al. 1979). Both latter studies used thin frozen alkaline phosphatase sections and electronic image analysis. Values for adult human hippocampus have not been encountered. Capillary densities measured here are lower than those reported in animal brains and children's hippocampi. Our values, however, seem reasonable when compared to those of Hunziker et al. (1977, 1978, 1979) for the human neocortex, if the principle established in the animal brain that the hippocampal capillary density is less than that in the neocortex also holds true for human CNS. Mao Tseng-jung's values for children suggest that it does. The few reported effects of ageing on neocortical capillary density and other derived parameters are consistent neither with one another nor with our own findings. Bär (1978, 1980) found in rat neocortex that capillary density and surface area increased with age, while capillary volume decreased. Hunziker et al. (1978, 1979) reported in human neocortex that capillary density and volume increased, while capillary surface area decreased (until age 74). In the present study, human hippocampal capillaries decrease in density and surface area but remain unchanged in volume.

Discussion

1. Effects of Age

(i) Diameters. When the three groups of patients are compared, the most significant difference is the increase in mean vascular diameter accompanying normal ageing (Tables 2, 3, 7, 8). The increment appears to affect both the capillary-sized vessels and the 10–30 μm arterioles, and is unlikely to be attributable entirely to the slightly larger percentage of arterioles present within the combined population in the old group (Table 14). The observation that human cerebral capillaries increase in diameter with age has also been made by Hunziker et al. (1979), who reported the largest capillary diameters in ages 55–74; diameters in older cases decreased again. Their samples were from precentral rather than hippocampal cortex, but their observation of an increase followed by a decrease is not unlike the possibly parabolic correlation with age suggested from our study (Fig. 10).

Why microvessels might enlarge with ageing is not clear, nor is the cause of the suggested later reduction. If the size and/or number of nerve cells or the neuropil surrounding the vascular net does actually shrink with age (Tomlinson and Henderson 1976), a reduction of the supporting tissue mass and pressure against the vascular walls might allow a passive expansion. This notion would agree with Dam's observation (1979) that in Ammon's horn the greatest neuronal dropout with age occurs in Rose's H_2, the zone showing our 2nd greatest increase in diameter (Tables 2, 3, 7, 8). [Our most severely affected zone, the subiculum, was not analyzed by Dam (1979)]. In time, endothelial cell dropout [noted by Bär (1978) in aged rats] might reverse this trend. Since the small arterioles in our material also seem to expand with age, some relaxation or attenuation of their smooth muscle cells might be postu-

lated. Yamaguchi et al. (1979) speculated that, in the larger cerebral vessels, a reduction with age in the vasoconstrictive response to hypocapnia might be due to "some biophysical change in the elasticity of the cerebral vessels". An increase in calibre may not mean an increase in metabolic exchange. Most of our capillaries have diameters smaller than the undeformed width of the red blood cell – approximately 7 μm in sections, 8.15 μm in the living state (Leeson and Leeson 1970). This discrepancy, usual in humans and animals, means that the highly plastic red cells must deform, commonly into a "bullet" or folded shape, to pass through the capillaries (Brånemark and Lindström 1963). The rheological consequences of this deformation are complex, but the larger surface area of a deformed red cell close to the endothelium increases the opportunity for transmural gaseous exchanges (Guest et al. 1963), and in animals, tissues such as muscle depending heavily on the red cell's exchange mechanisms have capillaries 25–45% smaller than those of parenchymatous organs like kidney and liver where such gaseous exchanges are less important (Sobin and Tremer 1977). Furthermore, reduction in the diameter of a capillary produces an increase in the surface area/blood volume ratio (S/V), providing an increased exchange area for a given amount of blood (Hunziker et al. 1974). Thus, the smaller calibre noted in younger subjects' capillaries (and in those of the H_2 zone in Alzheimer brains) may be associated with slightly increased exchanges, at least of the O_2 and CO_2 carried by erythrocytes. The effect upon nutrients, or metabolic and exogenous toxins, carried both into and away from the tissues by the plasma is even more difficult to propound.

(ii) Densities and Derived Parameters. The overall mean values of capillary density, surface area, and surface area/capillary volume ratio all decline with age; the capillary volume remains unchanged. These changes, especially when combined with the increase in capillary diameter, suggest that ageing results in a reduced capillary exchange capacity. The relationship between individual subjects' mean capillary densities and their ages is best described by a parabolic curve. This non-linear relationship, which resembles that of the diameters (Figs. 10 and 11), may reflect the tendency noted by Hunziker et al. (1979) for capillary density (as well as diameter and other parameters) to "peak" at ages 65–74. In individual zones, capillary density and surface area also decline in almost every instance; capillary volume increases as often as it decreases. There is no strong correlation between these changes and the occurrence of the relatively small numbers of neurofibrillary tangles and granulovacuoles that accumulate in normal ageing. There is, however, some indication that in aged brains these lesions might be associated with decreasing capillary exchange potentials: when the six zones are ranked for the magnitude of decrement with age in capillary density, surface and volume, those appearing most often in the top three positions (having the greatest loss of exchange potential) are presubiculum, H_1 and entorhinal cortex. The three zones most affected by neurofibrillary tangles in ageing are entorhinal cortex, presubiculum and H_1; by granulovacuoles, H_1, subiculum and entorhinal cortex.

2. *Effects of Alzheimer's Disease*

(i) Diameters. The lack of any significant difference between the mean diameters of the vessels in the old and the Alzheimer group might suggest that the pathogenetic process underlying senile dementia of the Alzheimer type is not associated with microvascular calibre. However, in Alzheimer's disease the hippocampal accumulations of tangles and granulovacuoles follow precisely the same pattern (Ball 1978a, b); for both, the increments attributable to Alzheimer's disease (beyond the incidence in normal ageing) are found in the six zones in the same rank order – H_2 > subiculum > H_1 > endplate > presubiculum > entorhinal cortex. Thus, H_2, the zone which suffers the greatest increase in the neuronal lesions, is the same zone that suffers the greatest reduction in diameter. (This relatively small mean diameter in H_2 of Alzheimer brains is even more remarkable in that, if the data of normal ageing were used for extrapolation, i.e., if the Alzheimer process were merely an exaggeration of normal old age, the vessels of this zone might have been expected to undergo the second greatest increase in diameter; Tables 3 and 8.) As with the relatively small capillaries of the young controls, these reduced capillaries may reflect an increased level of exchange.

(ii) Densities and Derived Parameters. When the overall mean values for capillary density, surface area, and volume in the Alzheimer brains are compared to those in the old, the differences are insignificant. However, associations between neuronal pathology and microvasculature do become significant when data from each of the six zones are compared separately. When the zones of the Alzheimer brains are ranked for capillary density, surface area, and volume, each of these orders has a significant positive correlation with the ranking order for the severity of affliction by tangles ($P < 0.05$). High values for capillary density, surface area, and volume (and hence potentially high levels of capillary exchange) are associated with high densities of tangles. There is also a positive significant correlation ($P < 0.05$)

between the average ranking order of all three of these capillary parameters and the order of tangles. The ranking order for capillary volume also has a positive significant correlation ($P < 0.05$) with the severity of granulovacuoles. Similar relationships emerge when the changes due to Alzheimer's disease are considered. Five capillary parameters are considered and ranked in the six zones according to the direction and magnitude of change that should increase exchange, i.e., rises in density, surface area, volume, and surface area/capillary volume ratio, and decreases in capillary diameter. In these five parameters' ranking orders, the three zones appearing most often in the first three positions, i.e., which show the largest increment of exchange potential, are H_2, subiculum and H_1. These are the same three zones that have, in that order, the highest increment of tangles and granulovacuoles in Alzheimer's disease over and above the levels of normal ageing (Ball 1978b). Both in measured values and in changes occurring in vessels there seems to be a clear indication that, in Alzheimer's disease, both neurofibrillary tangle and granulovacuolar severity are associated with putative high levels of capillary exchange. An unusual focal occurrence of neurofibrillary tangles has in fact been reported immediately adjacent to vascular malformations, where increased blood flow and exchange across altered permeability barriers might be anticipated (Johnson and Nielsen 1976). Actual physiologic levels of exchange cannot of course be assessed by a morphological study. Ultrastructural (e.g., basement membrane) changes in the vessels must also be considered (Kidd 1964; Regnault and Kern 1974; Mancardi et al. 1980).

The association of pathology with high levels of exchange in dementia contrasts with the reduced capillary exchange potential in ageing (Discussion, Section 1, above), suggesting a distinction between the formation of tangles and granulovacuoles in normal ageing, and their formation in Alzheimer's disease. Furthermore, a distinction can be drawn between these two types of neuronal lesion themselves. In the Alzheimer cases the comparison of high capillary exchange potentials with high levels of neuronal lesions, as well as the comparison described in ageing between falling capillary exchange potentials and increased numbers of lesions show that the correlations were closer for tangles than for granulovacuoles. The suggestion is that, in either condition, the tangles are more dependent on capillary exchange than are the granulovacuoles.

3. *Exchange Potentials in Different Zones*

A combined ranking order for the four parameters reflecting capillary exchange potentials in the six hippocampal zones of the ten normal subjects was obtained by averaging together the eight separate rank values of the young and old groups: (high) capillary density, surface area (S_v), volume (V_v), and surface area/capillary volume ratio (S/V, i.e., $2/r$). This ranking order was: entorhinal cortex > subiculum > H_1 > H_2 > presubiculum > endplate. The endplate, with the smallest capillary exchange potential, is commonly affected in conditions of hypoxia and ischemia. It also has relatively few arterioles, which arise distally in the hippocampal artery branching system. Conversely, the much more "favorable" capillary architecture of H_1 and subiculum may be associated with the peculiar susceptibility of these areas to the nerve cell lesions of Alzheimer's dementia. H_2 has a lower anatomical exchange potential than H_1 and subiculum, but it has better opportunities for collateral arteriolar supply (Uchimura 1928b). The high rank of the entorhinal cortex may reflect its embryologically transitional position between archicortex and the more vascular neocortex (Filimonoff 1947).

4. *Distribution of Arterioles*

No significant correlations were found between ranking orders of lesions' severities and arteriolar density in either ageing or demented subjects. The relative arteriolar contributions to the six zones in the ten normals may be calculated by averaging values for young and old groups, and ranked in two ways: by measured density (Table 12), for which the order is entorhinal cortex > presubiculum > subiculum > H_1 > endplate > H_2; and by percentage of total population (Table 14), for which the order is presubiculum > endplate > subiculum > H_1 > entorhinal cortex > H_2. The presubiculum, with its sparse total vascular population, has a large number of arterioles by either measure; this rich collateral supply, probably arising early from major branches of the posterior cerebral artery, might protect it in some hypoxic conditions, whereas its sparse capillary bed might be associated with its lack of involvement by the neuronal lesions of Alzheimer's disease. The H_2 zone appears strikingly low in arteriolar supply for both absolute density and proportion of population. Uchimura (1928b) pointed out, however, that this area receives arterioles from three different directions: through H_1, the dentate gyrus, and via the fimbrio-dentate sulcus. It may therefore be uniquely protected by collaterals. Since it receives only the terminals of arterioles that have previously passed through other zones, H_2 might be expected to contain shorter lengths and smaller calibres of arterioles; it is unlikely that this specialized arrangement deprives this region of adequate arteriolar supply. On the contrary: in Alzheimer's disease, the arteriolar

density of H_2 increases more (45.7%) than in any other zone. It may also be deduced, by comparing the diameters of capillaries alone with those of the combined arteriolar and capillary population, that arteriolar diameters increase in this zone with Alzheimer's disease. Increases in these two parameters indicate an "improved" supply of blood immediately proximal to the H_2 capillary bed. Since this is the zone with the greatest Alzheimer increment, the conclusion drawn from capillary data that lesions in Alzheimer's disease are associated with an increased blood supply is reinforced.

5. *Vascular Regression or Tissue Atrophy?*

(i) Capillaries. At least four processes could be influencing the capillary density in any cortical area in ageing or disease: the capillary network might proliferate; or it might regress; and/or the parenchyma might expand (by cellular proliferation or hypertrophy, or by edema); or it might shrink (by reduction in size or number of cells, or water loss). The capillary density at a given time will reflect the current balance of these influences. In the human hippocampus, as in the neocortex, an important effect of ageing and/or of Alzheimer's dementia on the parenchyma is possible atrophy of parenchyma and/or neuronal loss (Ball 1977; Dam 1979; Miller et al. 1980). Our finding of overall and zonal reductions in capillary density with ageing suggests that, in the hippocampus at least, regression of the capillary network exceeds the "condensing" effect of tissue shrinkage. This is true even in H_2, where Dam (1979) found the greatest loss of neurons with age. In the hippocampi of demented subjects, in which Ball (1977) has found a significant loss of neurons exceeding that observed in normal ageing, we find that tissue "condensation" outweighs any capillary regression in the three zones most severely afflicted by density of or increments in Alzheimer lesions (H_1, subiculum and H_2), so that capillary density rises (Table 10). In the other three zones, possible microvascular regression continues to lower the capillary density. It remains to be determined how capillary density changes with dementia in the neocortex, where Terry and Davies (1980) found no worse neuronal loss due to dementia than from ageing alone, and where accumulations of Alzheimer lesions are generally not as great as in the hippocampus (Dayan 1970), but where normal ageing has been shown to increase the capillary density (Hunziker et al. 1978, 1979).

(ii) Arterioles. The increase of overall mean arteriolar density with age (while the capillary density is reduced), and with Alzheimer's disease (while the overall capillary density remains unchanged), suggests that tissue "condensation" has a more important influence than vascular regression on arteriolar density. This is supported by the occurrence with Alzheimer's disease of the two greatest increases in arteriolar density in H_2 and H_1, – two zones sustaining large accumulations or increments of pathological lesions beyond the levels of normal ageing. Capillary density is also increased in these zones. Arteriolar condensation with tissue shrinkage in H_2 may however be difficult to predict or visualize, because this is the only zone not directly penetrated by arterioles from a pial surface. Instead, it receives only the terminal portions of arterioles that have already passed through H_1 (from the hippocampal sulcus) or the endplate or fimbria (from the medial dentate gyrus or fimbrio-dentate sulcus) (Uchimura 1928b). With ageing, the arteriolar density of H_2 appears to decrease slightly (Table 13), when it might have been expected to increase because of tissue shrinkage; Dam (1979) found that of the zones he studied the greatest loss of neurons with ageing occurred in H_2. Correlation data do not suggest that young "capillaries", enlarged by ageing to the size of "arterioles", contribute significantly to the increased measured arteriolar lengths observed in ageing and dementia.

6. *Temporal Relationships*

All of the Alzheimer patients included in this study were in advanced stages of dementia before death. It is therefore not possible to speculate on which change in the hippocampal cortex, vascular or neuronal, precedes the other, nor to attribute direct cause and effect. Angio-architectural changes promoting greater capillary exchange have, however, taken place in the same zones that have suffered the greatest increases in Alzheimer pathology relative to the normal old; and in the dements' brains, the areas showing high levels of Alzheimer lesions correlate well with those most likely to have highest capillary exchange potentials. Whether a regionally augmented blood flow bringing some etiological agent into greater contact with the neuronal population in such zones is actually operating as a pathogenetic mechanism for the dementia can only be substantiated by physiologic experiments.

7. *Ageing vs. Dementia*

Terry (1978) counted neurons in the neocortex of aged normal human brains and in brains from demented patients (though not in the hippocampal formation), and concluded that, while the nerve cell population may decline significantly with age, the dementing process apparently does not result in any additional significant neuronal decrease (Terry and Davies 1980).

McDermott et al. (1979), studying levels of aluminum accumulating in human brains, likewise found that while aluminum concentrations rise with age, the presence of dementia does not significantly affect the assayed values. Beskow et al. (1971) found that the number of deformities in intraparenchymal arterioles is greater with normal ageing but that the dementing process is not associated with any additional increase. Similarly, our data indicate that the most obvious change in arteriolar and capillary parameters accompanies ageing, rather than dementia. The presence of Alzheimer's disease is not associated with any significant alteration in the mean overall microvascular parameters of the hippocampal formation. Nevertheless, the peculiar zonal coindidences of alterations in capillary parameters relevant to increased exchange with the greatest increments or severities of tangles and granulovacuoles in the brains of demented people with Alzheimer's disease must be noted, even though the reason for these matching topographic propensities is not yet understood. Future morphometric studies of microvascular anatomy must in any case consider carefully the effect of the subjects' age upon the measurable parameters of the cortical blood supply.

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