PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 165, 327–329 (1980)

Mineral Composition of Brains of Normal and Multiple Sclerosis Victims (40980)

WILLIAM CRAELIUS, RICHARD M. JACOBS,* AND ANN O. LEE JONES*

*Department of Biology, Lafayette College, Easton, Pennsylvania 18042, and *Division of Nutrition, Food and Drug Administration, Washington, D.C. 20204*

Abstract. Elemental contents of human brain samples were determined using plasma emission spectroscopy. White matter samples from brains of multiple sclerosis (MS) victims had significantly greater concentrations of calcium, iron, manganese, and zinc, and significantly less phosphorus, as compared with controls. Whether these differences in elemental concentrations indicate an etiogenic or pathogenic relationship between these elements and MS is not known.

Accumulation of certain elements in the central nervous system has been implicated in the neuropathology of several disorders. Elevated brain concentrations of aluminum, calcium, copper, and iron have been associated, respectively, with Alzheimer's disease (1), amyotrophic lateral sclerosis (2), Wilson's disease (3), and hemochromatosis (4). Elemental brain composition in various other encephalopathies is not well known and data on the normal brain are fragmentary (5–9). In the present study, elemental analyses were performed on brains from deceased individuals who were either victims of multiple sclerosis (MS) or controls. The findings of this study reveal distinctive and consistent changes in brains of MS victims.

Materials and methods. Freshly frozen brain samples from victims that had clinically confirmed MS were prepared and supplied by neurospecimen banks.[1] Control brain samples were similarly prepared and supplied by pathologists; eight deaths were injury related, one was due to heart attack, and one was due to epilepsy. Ages ranged from 28 to 66 years for the MS victims and 16 to 80 years for the controls. Six males and five females comprised the MS group, and six males and four females comprised the control group. Frozen white matter from MS victims and controls and gray matter from MS victims, macroscopically free from lesions (plaques), were isolated from cerebral cortices by using acid-washed glass knives. Two plaques were isolated in a similar fashion. Utmost care was taken to prevent loss of moisture and prevent contamination. The sample size used for analysis of white and gray matter averaged approximately 7 and 4 g, respectively. The samples were wet-digested with 5:1 (v:v) HNO_3:$HClO_4$ (70%) and 1 ml of concentrated H_2SO_4. Reagent blanks and standard samples (National Bureau of Standards) of bovine liver and wheat flour were digested similarly. After digestion, samples contained only H_2SO_4 and mineral sulfates.

The digests were analyzed by inductively coupled argon plasma (ICAP) emission spectrometry (10). The argon plasma was produced by a high-frequency (27 MHz), 1.1-kW forward power radio frequency generator. The direct reading spectrometer (Jarrell–Ash Atom Comp Model 975) was equipped with a 0.75-m Pasche–Runge optical configuration and contained a spectrochemical controller. Background was corrected automatically by square wave modulation. Samples were introduced into the plasma source with a cross-flow pneumatic nebulizer. Elemental recovery was checked by ICAP analysis of samples to which internal standards of a few of the elements of interest had been added. Recoveries for copper, manganese, zinc, and iron were 91–94, 106–107, 102–103, and 100–102%, respectively. The elemental concentrations are expressed on a fresh weight basis.

[1] Human Neurospecimens Bank, Wadsworth Veterans Administration Hospital, Los Angeles, Calif.; Palo Alto Veterans Administration Hospital, Palo Alto, Calif.; and University of Pennsylvania Hospital, Philadelphia, Pa.

0037-9727/80/100327-03$01.00/0

Results and discussion. Table I presents the average concentrations and ranges of nine elements in brains from controls and MS victims. Eighteen additional elements were below the analytical limits. The maximal concentrations of elements that these tissues could have contained are shown in the footnote of the table. Concentrations of calcium, iron, manganese, and zinc were significantly higher in MS white matter than in controls. Phosphorus was significantly decreased in MS white matter, but the concentrations of the other four elements were unchanged. There have been reports of phospholipid loss in MS white matter from areas of the brain that appeared normal upon gross examination (11). The decrease in phosphorus content may reflect this fact. When MS gray matter was compared with MS white matter (groups MS-2 and MS-3), only phosphorus and zinc were significantly different; zinc was higher in the gray matter and phosphorus was lower. These elemental differences between gray and white matter have been described previously for control brains (6, 8, 9); however, in the present study, control gray matter was not sampled.

Two MS white matter samples contained lesions that appeared to be old, burned-out plaques, which were poorly vascularized. The two plaques, weighing 210 and 17 mg, both had concentrations of calcium, iron, manganese, and zinc that were above the range of those for control white matter. Concentrations of the other elements were within the normal range in the larger plaque, but all elements were increased in the smaller plaque. Dehydration may have accounted for some, but not all, of the increased elemental concentrations in the smaller plaque.

Aluminum was detected in the white matter of three brains from MS victims and one control brain, but was not detectable in their gray matter. Concentrations of aluminum were 5.3, 6.1, and 51.4 μg/g wet wt in the three samples from MS victims aged 41, 55, and 58 years, respectively, and was 10.2 μg/g wet wt in one 72-year-old accident victim. Aluminum levels of these

TABLE I. ELEMENTAL CONTENT OF HUMAN BRAINS[a]

Type of sample[b]	N	Ca (μg/g)	Cu (μg/g)	Fe (μg/g)	K (mg/g)	Mg (μg/g)	Mn (μg/g)	Na (mg/g)	P (mg/g)	Zn (μg/g)
Control	10	52.0 ± 5.0	3.81 ± 0.31	44.1 ± 2.6	2.84 ± 0.09	124 ± 4.1	0.258 ± 0.015	1.46 ± 0.06	4.21 ± 0.10	7.49 ± 0.15
		(38.5–85.1)	(2.45–5.81)	(31.7–58.2)	(2.40–3.28)	(111–150)	(0.171–0.324)	(1.13–1.89)	(3.66–4.59)	(6.74–8.29)
MS-1	11	65.4 ± 3.5[c]	3.12 ± 0.21	58.5 ± 3.0[d]	2.92 ± 0.12	137 ± 5.1	0.387 ± 0.056[c]	1.70 ± 0.11	3.75 ± 0.14[c]	10.9 ± 0.54[d]
		(50.2–91.0)	(2.24–4.53)	(43.2–72.4)	(2.35–3.65)	(111–156)	(0.152–0.812)	(1.16–2.50)	(2.56–4.45)	(7.71–16.0)
MS-2	7	67.4 ± 5.2	3.18 ± 0.20	59.7 ± 3.6	3.03 ± 0.17	144 ± 6.3	0.436 ± 0.080	1.75 ± 0.18	3.61 ± 0.19	11.7 ± 0.58
		(50.2–91.0)	(2.54–3.73)	(43.2–69.6)	(2.56–3.65)	(111–156)	(0.152–0.812)	(1.16–2.50)	(2.56–4.14)	(10.4–16.0)
MS-3	7	51.2 ± 11	3.87 ± 0.31	54.0 ± 3.2	3.05 ± 0.20	128 ± 5.3	0.284 ± 0.021	1.94 ± 0.12	2.83 ± 0.08[d]	14.1 ± 0.62[c]
		(54.5–106)	(2.74–5.27)	(43.5–65.2)	(2.53–3.83)	(107–146)	(0.203–0.366)	(1.67–2.58)	(2.47–3.11)	(11.8–15.4)
MS-4[e]	—	91.9, 287	5.51, 18.5	205, 308	2.67, 11.4	140, 552	0.495, 1.12	1.98, 5.13	3.07, 10.1	15.3, 90.2

[a] Mean values ± SE, with the range shown in parentheses. Values on a fresh weight basis. Statistical comparisons were made only between the control group and group MS-1 and between group MS-2 and group MS-3. The following elements were below the analytical range in all or a few samples (assay limit for size sample used and dilution, μg/g fresh wt): Ag < 0.075, Al < 0.075, As < 0.112, B < 0.015, Bi < 0.225, Cd < 0.0075, Cr < 0.015, In < 0.225, Li < 0.0075, Mo < 0.0375, Co < 0.015, Ni < 0.030, Pb < 0.112, Se < 0.112, Si < 0.038, Sn < 0.045, Sr < 0.038, Ti < 0.0075, V < 0.015.

[b] Controls, human brain samples from non-MS victims (white matter); MS-1, individuals with multiple sclerosis (white matter); MS-2, white matter samples from MS victims with correspondingly sampled gray matter; MS-3, gray matter; and MS-4, plaques.

[c] Values significantly different from controls or from group 2, Student's t test $P < 0.05$.

[d] Values significantly different from controls or from group 2, $P < 0.01$.

[e] Because only two plaque samples were analyzed and their mineral compositions varied so markedly, individual values are presented rather than the means.

magnitudes are all typical of those found in Alzheimer's disease and other senile dementias (1).

The greater metal concentrations found in brains from MS victims, not previously described, could be in the form of deposits. If deposits are present, they should be detectable histologically, and would be visible as electron-dense material in electron micrographs. Structures that are likely storage sites for the metals are astrocytes and macrophagous glial cells. Mineral deposits associated with degenerating myelin have been found in macrophages and astrocytes in the cerebellum of chicks with nutritional encephalopathy (12). Calcium-containing structures within human astrocytes in a case of leukoencephalitis have been described (13). Published fine-structural studies of MS plaques reveal that astrocytes and glial cells are often enlarged and usually dense near the lesions (14, 15). Enlarged, densely staining lysosomes and other inclusions are found within glial cells in perivascular areas near MS plaques (16). Since lysosomes are known to take up iron (17), they are possible subcellular storage sites for metals in MS plaques.

Whether the higher elemental concentrations found in brains of these MS victims are etiogenic or pathogenic cannot be determined from this study. The potential role of metals in the demyelinative process has been recently suggested by experiments in which small deposits of various metals were implanted into rabbit brains (18). Zinc, in particular, caused symptoms and pathology closely resembling those of encephalomyelitis, an animal model of MS. The pathology included perivascular cuffing and lymphocytic activation, which are distinctive features of MS (14, 15). It is thus possible that the peculiar elemental changes found in brains of MS victims may be causally related to demyelination. The altered brain chemistry may also be a factor in the enigmatic symptomatology of MS.

1. Crapper, D. R., Krishnan, S. S., and Dalton, A. J., Science **193**, 511 (1973).
2. Yase, Y., Yoshimashu, F., Uebayashi, Y., Iwata, S., and Sasajima, K., Folia Psychiatr. Neurol. Japon. **28**, 371 (1974).
3. Cartwright, G. E., Hodges, R. E., Gubler, C. J., Mahoney, J. P., Daum, K., Wintrobe, M. M., Bean, W. B., and Walshe, J. M., Brain **90**, 149 (1967).
4. Miyasaki, K., Murao, S., and Kozuni, N., J. Neuropathol. Exp. Neurol. **36**, 964 (1977).
5. Tipton, I. H., and Cook, M. J., Health Phys. **9**, 103 (1963).
6. Widdowson, E. M., and Dickerson, J. W. T., *in* "Mineral Metabolism" (C. L. Comar and F. Bronner, eds.), Vol. 2, Part A. Academic Press, New York (1964).
7. Smeyers-Verbeke, J., Bell, J., Lowenthal, A., and Massart, D. L., Clin. Chim. Acta **68**, 343 (1976).
8. Smeyers-Verbeke, J., Defrise-Gussenhoven, E., Ebinger, G., Lowenthal, A., and Massart, D. L., Clin. Chim. Acta **51**, 309 (1974).
9. Greiner, A. C., Chan, S. C., and Nicholson, G. A., Clin. Chim. Acta **61**, 325 (1975).
10. Fassel, V., Science **202**, 183 (1978).
11. Norton, W. T., *in* "Myelin" (P. Morrell, ed.), p. 383. Plenum, New York (1977).
12. Bubis, J. J., Sanbank, U., Bartov, I., Dror, Y., Budowski, P., and Wolman, M., Acta Neuropathol. **44**, 47 (1978).
13. Gambetti, P., Erulkar, S. E., Somlyo, A. P., and Gonatas, N. K., J. Cell Biol. **64**, 322 (1975).
14. Andrews, J. M., *in* "Multiple Sclerosis: Immunology, Virology, and Ultrastructure" (F. Wolfgram, ed.), p. 23. Academic Press, New York (1972).
15. Arstila, A. V., Riekkinen, A. V., Rinne, U. K., and Ritmer, L. L., Eur. Neurol. **9**, 1 (1973).
16. Prineas, J. W. and Wright, R. G., Lab. Invest. **38** 409 (1978).
17. Glaumann, H., Jansson, H., Arbogh, B., and Ericsson, J. L. E., J. Cell Biol. **67**, 887 (1975).
18. Levine, S., J. Neuropathol. Exp. Neurol. **37**, 471 (1978).

Received June 9, 1980. P.S.E.B.M. 1980, Vol. 165.