Metabolic Brain Disease, Vol. 3, No. 1, 1988

Effects of Aging and Alcohol on the Biochemical Composition of Histologically Normal Human Brain

Richard C. Wiggins,[1,5] Anne Gorman,[1] Carolyn Rolsten,[2] T. Samorajski,[2] William E. Ballinger, Jr.,[3] and Gerhard Freund[4]

Received September 28, 1987; accepted November 1, 1987

Human brains were removed at autopsy and examined grossly and histologically for any abnormality or evidence of disease. Sixty-two brains appearing normal by these criteria were examined further. First, a detailed record of alcohol consumption was obtained. Second, frozen punches of gray and white matter were used to determine the compositional change associated with age and drinking patterns. Increased age was associated with an increase in the water content, particularly in the white matter, a decline in RNA content in gray matter, a decline in total protein in white matter, and a decline in both myelin and the myelin-like subfraction. The loss of myelin membrane in white matter corresponded to a similar increase in water content, although there was an additional loss of some nonmyelin protein. There was no significant shift in the density between the myelin and the myelin-like membranes, and the protein composition of myelin was not significantly altered by age. A history of heavy alcohol consumption was associated with a relative increase in total protein in white matter even though heavy drinking accelerated the age-related loss of myelin. Presumably, alcohol produced a lag in the rate at which nonmyelin proteins are lost or accelerated the accumulation of abnormal protein. Alcohol consumption did not influence the myelin composition or the ratio of myelin and myelin-like membranes. The interval between patient death and autopsy was shown to have little or no effect on the samples used in this study. These data show that normal aging, uncomplicated by other disease processes, can have a significant effect on the composition of brain tissue, particularly the white matter, and that heavy alcohol consumption accelerates degenerative change, even in tissue appearing normal by histology.

KEY WORDS: aging; alcohol; DNA; myelin; myelin-like; protein; RNA; water.

[1]Department of Neurobiology and Anatomy, Box 20708, Houston, Texas 77025.
[2]Department of Psychiatry and the Mental Sciences, Box 20708, Houston, Texas 77025.
[3]Department of Pathology, University of Florida College of Medicine, Gainesville, Florida 32610.
[4]Department of Medicine, University of Florida College of Medicine, Gainesville, Florida 32610.
[5]To whom correspondence should be addressed.

0885-7490/88/0300-0067$06.00/0

INTRODUCTION

Myelination is a rapidly developing, progressive feature of brain development. It is associated with an opposite, or regressive, change in brain water content (Donaldson, 1924; Friede, 1966). With advancing old age, the composition of brain begins to change noticeably again but not as rapidly as in early development.

Although it seems accepted that the water content of rodent brain gradually declines during advancing age (Friede, 1966; Himwich, 1973), the water content of human brain has been described as either declining gradually from birth to age 90 years (Ordy and Kaack, 1975) or increasing slightly again, especially between 80 and 90 years of age (Davis and Himwich, 1975; Samorajski and Rolsten, 1973; Samorajski, 1980).

The concentration of brain myelin, or myelin constituents, measured as a percentage of wet weight (Ansari and Loch, 1975; Berlet *et al.*, 1982; Berlet and Volk, 1980; Horrocks *et al.*, 1981; Malone and Szoke, 1985; Reiderer *et al.*, 1984) or estimated by histological staining (Lintl and Braak, 1983), decreases in human old age. However, the relative contents of water and other constituents have not been examined in relation to myelin loss. Possibly the loss of myelin during aging corresponds to the increase in water, just as the gain in myelin during postnatal development is related to a loss of water.

The purpose of this study was to use a large, well-documented sample of human brains to determine major changes in the composition of gray and white matter (separately) during aging. The availability of an accurate history of the patient's alcohol consumption was especially critical, as alcohol is known to influence myelin metabolism during early development (Wiggins, 1986) and could possibly influence the myelin loss observed in aging.

METHODS

Human Brain Samples. Brains were obtained at autopsy from the Shands Teaching Hospital, University of Florida, and the Veterans Administration Medical Center, Gainesville, Florida. The brains were removed and examined using a systematized protocol for documenting brain weight and any gross brain changes, i.e., the amount of cerebral edema, cerebral atrophy, and ventricular dilatation, and the extent of atherosclerotic vascular disease. Sections were taken for histologic examination from standardized locations including frontal and temporal cerebral cortex, amygdala, caudate, thalamus, hippocampus, putamen, globus pallidus, cerebellar hemispheres, cerebellar vermis, pons, and midbrain. Eight specific pathological changes were examined for and documented in each tissue section including neurofibrillary tangle change, senile plaques, acute neuronal death, gliosis, and vascular changes, with special attention to the mammillary bodies and basis pontis to rule out Wernicke's encephalopathy and central pontine myelinolysis, respectively. The remainder of the fresh tissue samples was then frozen and maintained in an Ultra-low freezer at $-70°C$. Only brains that were grossly and histologically normal were shipped periodically from Gainesville to Houston. These were packed in dry ice during transport, numbered serially upon arrival, and stored at $-70°C$.

The time between death and autopsy was noted in all cases. Detailed alcohol consumption histories were obtained from current and past medical records and were verified by contacting the nearest living relative or friend. Alcohol consumption was classified as nondrinkers, light drinkers (i.e., infrequent), moderate drinkers (fewer than 5 drinks per day), and heavy drinkers (5 or more drinks per day for at least 10 years). Rather than work samples up individually upon arrival, the collection was allowed to reach a size representing 62 brains before initiating quantitative analysis.

A cork borer was used to obtain punches of similar size from frozen slices. White matter punches were taken at the confluence of the corpus callosum and the corona radiata. Gray matter was taken from the medial and superior frontal gyri.

Myelin Purification. The quantification of myelin proceeded under "double-blind" circumstances. Everyone operationally involved in myelin recovery was kept "blind" to all matching clinical information, including age and the conditions of autopsy. Individuals having access to patient information were kept blind to the biochemical data until the results for all samples were complete. Myelin purification was completed on all available samples within a 3-week period to minimize procedural variance.

Myelin was prepared by quantitative ultracentrifugation using an L8M ultracentrifuge (Beckman Instruments, Palo Alto, Calif.) and minor modifications of the procedure of Norton and Poduslo (1973). Anticipating the possibility of appreciable myelin in "floating" fractions or other lighter-than-usual fractions in senescent brain (Berlet *et al.*, 1982), the sedimentation time on discontinuous sucrose gradients was increased from 30 to 60 min to separate maximally fractions differing by bouyant density. Also, acceleration, run, and deceleration parameters were automated (Memory-Pac, Beckman Instruments) to standardize total centrifugal effects (shown as rad^2/sec).

Frozen white matter punches weighing about 1 g were homogenized in 19 vol of 0.32 *M* sucrose to yield 5% homogenates. The same homogenization vessel (glass with Teflon pestle) was used for all samples. Homogenization was timed to a constant 1.5 min, using 10 up-and-down strokes around a slowly rotating pestle (fractional horse-power motor, A. H. Thomas, Philadelphia; variable transformer) and the vessel was chilled with ice throughout.

Homogenate (20 ml) was layered over 0.88 *M* sucrose (10 ml) using polycarbonate ultracentrifuge tubes. These were centrifuged at 25,000 rpm for 60 min, using the SW27 rotor and buckets ($\omega^2 t = 2.47 \times 10^{10}$). The crude myelin interface was removed in a total volume of 7 ml, dispersed in cold water, and pelleted by centrifugation for 30 min (25,000 rpm; SW27 rotor). The pellet of crude myelin was dispersed in cold water in capped ultracentrifuge tubes and osmotically shocked for 20 min.

The osmotic shock step is an especially critical point where the myelin and myelin-like membranes are separated with a brief, low-speed centrifugation. This step was carried out with the samples already loaded into a Type 30 rotor (Beckman), and the centrifuge chamber, and already under vacuum. A preprogrammed setting of 20 min at a speed of 0 rpm provided the osmotic shock, which was followed by automatic acceleration to 10,000 rpm and centrifugation for 10 min (type 30 rotor; $\omega^2 t = 1.58 \times 10^8$). This procedure was essential using the L8: otherwise acceleration holds at a maximum speed of 3000 rpm until the vacuum reaches 750 μm, and this can

be an appreciable part of the total run time. The supernatant containing the myelin-like membranes was removed and this fraction was pelleted by centrifugation in water for 60 min (28,000 rpm; Type 30 rotor) and freeze-dried.

The myelin pellet was resuspended in 0.32 *M* sucrose and a second sucrose gradient, identical to the first, was run. The twice-purified myelin was finally pelleted in water (25,000 rpm; 30 min) and washed twice by resedimentation in water (25,000 rpm; 15 min).

Compositional Analysis. Concentrations of protein, RNA, and DNA were determined in individual samples of gray and white matter using a differential extraction procedure and spectrophotometric analysis (Wannemacher *et al.*, 1965). For water content, tissues were placed in preweighed glass vials, reweighed, placed in a 60°C oven for 96 hr, and weighed again. Protein was measured by a procedure based on Lowry *et al.* (1951).

Electrophoretic Analysis. Proteins of the myelin and myelin-like membranes were separated on polyacrylamide slab gels prepared and run as we have described before (Greenfield *et al.*, 1971; Morrell *et al.*, 1975). About 1 mg of freeze-dried membrane was delipidated using ether:ethanol (3:2). Delipidated protein was solubilized overnight in 1% sodium dodecyl sulfate (Wiggins and Fuller, 1981) for protein determination. A 10-μg aliquot was solubilized in the concentrated sample buffer (3:1) and applied to the gels. Coomassie brilliant blue was used as the stain and the gels were scanned using a Corning 750 densitometer.

Data Analysis. Values are presented in "scatter diagrams" to avoid bias. However, several statistical procedures were carried out using SAS (SAS Institute, Cary, N.C.) statistical programs. Regression analyses (PROC REG) were done for all subjects combined and separately for groups of alcoholics and controls. The *P* values in this procedure were calculated using ANOVA.

RESULTS

Retrospective Sampling Adjustments

In no case were results for light or medium drinkers meaningfully different from those for nondrinkers, whereas data for heavy drinkers did show indications of departure from "normal" values. Consequently, "controls" as shown in the following figures include results for nondrinking individuals, light drinkers, and moderate drinkers. This practice would moderate differences between the two test groups (controls and heavy drinkers), bias the data in a conservative direction, and increase confidence where differences between heavy drinkers and controls are noted.

However, there was not a sufficient sample of heavy drinkers in the group over age 70 years. Consequently, the combination of advanced age (over 70 years) and heavy drinking could be compared directly with the controls available in this age group.

Water (Fig. 1)

The water content in white matter averaged 69.9 $\pm$ 1.9% (N = 36) and 69.9 $\pm$ 1.8% (N = 29) in controls and heavy drinkers, respectively. However,

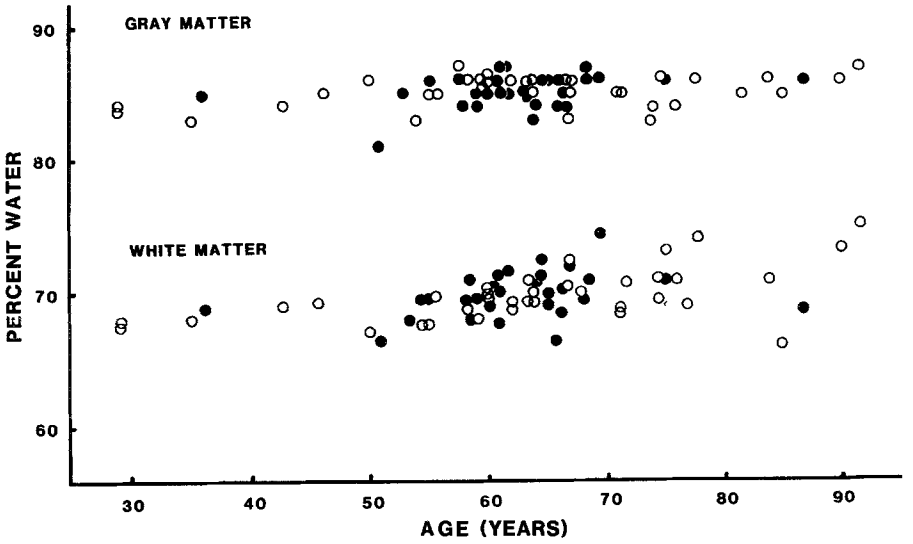

Fig. 1. A scatter plot showing the relationship between age and water content in gray matter (top panel) and white matter (bottom panel) in controls (open circles) and heavy drinkers (filled circles). Each point represents an individual sample from frozen human brain.

Table I. White Matter Constituents of Normal Brains[a]

	Mean ± SE at age		
	Under 50 years	50–70 years	Over 70 years
Water (%)			
Controls ($P < 0.05$; ANOVA)	68.6 ± 0.2	69.5 ± 0.3	71.0 ± 0.7
Heavy drinkers (NS; t test)		70.0 ± 0.4	
Total protein (mg/g)			
Controls (NS; ANOVA)	141.3 ± 7.6	135.6 ± 3.8	131.5 ± 4.6
Heavy drinkers (NS; t test)		143.9 ± 3.6	
Myelin protein (mg/g)			
Controls ($P < 0.025$; ANOVA)	62.1 ± 2.8	47.5 ± 2.7	40.8 ± 3.3
Heavy drinkers ($P < 0.05$; t test)		40.5 ± 2.5	
Myelin protein/total protein (%)			
Controls ($P < 0.05$; ANOVA)	45.3 ± 4.2	34.7 ± 2.1	30.3 ± 2.6
Heavy drinkers (NS; t test)		29.4 ± 2.4	

[a]In addition to the statistical analysis provided in the text, samples were arbitrarily combined according to the age groups shown here to determine if the age-related changes described in the text accelerated after age 70 years.

regression analysis (all samples) revealed an increase of 5% with advancing old age ($P = 0.0001$), from 67% at age 30 to 72% at age 90 years. Although sample variance increased with age, especially above 70 years, the Pearson correlation between water content (controls) and age was ($P = 0.0002$).

Samples were arbitrarily grouped according to the ages under 50, 50–70, and over 70 years to determine if the age-related increase in water content was relatively constant or accelerated after age 70 years. The results, shown in Table I, support the

evidence from regression analysis. These data show that the increase in water is gradual over a period of several decades, although the magnitude of increase observed by arbitrarily lumping all "under 50" samples is not as great as the increase indicated by regression analysis from 30 to 90 years. Table I also shows that the difference between heavy drinkers and controls at matched ages is not significant.

An increase of only 1.5% was observed in gray matter water, and this was not statistically significant. The mean water content for gray matter was 85.1 ± 1.1% controls (N = 35) and 85.1 ± 1.2% (N = 29) among heavy drinkers.

RNA/DNA (Figs. 2 and 3)

Gray matter RNA appears to decline from about 2.0 mg/g at age 30 years to about 1.5 mg/g by age 90 years, and regression analysis indicates that the 25% change is highly significant (P = 0.015). Heavy drinking only slightly lowered the overall RNA concentration; sample means were 1.76 ± 0.32 and 1.73 ± 0.30 mg/g in controls (N = 35) and heavy drinkers (N = 29), respectively. White matter RNA averaged 1.63 ± 0.25 mg/g wet weight at all ages in controls (N = 35) and 1.59 ± 0.27 in heavy drinkers (N = 29).

Gray matter DNA averaged 0.40 ± 0.08 mg/g in controls (N = 35) and 0.40 ± 0.07 mg/g among heavy drinkers (N = 29). These did not appear to vary with age. Consequently, the RNA/DNA ratio declined by 30%, which is consistent with the loss of RNA described above. White matter DNA averaged 0.68 ± 0.09 mg/g in controls (N = 34) and 0.60 ± 0.12 mg/g in heavy drinkers (N = 29). Advancing old age did not appear to affect white matter RNA or DNA values.

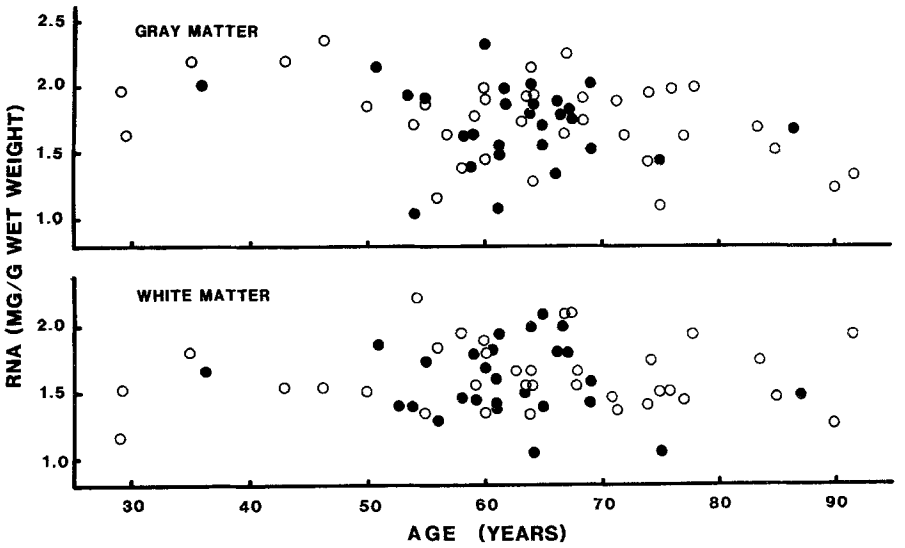

Fig. 2. The relationship between RNA and aging in human brain (see the legend to Fig. 1 for details).

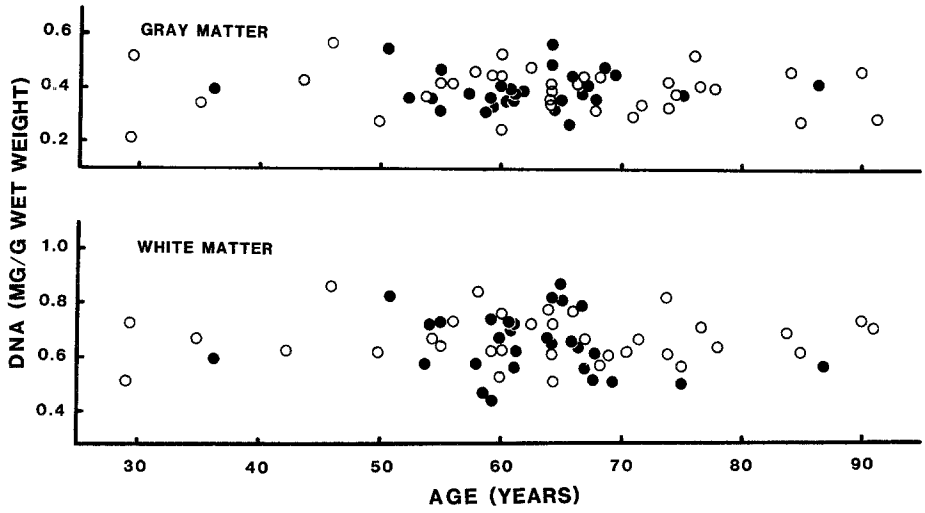

Fig. 3. The relationship between DNA and aging in human brain (see the legend to Fig. 1 for details).

Total Protein (Fig. 4)

Regression analysis indicates that the total protein content of white matter declined about 20% from age 30 to age 90 years, from about 155 to about 125 mg/g. The Pearson correlation between total protein and age was $P = 0.0245$, and Table I confirms the gradual decline in protein concentration.

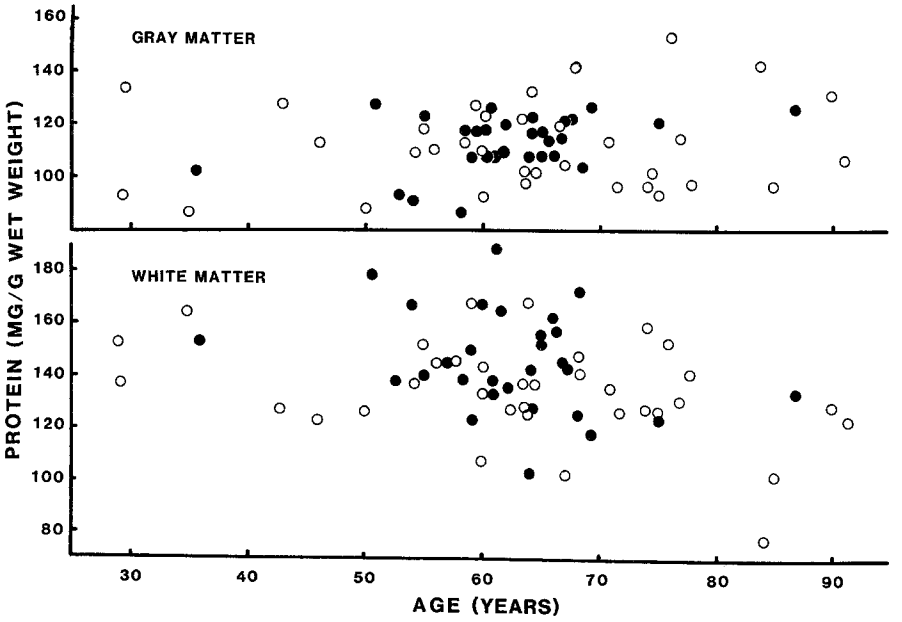

Fig. 4. The relationship between total protein and aging in human brain (see the legend to Fig. 1 for details).

The total protein content of white matter was 9% higher in heavy drinkers (146 ± 19 mg/g; $N = 29$) than in controls (134 ± 19 mg/g; $N = 35$) ($P = 0.02$). Table I confirms a higher protein concentration in heavy drinkers within a more favorably matched age group, although the magnitude of the elevation is only 6.1% when compared this way.

The total protein content of gray matter did not change with age or alcohol consumption. The concentration in controls ($N = 35$) and heavy drinkers ($N = 29$) was 112 ± 17 and 113 ± 11 mg/g, respectively.

Myelin and the Myelin-like Fraction (Figs. 5 and 6)

Regression analysis revealed a significant decline in the white matter concentration of myelin from age 30 to age 90 years, amounting to about 20% ($P = 0.02$), from 176 to 133 mg/g. The Pearson correlation between myelin yield and age was $P = 0.0003$. Table I reports myelin yields as the recovery of myelin protein, and these data, too, show a gradual loss of myelin spanning a period of several decades.

Comparing the ratio of myelin protein to total white matter protein (Table I) indicates that the rate of loss of myelin is slightly greater than the loss of total protein, as the contribution of myelin to total protein declines with age.

Table I also shows that the recovery of myelin protein from heavy drinkers was only 85% of normal in the 50- to 70-year age group. The ratio of myelin protein to total protein was only 83.9% of normal in heavy drinkers as a result of a slightly higher overall total protein concentration in this group.

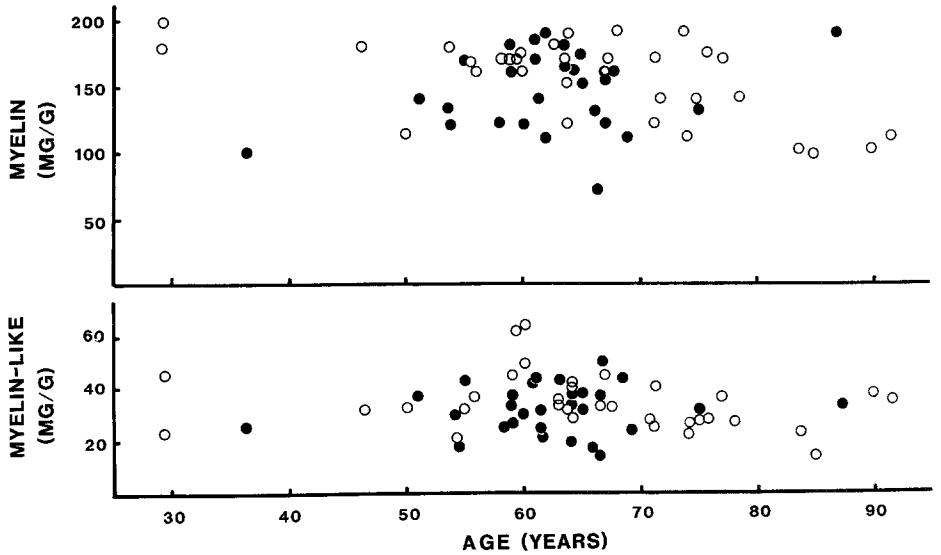

Fig. 5. The relationship between myelin (top panel) and myelin-like membrane (bottom panel) in human white matter during aging (see the legend to Fig. 1 for other details).

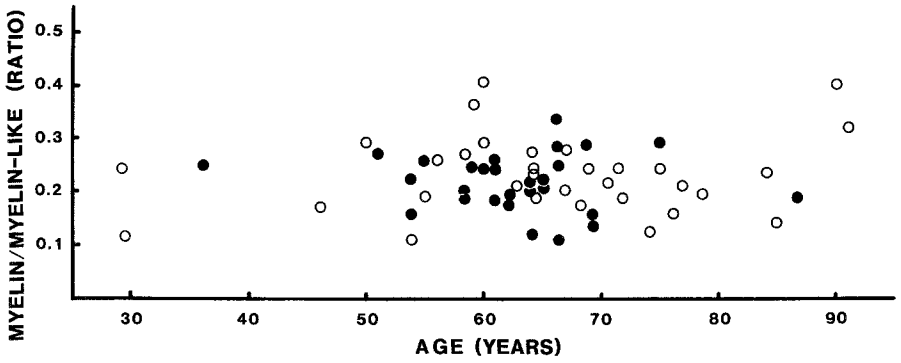

Fig. 6. The ratio of myelin to myelin-like membranes in human brain white matter during aging (see the legend to Fig. 1 for other details).

The recovery of myelin-like membranes followed the same trend as the myelin membranes. Consequently, the myelin-like/myelin ratio (Fig. 6) remained constant with advancing age in controls (0.230 ± 0.073; $N = 32$) and heavy drinkers (0.214 ± 0.051).

Composition of Myelin and Myelin-like Membranes (Figs. 7 and 8)

With advancing age, no statistically significant change was observed in the protein content of myelin membrane in controls ($29.3 \pm 4.3\%$; $N = 32$) and heavy drinkers alike ($27.0 \pm 5.2\%$; $N = 28$). Similarly, no effect of age was found on the

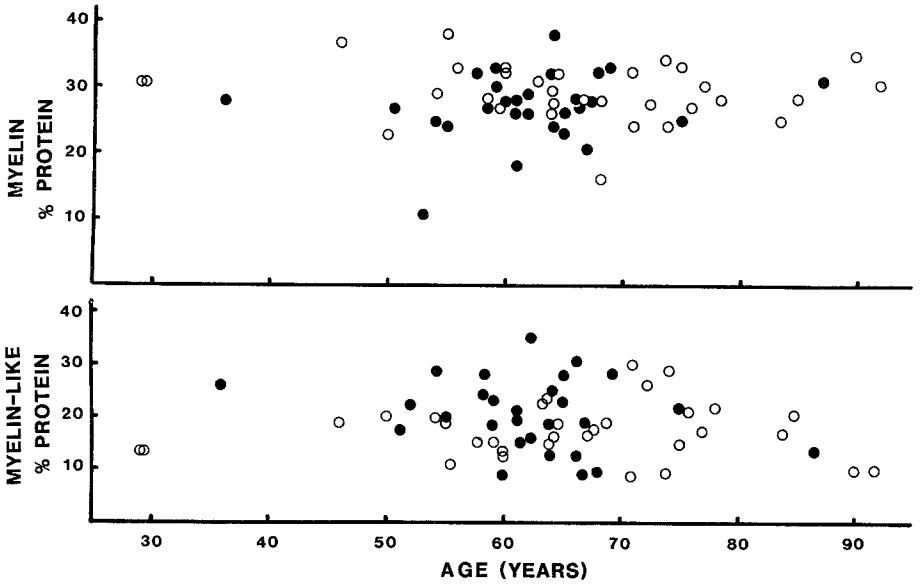

Fig. 7. The proportion of myelin (top panel) and of myelin-like membranes (bottom panel) comprised of protein during aging (see the legend to Fig. 1 for other details).

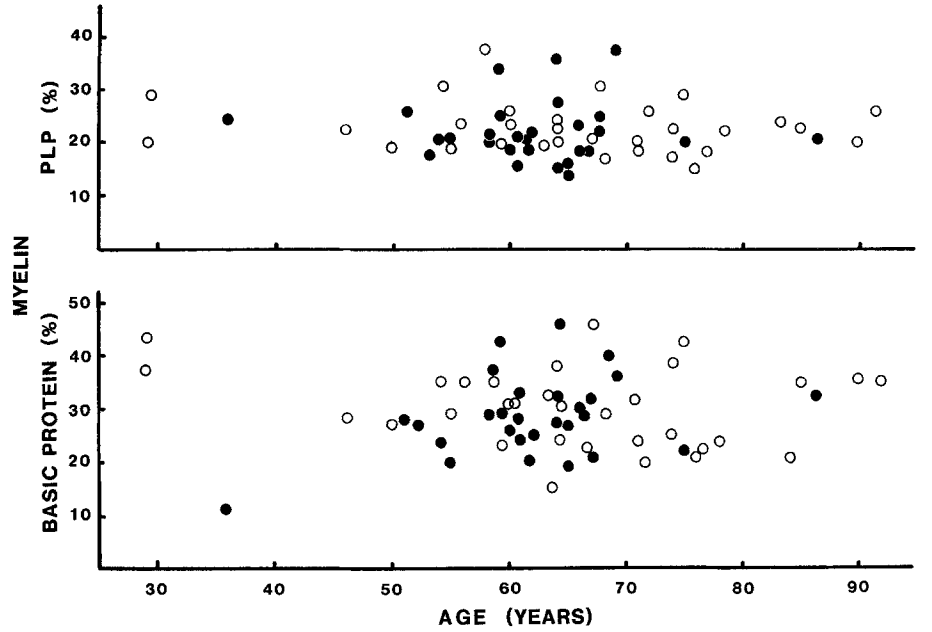

Fig. 8. The proportion of Folch-Lees proteolipid protein (top panel) and of basic protein (bottom panel) in human myelin during aging (see the legend to Fig. 1 for other details).

percentage of the myelin-like membrane comprised of protein. However, protein comprised a slightly higher proportion of the myelin-like fraction in heavy drinkers (control, 17.3 ± 5.3%, $N = 32$; heavy drinkers, 20.5 ± 7.0%, $N = 28$).

Using estimates based on electrophoretic separation and Coomassie blue binding, the proportions of myelin protein comprised of Folch-Lees proteolipid protein (22.4 ± 5.1%, controls, $N = 32$; 22.2 ± 5.6%, heavy drinkers, $N = 28$) and of basic proteins (30.2 ± 7.5%, controls, $N = 32$; 28.4 ± 7.3%, heavy drinkers, $N = 28$) were not significantly affected by increasing age. The composition of myelin-like proteins was not determined, since relatively little of the protein in this fraction consists of the well-characterized proteolipid and basic proteins (Benjamins *et al.*, 1976).

Autopsy Interval (Fig. 9)

Within the time during which most of the brains employed here were removed and frozen, the autopsy interval had little effect (Pearson correlation statistics). Two features that would intuitively cause concern (given a long autopsy interval) are the myelin-like/myelin ratio and the percentage of myelin comprised of basic protein. The former was absolutely constant. Regression analysis indicated that about one-third to one-half of the basic protein would be gradually lost from the myelin fraction over a 90-hr interval ($P = 0.034$), but the mean autopsy times of samples employed here were 20 ± 17 hr for heavy drinkers and 17 ± 11 hr for controls.

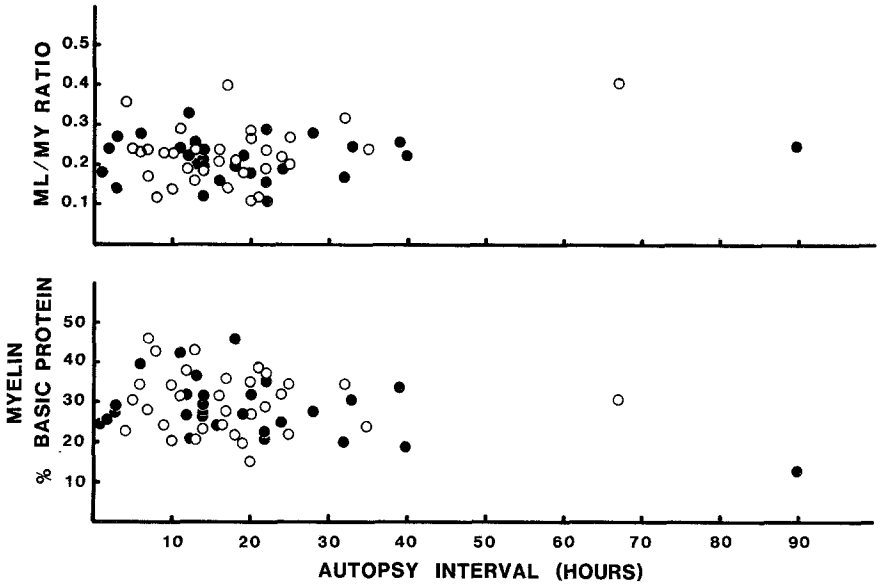

Fig. 9. The ratio of myelin to myelin-like membranes (top panel) and the content of basic protein (bottom panel) in human myelin during the interval between death and autopsy (see the legend to Fig. 1 for other details).

DISCUSSION

Regression analysis shows that the water gain from age 30 to 90 years amounts to about 50 mg/g brain. Likewise, the loss of myelin membrane amounts to about 43 mg/g. This agreement indicates that most of the gain in water results from a nearly quantitative replacement resultant from myelin degeneration. Since about 30% of human myelin is comprised of protein (Norton, 1981), the loss of myelin membrane accounts for about 13 of the 30 mg/g of total protein lost. Thus, there is an additional loss of nonmyelin protein during aging. Combining the loss of myelin membrane (43 mg/g) with the loss of nonmyelin protein (about 17 mg/g), the total loss amounts to about 60 mg/g, which is still in close agreement with the gain in water content.

Since the protein content of myelin does not change appreciably with increasing age, the decline in myelin protein as a percentage of the total protein (Table I) indicates that myelin membrane is lost from white matter at a faster rate than the loss of additional nonmyelin protein. One can speculate that the relatively greater loss of myelin may be associated with a number of contributing factors, such as vulnerability to reactive oxygen species (Konat and Wiggins, 1985; Konat *et al.*, 1986) and increased peroxidation during aging (Chia *et al.*, 1983), possible differences in the relative loss of myelinated and nonmyelinated axons during aging (Sturrock, 1987), and other factors producing a relatively selective demyelination.

In heavy drinkers, the loss of total protein actually appears to lag the normal age-related loss that would be expected (Table I). Since degenerative changes in the myelin of alcoholics is actually faster than in control groups, it is clear that heavy

drinking promotes age-related degeneration. These data indicate either a lag in the normal loss of nonmyelin protein or an accumulation of abnormal protein in heavy drinkers. If the former case is correct, then the losses of myelin and nonmyelin protein in white matter are not necessarily coupled events.

Since protein recovery did not change in gray matter, although RNA declined, it is possible that much of the nonmyelin protein lost from white matter was originally synthesized in gray matter, presumably in neuronal perikarya. The loss of myelin would not be expected to correlate with a measurable loss of white matter RNA, since myelin proteins have unusually long half-lives and the proportion of myelin membrane comprised by protein is much lower than for other types of subcellular membranes.

The ratio of myelin and myelin-like membranes remained constant during aging (Fig. 6) and during the interval between death and autopsy (Fig. 9). Since the rate of loss of both subfractions was equal during aging, and since both membranes remained stable post mortem, it is clear that myelin loss is not associated with an increase in the myelin-like fraction. Since myelin-like membranes do not appear to represent degraded myelin, the two fractions may be metabolically related.

The autopsy time did not significantly influence any of the results reported here, which is consistent with the postmortem stability of white matter reported by Rand *et al.*, (1979). However, regression analysis and Fig. 9 indicate that long intervals (more than 24 hr; certainly 40 hr) between death and autopsy would result in a measurable loss of basic protein from the myelin composition. Myelin basic protein is known to be especially labile, particularly when brain samples have been frozen and thawed (Ansari *et al.*, 1975; Martinez, 1986).

In conclusion, we confirm previously documented increases in brain water with aging, and we show that the increase is confined largely to the white matter. We also confirm the loss of myelin membrane from aging white matter, and we show that there is an additional, slower loss of nonmyelin protein. We find that the increase in water is quantitatively similar to the concomitant loss of myelin membrane and nonmyelin proteins, and we hypothesize the increase in brain water with aging is a consequence primarily of degenerative changes in white matter: particularly demyelination and, also, the loss of nonmyelin proteins. The only comparable change observed in aging gray matter consisted of a decline in RNA, and it is tempting to speculate that the loss of non-myelin proteins in the white matter is related to declining synthesis in the gray matter. Myelin is lost at an accelerated rate from white matter of alcoholics, but in contrast to the normal aging process, the total white matter protein increases slightly in alcoholics. Mechanisms underlying the degenerative changes associated with normal aging of white matter and with alcoholism are unknown, but they are not related to the autopsy time interval, causes of death, or alcohol-related diseases.

ACKNOWLEDGMENTS

This research was supported in part by a grant from the National Multiple Sclerosis Society and by Public Health Service Grants NS-13799 and DA-04072 (R.W.).

REFERENCES

Ansari, K. A., and Loch, J. (1975). Decreased myelin basic protein content of the aged human brain. *Neurology* **25**: 1045–1050.

Ansari, K. A., Hendrickson, H., Sinha, A. A., and Rand, A. (1975). Myelin basic protein in frozen and unfrozen bovine brain: A study of autolytic changes in situ. *J. Neurochem.* **25**: 193–195.

Benjamins, J. A., Gray, M., and Morell, P. (1976). Metabolic relationship between myelin subfractions: Entry of proteins. *J. Neurochem.* **27**: 571–575.

Berlet, H. H., and Volk, B. (1980). Studies of human myelin proteins during old age. *Mech. Aging Dev.* **14**: 211–222.

Berlet, H. H., Ilzenhoffer, H., Echtenacher, B., and Volk, B. (1982). Old age alters density of myelin isolated from human brain. *Exp. Brain Res.* **5** (Suppl.): 167–174.

Chia, L. S., Thompson, J. E., and Moscarello, M. A. (1983). Changes in lipid phase behaviour in human myelin during maturation and aging. *FEBS Lett.* **157**: 155–158.

Davis, J. M., and Himwich, W. A. (1975). Neurochemistry of the developing and aging mammalian brain. In Ordy, J. M., and Brizzee, K. R. (eds.), *Neurobiology of Aging*, Plenum Press, New York, pp. 329–357.

Donaldson, H. H. (1924). The rat. Data and reference tables for the albino rat and the Norway rat. *Mem. Wistar Inst. Anat. Biol.* **6**: 276–280.

Friede, R. H. (1966). *Topographic Brain Chemistry*, Academic Press, New York, pp. 464–465.

Greenfield, S., Norton, W. T., and Morell, P. (1971). Quaking mouse: Isolation and characterization of myelin protein. *J. Neurochem.* **18**: 2119–2128.

Himwich, H. E. (1973). Early studies of the developing brain. In Himwich, W. (ed.), *Biochemistry of the Developing Brain*, Marcel Dekker, New York, pp. 1–53.

Horrocks, L. A., Van Rollins, M., and Yates, A. J. (1981). Lipid changes in the aged brain. In Thompson, R. H. S., and Davidson, A. N. (eds.), *The Molecular Basis of Neuropathology*, Edward Arnold, London, pp. 601–630.

Konat, G. W., and Wiggins, R. C. (1985). The effect of reactive oxygen species on myelin membrane proteins. *J. Neurochem.* **45**: 1113–1118.

Konat, G. W., Gantt, G., Gorman, A., and Wiggins, R. C. (1986). Peroxidative aggregation of myelin membrane protein. *Metab. Brain Dis.* **1**: 157–164.

Lintl, P., and Braak, H. (1983). Loss of intracortical myelinated fibers: A distinctive age-related alteration in the human striate area. *Acta Neuropathol. (Berl.)* **61**: 178–182.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. (1951). Protein measurements with the Folin-phenol reagent. *J. Biol. Chem.* **193**: 265–275.

Malone, J. J., and Szoke, M. C. (1985). Neurochemical changes in white matter. Aged human brain and Alzheimer's disease. *Arch. Neurol.* **42**: 1063–1066.

Martinez, M. (1986). Myelin in developing human cerebellum. *Brain Res.* **364**: 220–232.

Morell, P., Wiggins, R. C., and Gray, J. (1975). Polyacrylamide gel electrophoresis of myelin proteins: A caution. *Anal. Biochem.* **68**: 148–154.

Norton, W. T. (1981). Formation, structure and biochemistry of myelin. In Siegel, G. J., Albers, R. W., Agranoff, B. W., and Katzman, R. (eds.), *Basic Neurochemistry*, 3rd ed., Little, Brown, Boston, pp. 63–92.

Norton, W. T., and Poduslo, S. E. (1973). Myelination in rat brain: Method of myelin isolation. *J Neurochem.* **21**: 749–757.

Ordy, J. M., and Kaack, B. (1975). Neurochemical changes in composition, metabolism and neurotransmitters in the human brain with age. In Ordy, J. M., and Brizzee, K. R. (eds.), *Neurobiology of Aging*, Plenum, New York, pp. 253–285.

Rand, A., Ansari, K. A., and Loch, J. (1979). 2′,3′-Cyclic nucleotide 3′-phosphodiesterase activity of human white matter and time interval between death and autopsy. *J. Neurochem.* **32**: 627–628.

Riederer, B., Honegger, C. G., Tobler, H. J., and Ulrich, J. (1984). The effect of age on the microheterogeneous pattern of human myelin basic protein. *Gerontology* **30**: 234–239.

Samorajski, T. (1980). Neurochemical changes in the aging human and nonhuman primate brain. In Eisdorfer, C., and Fann, W. E. (eds.), *Psychopharmacology of Aging*, Spectrum, Jamaica, N.Y., pp. 145–169.

Samorajski, T., and Rolsten, C. (1973). Age and regional differences in the chemical composition of brains of mice, monkeys and humans. *Prog. Brain Res.* **40**: 253–265.

Sturrock, R. R. (1987). Age-related changes in the number of myelinated axons and glial cells in the anterior and posterior limbs of the mouse anterior commissure. *J. Anat.* **150**: 111–127.

Wannemacher, R. W., Banks, W. L., and Wunner, W. H. (1965). Use of a single tissue extract to determine protein, nucleic acid concentrations and rate of amino acid incorporation. *Anal. Biochem.* **11**: 320–326.

Wiggins, R. C. (1986). Myelination: A critical stage in development. *Neurotoxicology* **7**: 102–120.

Wiggins, R. C., and Fuller, G. N. (1981). Analysis of the distribution of rat sciatic nerve protein among soluble, insoluble, and myelin subfractions. *Neurochem. Res.* **6**: 719–727.