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Regional Distribution of Potassium, Calcium, and Six Trace Elements in Normal Human Brain

H. Duflou[1], W. Maenhaut[1,3], and J. De Reuck[2]

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Eight elements (i.e. K, Ca, Mn, Fe, Cu, Zn, Se, and Rb) were measured in 50 different regions of 12 normal human brains by particle-induced X-ray emission (PIXE) analysis. The dry weight concentrations of K, Fe, Cu, Zn, Se, and Rb were consistently higher for gray than for white matter areas. The K, Zn and Se concentrations for the regions of mixed composition and, to some extent, also the Rb concentrations, were intermediate between the gray and white matter values, and they tended to decrease with decreasing neuron density. The mean dry weight concentrations of K, Ca, Zn, Se, and Rb in the various brain regions were highly correlated with the mean wet-to-dry weight ratios of these regions. For Mn, Fe, and Cu, however, such a correlation was not observed, and these elements exhibited elevated levels in several structures of the basal ganglia. For K, Fe, and Se the concentrations seemed to change with age. A hierarchical cluster analysis indicated that the structures clustered into two large groups, one comprising gray and mixed matter regions, the other white and mixed matter areas. Brain structures involved in the same physiological function or morphologically similar regions often conglomerated in a single subcluster.

KEY WORDS: Particle-induced X-ray emission; elements; age; human brain.

INTRODUCTION

Various trace elements unquestionably play an important role in the physiology and pathology of the central nervous system (1-3). Some elements (e.g., Mn, Fe, Cu, Zn, and I) are essential for brain growth and functioning, whereas others, such as Pb and Hg may cause severe brain damage. However, the essential trace elements may also impair brain function, when they are present at elevated levels. For instance, it has been demonstrated that chronic exposure to manganese ores can result in Parkinsonism and dementia (1-3).

Although the biochemical function of the trace elements is still poorly understood, studies on the effect of altered trace element levels have resulted in methods for curing and prevention of disease (1-3). In psychiatry, Li is administered to patients who suffer from mania or depression (4). More recently, the effect of Rb as antidepressant has been studied (4).

The growing awareness of the importance of the trace elements for the working of the central nervous system has also stimulated research on their concentrations in normal and pathological brain. The analysis of various brain regions has revealed a heterogeneous distribution of several elements (5-8). Moreover, the distribution seems to reflect functional differences of the various brain regions. However, in order to gain a deeper understanding of the specific functions of the trace elements in the brain, more extensive trace elemental mappings are needed.

In the present study we measured 8 elements (i.e. K, Ca, Mn, Fe, Cu, Zn, Se, and Rb) in up to 50 different

[1] Laboratory of Analytical Chemistry, Institute for Nuclear Sciences, Rijksuniversiteit Gent, Proeftuinstraat 86, B-9000 Gent, Belgium.
[2] Kliniek voor Neurologie, Universitair Ziekenhuis, De Pintelaan 185, B-9000 Gent, Belgium.
[3] To whom to address reprint request.

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areas of 12 normal human brains by means of particle induced X-ray emission (PIXE). PIXE is a fast, multi-element analysis technique, which allows measuring μg/g levels of several elements in small biological samples approximating a few mg. It is therefore very suitable for studying the regional distribution of trace elements in the brain.

EXPERIMENTAL PROCEDURE

The brains originated from 12 persons with ages between 7 and 69 years (see Table I). They were removed at autopsy, within 72 hours after death, by means of standard procedures. Subsequently, all brains were superficially rinsed with cold double distilled water. Tissue samples were dissected from 50 different brain regions (Table II) by means of molybdenum knives, as described elsewhere (9). Samples were taken from corresponding regions in both the left and the right cerebral hemisphere, the brainstem and the cerebellum. Macroscopic examination of the brains revealed no abnormalities. The samples were weighed to determine their wet weight, and then were freeze-dried for 72 h in a Leybold-Heraeus vacuum apparatus, and oven dried for 12 h at 75°C. The weights of the dried tissues ranged from 3 to 800 mg. The further sample preparation and PIXE analysis were limited to the tissues from the left cerebral hemisphere, the brainstem and cerebellum of each brain. However, for two brains (A and K), we also analyzed corresponding regions from both hemispheres. Typically, 4 PIXE targets were prepared and analyzed for each brain sample. The PIXE analysis method, experimental set-up, and target preparation procedure are described in detail elsewhere (10-12, 9).

RESULTS

Trace Element Levels in Corresponding Regions of the Two Brain Hemispheres. Samples of 61 corresponding regions from both hemispheres were analyzed (i.e., of 23 regions of brain A and 38 regions of brain K). The dry weight concentration data set obtained was used to calculate the correlation coefficient between the right side and left side concentration of each element. Furthermore, dry weight elemental concentration ratios (right side)/(left side) were calculated for each of the 61 brain regions, and then averaged over all regions. The results are listed in Table III. All correlation coefficients are highly significant ($P < 0.0005$, one-sided test), and the average ratios are close to one. These findings indicate that there are no systematic concentration differences between the two brain sides. A scatter diagram, which demonstrates the good correlation between the Rb concentrations of both hemispheres, is shown in Figure 1.

Wet-to-Dry Weight Ratio (Reduction Factor) and Elemental Concentrations for 50 Different Brain Regions. The mean concentration and associated standard deviation (both expressed in μg/g dry weight) were calculated for each element in each of the 50 different brain regions, by averaging the individual results of the 12 brains. The Rb data of brain G were excluded, however, as they were substantially higher than for the other 11 brains. Also, for brains A and K, the data of the left and right sides were first averaged before calculating the overall means. The mean wet-to-dry weight ratio (reduction factor) for each brain region was obtained in a similar way. The 50 brain regions studied were classified into 4 groups, i.e. one comprising gray matter areas, a second of mixed composition, a third comprising white matter, and a fourth of samples which do not fit into the first three groups. The results for each individual region are listed in Table IV and shown in Figures 2, 3, and 4. The regions of the mixed matter group are essentially ordered on the basis of decreasing neuron density in the table and the figures.

Table IV and Figures 2, 3 and 4 show that the reduction factor and the dry weight concentrations of K,

Table I. Age, Sex, Brain Weight, and Cause of Death for Patients A to L

Patient	Age	Sex	Brain weight	Diagnosis
A	7 y	M	1493 g	Acute respiratory distress
B	69 y	F	1220 g	Acute cardiac failure
C	15 y	F	1490 g	Acute respiratory distress
D	9 y	F	1230 g	Acute respiratory distress
E	59 y	F	1150 g	Bronchus carcinoma
F	21 y	M	1467 g	Acute respiratory distress
G	39 y	F	1230 g	Anemic anoxia
H	33 y	F	1470 g	Anemic anoxia
I	43 y	M	1500 g	Anemic anoxia
J	62 y	M	1475 g	Anemic anoxia
K	33 y	F	1325 g	Acute respiratory distress
L	42 y	F	1170 g	Anemic anoxia

Table II. Brain Regions Studied, as Classified on the Basis of Gross Morphology

Cerebral hemispheres:
- Cerebral cortex
  - (1)[a](G)[b]gyrus frontalis superior
  - (2) (G) gyrus cinguli
  - (4) (G) gyrus rectus
  - (9) (G) gyrus temporalis inferior
  - (10) (G) insula
  - (17) (M) uncus hippocampi
  - (18) (G) gyrus parahippocampalis
  - (23) (G) gyrus precentralis
  - (24) (G) gyrus postcentralis
  - (38) (G) gyrus lingualis
- Cerebral white matter
  - (3) (W) genu corporis callosi
  - (5) (W) septum pellucidum
  - (11) (W) chiasma opticum
  - (14) (W) corpus fornicis
  - (21) (W) fasciculus mamillothalamicus
  - (25) (W) capsula interna
  - (37) (W) radiatio optica
- Basal ganglia
  - (6) (M) nucleus caudatus
  - (7) (M) putamen
  - (8) (M) globus pallidus
  - (12) (M) corpus amygdaloideum
  - (13) (M) infundibulum
  - (15) (M) claustrum
  - (19) (M) nucleus ventralis anterior thalami
  - (20) (M) corpora mamillaria
  - (27) (M) nucleus ruber
  - (28) (M) nucleus centromedianus thalami
  - (29) (M) nucleus medialis dorsalis thalami
  - (30) (M) nucleus lateralis posterior thalami
  - (31) (M) nucleus ventralis lateralis thalami
  - (32) (M) nucleus pulvinaris thalami

Brainstem :
- (22) (M) substantia nigra
- (26) (W) pedunculus cerebri
- (33) (M) anulus aquaeductus cerebri
- (34) (M) colliculi superiores
- (35) (M) colliculi inferiores
- (39) (M) tractus corticospinalis
- (40) (M) dorsum pontis
- (41) (M) nucleus olivaris inferior
- (42) (W) pyramid
- (43) (M) dorsum medullae

Cerebellum :
- (44) (G) paraflocculus ventralis cerebelli
- (45) (G) lobulus flocculonodularis
- (46) (G) vermis cerebelli
- (47) (M) nucleus dentatus
- (48) (W) corpus medullare cerebelli

Other brain areas :
- (16) plexus choroideus
- (36) corpus pineale
- (50) blood vessel
- (51) meninx

[a] The numbers in parentheses indicate the dissection numbers and are used to indicate the regions throughout the text.
[b] G, M or W indicate that the region has been classified as gray matter, as a structure of mixed composition or as white matter, respectively.

Table III. Correlation Coefficients and Dry Weight Concentration Ratios (Right Side)/(Left Side) for 8 Elements in Corresponding Structures of the Right and Left Side of Human Brains A and K[a]

Element	Correlation coefficient between the right and left side concentration	Average ratio ± standard deviation
K	0.84	0.96 ± 0.15
Ca	0.64	1.00 ± 0.28
Mn	0.84	0.96 ± 0.18
Fe	0.96	1.03 ± 0.20
Cu	0.92	0.94 ± 0.14
Zn	0.93	0.95 ± 0.14
Se	0.78	1.01 ± 0.30
Rb	0.91	0.97 ± 0.10

[a] Regions analyzed for brain A were nos. : 1, 2, 4, 6, 7, 9, 10, 15, 17, 18, 20, 23, 24, 28, 29, 31, 32, 37, 38, 41, 42, 44, 48; Regions analyzed for brain K were nos. : 1, 2, 3, 4, 6, 7, 8, 9, 10, 12, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 37, 38, 39, 40, 41, 42, 44, 47, 48.

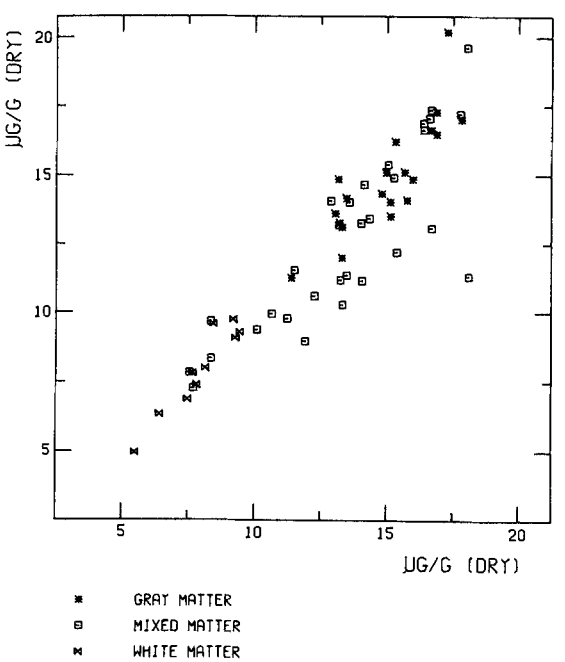

Fig. 1. Scatter plot of the Rb concentration in the left hemisphere versus the Rb concentration in the right hemisphere for 61 corresponding regions of brains A and K.

Fe, Cu, Zn, Se and Rb are consistently higher for gray than for white matter areas. The difference is particularly pronounced for Cu and Zn, for which the mean gray matter concentrations are resp. 2.6 and 2.5 times higher than the mean white matter levels. The K, Zn, and Se concentrations for the regions of mixed composition and, to a lesser extent, also the Rb concentration and the

Table IV. Wet-to-Dry Weight Ratio (Reduction Factor) and Elemental Concentrations[a] for 50 Different Brain Regions

Region	Reduction Factor $\bar{x} \pm s$[b]	N[c]	K $\bar{x} \pm s$	N	Ca $\bar{x} \pm s$	N	Mn $\bar{x} \pm s$	N	Fe $\bar{x} \pm s$	N
Gray matter										
1	6.09 ± 0.47		17200 ± 1900		520 ± 380		1.24 ± 0.13		238 ± 58	
2	6.14 ± 0.43		17900 ± 1900		510 ± 300		1.30 ± 0.12		199 ± 39	
4	5.96 ± 0.37		16900 ± 1100		440 ± 190		1.220 ± 0.053		228 ± 51	
9	6.03 ± 0.30		17500 ± 1500		420 ± 190		1.19 ± 0.14		234 ± 52	
10	6.15 ± 0.59		19000 ± 2200		314 ± 57		1.28 ± 0.15		237 ± 46	
18	5.94 ± 0.41		17000 ± 2200		366 ± 89		1.22 ± 0.14		232 ± 48	
23	5.38 ± 0.53		16000 ± 2200		302 ± 45		1.22 ± 0.16		257 ± 71	
24	5.92 ± 0.59		16400 ± 2200		357 ± 60		1.38 ± 0.21		277 ± 74	
38	5.40 ± 0.39		17500 ± 2300		410 ± 280		1.40 ± 0.27		266 ± 74	
44	5.91 ± 0.29		17700 ± 1200		352 ± 74		1.82 ± 0.25		249 ± 53	
45	5.89 ± 0.43		17600 ± 1800		420 ± 300		1.96 ± 0.18		263 ± 70	
46	6.36 ± 0.76		17300 ± 1300		390 ± 180	11	1.69 ± 0.17		229 ± 70	
Mixed matter										
15	4.96 ± 0.91	10	14600 ± 2000		340 ± 190		1.17 ± 0.15		253 ± 57	
17	5.80 ± 0.70	11	17000 ± 2400	11	307 ± 39	11	1.54 ± 0.29	11	225 ± 54	11
12	5.79 ± 0.40		16600 ± 1000		305 ± 71		1.16 ± 0.17		227 ± 55	
6	5.96 ± 0.62		16800 ± 1000		321 ± 40		2.48 ± 0.26		520 ± 180	
7	5.12 ± 0.43		18100 ± 1500		260 ± 17		2.67 ± 0.33		610 ± 240	
8	4.48 ± 0.64		15300 ± 2500		309 ± 89		2.18 ± 0.55		820 ± 200	
28	4.64 ± 0.36	11	14500 ± 1300	11	259 ± 32	11	1.62 ± 0.25	11	280 ± 82	11
29	4.59 ± 0.73		13700 ± 1200		276 ± 61		1.58 ± 0.24		283 ± 81	
30	4.17 ± 0.44		13500 ± 1200		242 ± 60		1.60 ± 0.27		266 ± 80	
31	4.11 ± 0.47		13500 ± 1400		249 ± 75		1.50 ± 0.29		261 ± 91	
32	5.01 ± 0.66	11	14700 ± 1800		305 ± 73		1.72 ± 0.26		279 ± 76	
19	5.18 ± 0.61		15000 ± 2100		380 ± 140		1.84 ± 0.26		306 ± 74	
22	4.93 ± 0.80	11	14500 ± 2500		259 ± 59		1.74 ± 0.47		710 ± 320	
27	3.79 ± 0.94	7	13000 ± 3800	8	218 ± 78	8	1.48 ± 0.51	8	230 ± 130	8
20	6.02 ± 0.51	9	12500 ± 2500	11	444 ± 69	11	1.59 ± 0.20	11	372 ± 90	11
13	6.29 ± 0.76	8	14400 ± 3500	10	430 ± 130	10	1.88 ± 0.52	10	260 ± 110	10
33	6.1 ± 1.1	10	16000 ± 2700	11	313 ± 72	10	2.01 ± 0.47	11	167 ± 40	11
34	5.69 ± 0.99	10	13800 ± 2400	11	349 ± 46	11	1.65 ± 0.19	11	248 ± 73	11
35	5.7 ± 1.2	9	14100 ± 2100	11	390 ± 120	11	1.94 ± 0.39	11	240 ± 65	11
40	4.02 ± 0.21		12500 ± 1300		222 ± 35		1.13 ± 0.18		123 ± 21	
41	4.36 ± 0.37	11	10800 ± 1100	11	350 ± 130	11	1.05 ± 0.14	11	80 ± 19	11
43	4.35 ± 0.28		11800 ± 1200	11	277 ± 64	11	1.24 ± 0.26	11	110 ± 32	11
47	3.70 ± 0.24		11200 ± 2000		200 ± 110		1.02 ± 0.24		350 ± 170	
39	3.76 ± 0.16		10770 ± 920		243 ± 71		0.85 ± 0.14		78 ± 16	
White matter										
21	4.47 ± 0.55	3	12900 ± 4100	4	380 ± 180	4	1.28 ± 0.41	4	210 ± 120	4
11	4.52 ± 0.47	11	8900 ± 1300	10	1020 ± 840	10	1.02 ± 0.18	10	93 ± 18	10
3	3.45 ± 0.17		9700 ± 1300		209 ± 88		0.84 ± 0.20		133 ± 32	
5	5.7 ± 1.6		12900 ± 3700		330 ± 140		1.24 ± 0.39		153 ± 33	
14	4.66 ± 0.64		11100 ± 1800		370 ± 140		1.05 ± 0.24		144 ± 36	
25	3.30 ± 0.21		10900 ± 1100		203 ± 72		1.05 ± 0.21		136 ± 33	
37	3.37 ± 0.31		9890 ± 730		181 ± 42		1.08 ± 0.13		125 ± 29	
26	3.58 ± 0.27	11	9600 ± 1200	11	231 ± 94	11	0.80 ± 0.11	11	135 ± 54	11
42	3.97 ± 0.33	10	8900 ± 1900		330 ± 190		0.86 ± 0.38		71 ± 40	
48	3.44 ± 0.15		12500 ± 2400		196 ± 93		0.80 ± 0.18		133 ± 38	
Other brain areas										
16	6.16 ± 0.66		14400 ± 2400		15000 ± 19000		1.76 ± 0.79		830 ± 340	
36	6.5 ± 1.9	9	13900 ± 5600	9	53000 ± 68000	9	3.5 ± 2.8	9	410 ± 200	9
50	5.06 ± 0.77	9	11200 ± 1900	9	2800 ± 2500	9	0.68 ± 0.30	9	560 ± 570	9
51	6.2 ± 1.2	8	13900 ± 2600	9	3200 ± 2900	9	1.05 ± 0.36	9	370 ± 220	9

Region	Cu $\bar{x} \pm s$[b]	N[c]	Zn $\bar{x} \pm s$	N	Se $\bar{x} \pm s$	N	Rb $\bar{x} \pm s$	N
Gray matter								
1	24.1 ± 4.2		68.7 ± 4.3		0.93 ± 0.15		14.2 ± 2.3	11
2	24.9 ± 5.9		74.3 ± 7.9		0.90 ± 0.19		17.0 ± 2.6	11
4	24.0 ± 4.2		73 ± 12		0.91 ± 0.18		14.6 ± 2.4	11
9	24.3 ± 4.3		79.6 ± 6.6		0.91 ± 0.13		14.5 ± 2.4	11
10	24.7 ± 4.7		77.4 ± 9.5		0.96 ± 0.15		18.0 ± 2.8	11
18	23.1 ± 4.7		79.2 ± 8.4		0.93 ± 0.14		15.1 ± 2.2	11
23	23.3 ± 5.4		59.8 ± 9.8		0.82 ± 0.17		14.5 ± 3.2	11
24	25.5 ± 5.1		63.1 ± 4.5		0.87 ± 0.10		14.8 ± 2.9	11

Table IV. Continued

Region	$\bar{x} \pm s^b$	N[c]	Cu $\bar{x} \pm s$	N	Zn $\bar{x} \pm s$	N	Se $\bar{x} \pm s$	N	Rb
Gray matter									
38	25.4 ± 5.1		67.2 ± 9.1		0.92 ± 0.21		16.1 ± 4.2	11	
44	33.5 ± 6.3		69.2 ± 3.8		0.99 ± 0.11		13.5 ± 2.5	11	
45	35.2 ± 6.1		70.9 ± 3.2		1.01 ± 0.13		14.6 ± 2.3	11	
46	31.1 ± 5.4		66.7 ± 4.1		0.96 ± 0.11		14.2 ± 2.2	11	
Mixed matter									
15	22.0 ± 6.2		56 ± 11		0.79 ± 0.24		14.1 ± 2.3	11	
17	20.1 ± 4.2	11	85 ± 19	11	0.83 ± 0.14	11	16.9 ± 3.1	10	
12	22.0 ± 4.2		83.1 ± 5.6		0.92 ± 0.10		15.8 ± 3.0	11	
6	31.3 ± 3.8		73.3 ± 4.0		1.03 ± 0.16		18.4 ± 2.9	11	
7	33.9 ± 5.2		67.9 ± 7.5		1.07 ± 0.23		18.7 ± 2.9	11	
8	30.6 ± 5.4		53 ± 11		0.89 ± 0.20		15.7 ± 3.2	11	
28	19.8 ± 4.5	11	52.8 ± 4.4	11	0.83 ± 0.14	11	15.4 ± 2.8	10	
29	18.6 ± 4.1		48.7 ± 8.3		0.80 ± 0.13		14.5 ± 2.4	11	
30	18.6 ± 4.3		44.1 ± 7.8		0.73 ± 0.16		14.0 ± 2.1	11	
31	17.3 ± 4.9		43.1 ± 7.2		0.73 ± 0.11		14.5 ± 2.6	11	
32	18.5 ± 2.9		53.9 ± 9.3		0.87 ± 0.13		15.2 ± 2.5	11	
19	21.2 ± 6.7		56.5 ± 5.8		0.87 ± 0.19		16.4 ± 3.4	11	
22	68 ± 32		61 ± 14		0.98 ± 0.24		14.1 ± 2.6	11	
27	27.2 ± 7.9	8	38 ± 13	8	0.59 ± 0.12	8	12.8 ± 2.8	7	
20	19.3 ± 3.9	11	52.0 ± 3.8	11	0.85 ± 0.12	11	12.7 ± 2.2	10	
13	23.1 ± 7.6	10	55 ± 13	10	0.88 ± 0.17	10	15.4 ± 3.7	9	
33	32 ± 13	11	63 ± 11	11	0.88 ± 0.13	11	15.6 ± 2.0	9	
34	29.0 ± 5.3	11	57.0 ± 7.3	11	0.78 ± 0.15	11	13.1 ± 2.2	9	
35	30.5 ± 5.5	11	57.1 ± 9.3	11	0.83 ± 0.28	11	13.6 ± 3.2	9	
40	29 ± 12		36.7 ± 4.8		0.56 ± 0.13		11.1 ± 1.7	11	
41	15.8 ± 3.4	11	44.2 ± 4.0	11	0.62 ± 0.12	11	9.7 ± 1.6	10	
43	18.6 ± 6.5	11	43.6 ± 7.8	11	0.619 ± 0.094	11	10.4 ± 2.0	10	
47	36.6 ± 8.2		42.2 ± 7.6		0.56 ± 0.13		8.9 ± 1.7	11	
39	6.6 ± 1.6		29.4 ± 1.9		0.525 ± 0.089		9.5 ± 1.8	11	
White matter									
21	17 ± 12	4	41 ± 14	4	0.59 ± 0.21	4	11.1 ± 2.2	3	
11	3.8 ± 1.4	10	27.6 ± 2.8	10	0.46 ± 0.11	10	8.6 ± 1.4	9	
3	10.2 ± 2.7		25.7 ± 3.8		0.39 ± 0.11		9.4 ± 1.4	11	
5	8.2 ± 3.2		42 ± 12		0.56 ± 0.11		13.7 ± 5.0	11	
14	7.0 ± 2.8		31.3 ± 7.0		0.48 ± 0.11		11.6 ± 2.4	11	
25	12.2 ± 2.6		25.2 ± 5.8		0.42 ± 0.12		11.1 ± 2.4	11	
37	10.6 ± 2.6		24.6 ± 2.6		0.413 ± 0.093		9.7 ± 1.7	11	
26	9.8 ± 2.7	11	24.7 ± 3.8	11	0.459 ± 0.095	11	9.3 ± 1.6	10	
42	7.8 ± 7.3		31 ± 12		0.48 ± 0.13		7.8 ± 1.6	11	
48	19.2 ± 5.8		30.0 ± 6.8		0.49 ± 0.19		9.6 ± 3.0	11	
Other brain areas									
16	8.8 ± 2.2		83 ± 31		1.11 ± 0.29		15.6 ± 3.5	11	
36	16.1 ± 9.1	9	160 ± 100	9	0.68 ± 0.53	9	14.7 ± 5.7	8	
50	5.1 ± 2.0	9	84 ± 22	9	0.643 ± 0.088	9	10.6 ± 2.4	8	
51	8.5 ± 3.2	8	55 ± 20	9	0.98 ± 0.35	9	12.2 ± 2.6	8	

[a]Concentrations are given in μg/g dry weight.
[b]Average and standard deviation, based on N individual results.
[c]Number of individual results, N = 12 unless indicated.

reduction factor are intermediate between the gray and white matter values, and they tend to decrease with decreasing neuron density. Mn, Fe, and Cu, on the other hand, show a clearly different picture, and have the highest concentration in regions with mixed composition.

In order to assess the overall elemental variability over the 12 brains, the percentage standard deviations of all 50 regions were averaged for each element. Ca (with a percentage standard deviation of 40.3%) shows the highest variability over the 12 brains. The variability is about 10% smaller for Fe (31.8%) and Cu (28.1%), while Se (21.6%), Mn (20.4%), Rb (19.3%), Zn (17.6%) and K (14.4%) exhibit the lowest variability. As could be expected, the plexus choroideus (no. 16) and the cor-

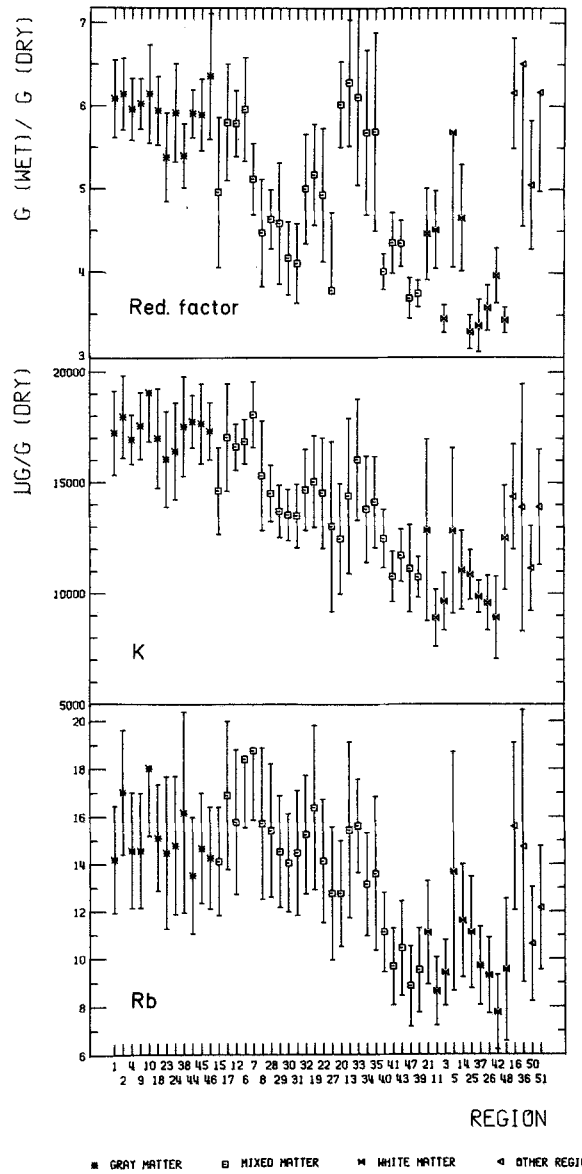

Fig. 2. Wet-to-dry weight ratio (reduction factor), and K and Rb concentration of 50 brain regions. Data points and error bars indicate averages and standard deviations, usually based on 12 individuals (A-L).

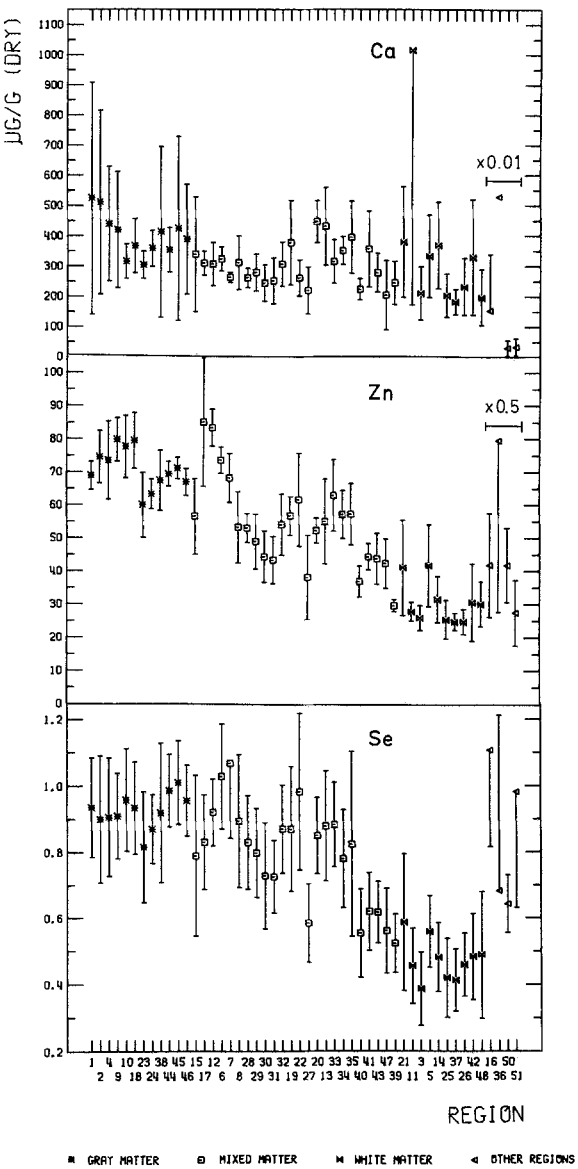

Fig. 3. Concentrations of Ca, Zn, and Se in 50 brain regions. Data points and error bars indicate averages and standard deviations, usually based on 12 individuals (A-L). The Ca and Zn concentrations for regions nos. 16, 36, 50, and 51 were multiplied by 0.01 and 0.5, respectively.

pus pineale (no. 36) exhibit a much higher variability for various elements than the other brain areas, due to their frequent degenerative changes (13, 14).

Mean Elemental Concentrations in Whole Brain, Cerebral Cortex, Cerebral White Matter, Basal Ganglia, Brainstem, and Cerebellum. In the open literature elemental concentrations have often been reported for the whole brain, and for large parts of the brain, such as the cerebral cortex, cerebral white matter, basal ganglia, brainstem, and cerebellum. In order to be able to compare our results with these values, mean concentration data were calculated based on the elemental concentrations for the various analyzed brain areas, and the weight fractions of each brain region relative to the whole brain. The weight fractions used were based on volume measurements of Schlenska (15), Lange (16), Stephan et al. (17), Stephan (18), and Eggers et al. (19), and on

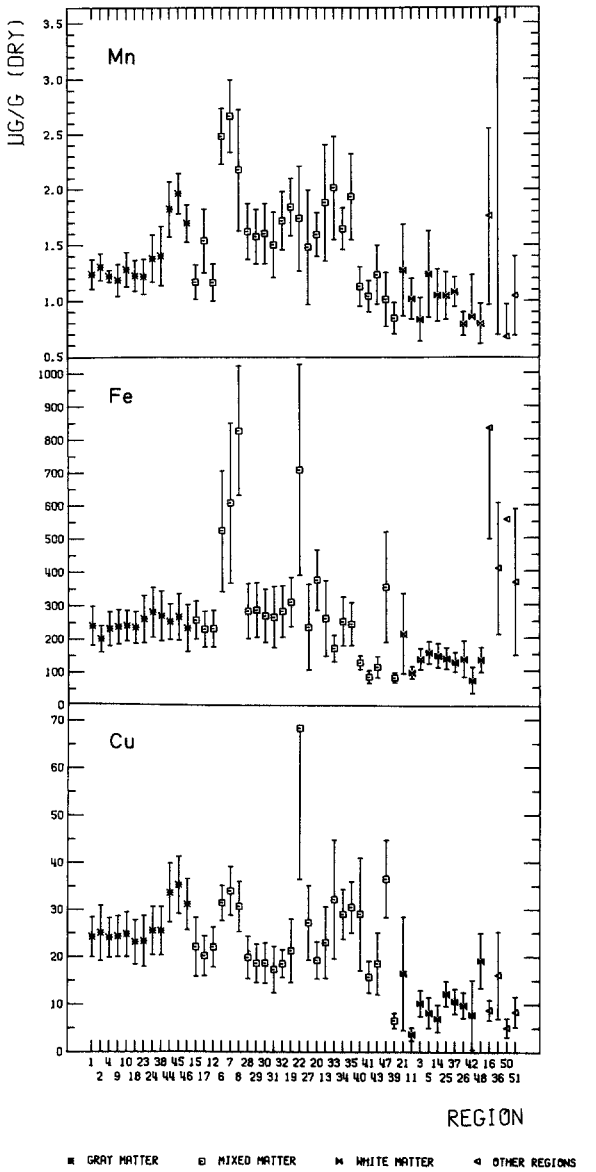

Fig. 4. Concentrations of Mn, Fe, and Cu in 50 brain regions. Data points and error bars indicate averages and standard deviations, usually based on 12 individuals (A-L).

weights reported by Blinkov and Glezer (20). Since the density of the gray and white matter of the brain are close to each other (1.0433 g/cm^3 and 1.0385 g/cm^3, respectively) (21), volume fractions could be used equally well as weight fractions. The average concentrations and standard deviations are listed in Table V. These results are based on 12 different brains, except for Rb for which the data of brain G were excluded.

Similarities Between the Elemental Profiles and the Profile of Wet-to-Dry Weight Ratio (Reduction Factor). Table IV and Figures 2 and 3 indicate that the concentrations of various elements vary in a way rather similar to the wet-to-dry weight ratio (reduction factor). Linear correlation coefficients between the mean elemental concentrations in 46 brain regions (regions nos. 16, 36, 50 and 51 were excluded) and the mean reduction factors were calculated, and the results are listed in Table VI. K, Ca, Zn, Se, and Rb are very significantly correlated with the reduction factor ($P < 0.0005$, one-sided test), so that the concentration variability of these elements over the different brain areas may to a large extent be explained by variations in the fluid content of the brain regions. For these same elements, a linear regression analysis was performed in order to estimate the concentration in the liquid and in the solid phase of the brain. The concentration of metal per unit dry weight was used as dependent variable and the ratio between the weight of the water in the sample and the dry sample weight (reduction factor − 1) as independent variable, so that the intercept equals the concentration in the solid phase and the slope equals the concentration in the liquid phase of the brain. The results of the the regression analysis are given in Table VI.

Similarities Between Brain Regions, Based on Similar Elemental Profiles. The data set of mean concentrations of 8 elements in 46 brain regions (nos. 16, 36, 50, and 51 were excluded) was subjected to a hierarchical cluster analysis. Standardized concentration data were used and the furthest neighbor strategy was selected (22, 23). Figure 5 shows the resulting clustering diagram. The 46 brain regions conglomerate in two main clusters, containing resp. 30 and 16 structures. The first cluster includes all gray matter areas, while the second contains all white matter. It can further be noticed that all gray matter regions, except no. 23, are included in one subcluster which begins with region no. 1 and ends with no. 45. This subcluster is itself built up of two subsubclusters. The first of these contains all gray matter of the cerebrum (nos. 1, 4, 9, 2, 24, 38, 10, 12 and 18), with the exception of structure no. 23, while the other comprises the gray matter of the cerebellum (nos. 44-46).

Changes in Trace Elemental Concentrations with Age. The reduction factors and elemental concentrations of 46 brain regions (nos. 16, 36, 50, and 51 were excluded) were averaged for each brain, and classified according to the age of the brains. Scatter plots of K, Fe and Se versus age are given in Figures 6, 7, and 8. They show that the K concentration decreases with age, while Fe and Se increase. For the other elements and for the reduction factor, no clear variation with the age could be observed.

Table V. Wet-to-Dry Weight Ratio (Reduction Factor) and Elemental Concentrations[a] for the Whole Brain, Cerebral Cortex, Cerebral White Matter, Basal Ganglia, Brainstem and Cerebellum

Element	Whole Brain $\bar{x} \pm s^b$	Cerebral Cortex $\bar{x} \pm s$	Cerebral White Matter $\bar{x} \pm s$	Basal Ganglia $\bar{x} \pm s$	Brainstem $\bar{x} \pm s$	Cerebellum $\bar{x} \pm s$
K	14610 ± 900	17100 ± 980	10500 ± 1100	15730 ± 950	13200 ± 1500	15720 ± 760
Ca	353 ± 69	430 ± 140	270 ± 68	294 ± 25	293 ± 48	325 ± 95
Mn	1.297 ± 0.094	1.252 ± 0.068	1.06 ± 0.13	2.03 ± 0.21	1.53 ± 0.20	1.53 ± 0.15
Fe	224 ± 47	239 ± 54	135 ± 25	440 ± 130	286 ± 87	246 ± 55
Cu	21.7 ± 3.6	24.2 ± 4.3	9.0 ± 1.9	26.0 ± 3.6	32.6 ± 7.6	31.5 ± 4.6
Zn	54.6 ± 2.9	71.3 ± 4.5	27.9 ± 4.0	60.4 ± 3.6	49.6 ± 5.7	58.3 ± 2.6
Se	0.749 ± 0.084	0.90 ± 0.12	0.446 ± 0.073	0.92 ± 0.13	0.759 ± 0.084	0.835 ± 0.082
Rb	13.2 ± 2.0	14.8 ± 2.4	10.7 ± 1.7	16.5 ± 2.4	12.5 ± 2.0	12.5 ± 1.9
Reduction Factor	5.16 ± 0.20	5.92 ± 0.26	3.99 ± 0.37	5.02 ± 0.30	4.88 ± 0.63	5.25 ± 0.27

[a]Concentrations in μg/g dry weight.
[b]Mean and standard deviation, usually based on the results for 12 brains; the Rb concentrations of brain G were excluded, however.

Table VI. Linear Correlation Coefficients Between the Mean Elemental Concentrations and the Mean Reduction Factors; Coefficients of the Linear Regression Between the Mean Elemental Concentrations and the Mean Values of the (Reduction Factor - 1)[a]

Element	Correlation coefficient	Coefficients of the linear regression	
		Intercept ± St. Dev.	Slope ± St. Dev.
K	0.80	4900 ± 1100	2340 ± 270
Ca[b]	0.78	50 ± 34	67.6 ± 8.2
Mn	0.48		
Fe	0.22		
Cu	0.37		
Zn	0.86	−10.0 ± 5.8	15.8 ± 1.4
Se	0.82	0.086 ± 0.073	0.168 ± 0.018
Rb	0.71	5.2 ± 1.3	2.07 ± 0.31

[a]The correlation coefficient calculations and the linear regression analysis were based on 46 brain regions; regions nos. 16, 36, 50, and 51 were excluded.
[b]Region no. 11 was excluded because it showed a much higher average Ca concentration and associated standard deviation than the other brain regions.

DISCUSSION

The analysis of samples from 61 corresponding regions of brain A and K revealed similar concentrations for each of the 8 elements. This close resemblance of the trace element distribution in both sides of the human brain has already been reported by other investigators (5, 24-26). It indicates that the significant concentration differences for the various brain regions are not at all accidental. Moreover, there seems to be a relation between the trace element profile of a brain region and its function. This is suggested by the conglomeration in one subcluster (see Figure 5) of regions involved in the same physiological function (e.g., the subcluster with regions 6-8 and 22), and of morphologically similar regions (e.g., a subcluster with the cerebellar gray matter structures 44-46, and the subcluster comprising the nuclei of the thalamus, nos. 28-32).

The distribution of the 8 measured elements in the 50 different brain regions and some possible relations with the function of these various brain structures, will now be discussed in some detail.

Potassium. Potassium ions are essential for the propagation of the action potential in the neurons (27).

The K concentration is found to be the highest in gray matter, decreases with decreasing neuron density in mixed matter areas, and is the lowest in white matter. This pattern is probably, to a large extent, due to a decrease in total water content, as is suggested by the correlation with the reduction factor. It may even be concluded that the K concentration of the various brain structures is closely linked with their intracellular fluid content, since the intracellular fluid represents about 80% of the total water amount of the brain and has a 10 times higher K concentration than the extracellular fluid (27). The insula (no. 10) exhibits the highest K level, while

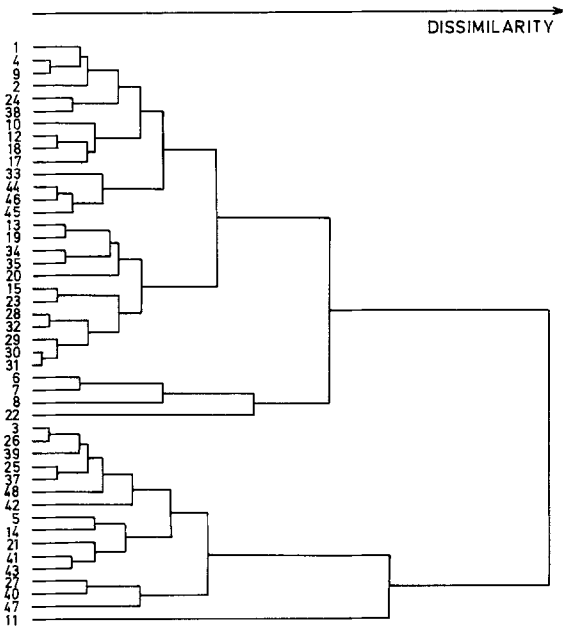

Fig. 5. Dendrogram, obtained by applying a hierarchical cluster analysis on the data matrix with mean concentrations of 8 elements in each of the 46 brain regions of gray, mixed or white matter.

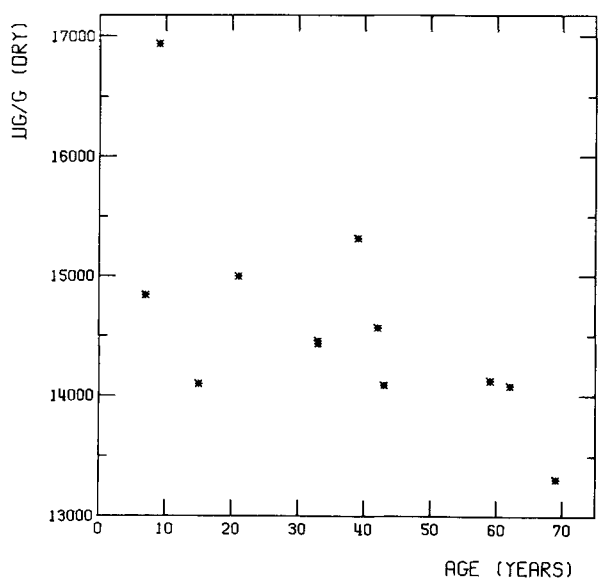

Fig. 6. Scatter plot of the average K concentration in the 12 brains versus age.

the lowest concentrations are found in the chiasma opticum (no. 11) and the pyramid (no. 42). The decrease of the K with age, as suggested by Fig. 6, has also been reported by Markesbery et al. (28).

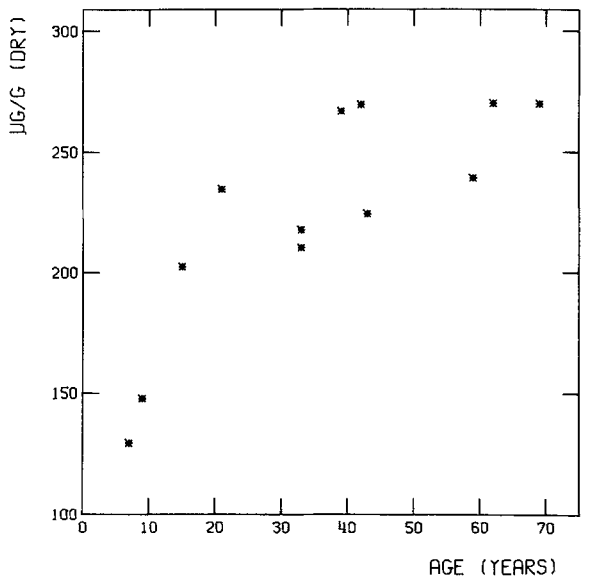

Fig. 7. Scatter plot of the average Fe concentration in the 12 brains versus age.

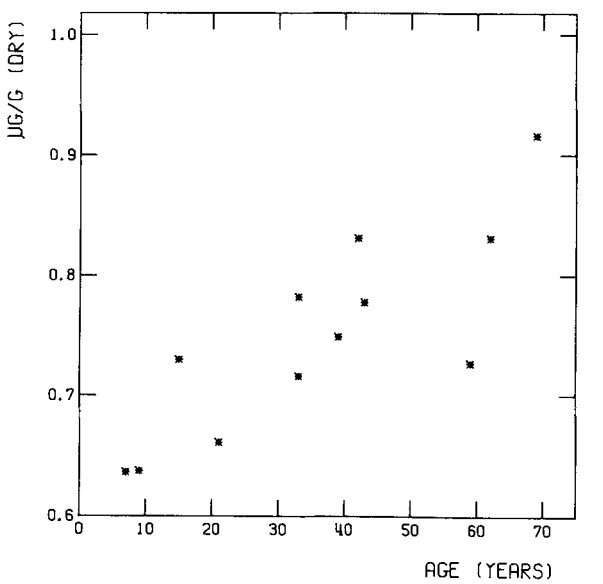

Fig. 8. Scatter plot of the average Se concentration in the 12 brains versus age.

Our K data for the whole brain in Table V are in close agreement with the values of Hamilton et al. (29) and Ehmann et al. (30). Assuming a wet-to-dry weight ratio of 5.16 (see Table V), their wet weight concentrations can be converted to dry weight concentrations of 12900 μg/g and 13300 μg/g, respectively. Hamilton et al. (29) also reported a separate K value for the basal

ganglia, which amounts to 15800 μg/g after performing a similar conversion to dry weight concentration. This value compares well with our data for the same region. Comparable K concentrations have also been published for the cerebral cortex (i.e., of 18680 μg/g) and for the hippocampus (17760 μg/g) (31).

Calcium. Calcium fulfills an important role in the transmission of nerve pulses by triggering the release of neurotransmittors. It furthermore modulates numerous cellular reactions when entering from the extracellular fluid or when being released from intracellular stores (32). In chronic epileptic brain tissue the extracellular Ca^{2+} concentration was found to be reduced (33). Elevated Ca levels could possibly have depressogenic effects (34).

The good correlation between Ca and the reduction factor (see Table VI) indicates that, as for K, the concentration of Ca in the various brain structures is closely related to their fluid content. The Ca concentration in the chiasma opticum (region no. 11) is, however, much higher than in the other white matter areas and varies highly over the different brains. Also, in a number of other brain regions (i.e., nos. 1, 2, 15, 16, 36, 38, 42, 45, 47, 50, and 51), the Ca concentration shows a high standard deviation over the 12 analyzed brains of more than 50%. The elevated Ca levels for the plexus choroideus (no. 16) and the corpus pineale (no. 36) are probably due to the presence of calcareous deposits. The calcification does not seem to be correlated with age (linear correlation coefficients of 0.25 and 0.04, respectively). This is confirmed by Nathan et al. (35), who analyzed 100 human pineal bodies. According to Collard and Collard (36), Krstić (37) and Michotte et al. (38), however, the incidence of pineal gland calcification is proportional to age.

The lowest Ca levels are found in the genu corporis callosi (no. 3), capsula interna (no. 25), radiatio optica (no. 37), nucleus dentatus (no. 47), and corpus medullare cerebelli (no. 48).

The results of Hamilton et al. (29) for the whole brain (290 μg/g, after conversion to dry weight) and the basal ganglia (290 μg/g) compare well with our data. Also, for the cerebral cortex and the hippocampus, similar concentrations are reported by Ward and Mason (31) (382 μg/g and 352 μg/g, respectively). The Ca data of Greiner et al. (25), who measured Mg, Ca, Cu and Zn in different areas of normal human brains, are systematically higher than ours, however.

Manganese. Although manganese is among the least toxic of the trace elements, manganese poisoning frequently occurs among miners, who are chronically exposed to manganese ores (1-3), and it may lead to Parkinsonism and dementia. The element probably interacts with certain catecholamines, as has been suggested by clinical observations, therapies and experiments (2, 3, 39-41).

Manganese intoxication seems to result in a preferential damage of the globus pallidus, hypothalamus, nucleus caudatus and putamen (42). Fig. 4 shows that even for normal human brain, Mn is concentrated in these same regions. Raised Mn levels are also found in the corpus pineale (no. 36), indicating that the element could play a role in the endocrine working of the brain. It can further be noticed that the gray matter regions of the cerebellum (nos. 44-46) contain more Mn than the cerebral gray matter structures (nos. 1, 2, 4, 9, 10, 18, 23, 24 and 38). The genu corporis callosi (no. 3), pedunculus cerebri (no. 26), tractus corticospinalis (no. 39), pyramid (no. 42), and corpus medullare cerebelli (no. 48) exhibit the lowest Mn concentration.

Our Mn data for the whole brain agree with the results of Hamilton et al. (29) (1.0 μg/g, after conversion to dry weight) and Ehmann et al. (30) (1.35 μg/g, after conversion). Larsen et al. (6) and Bonilla et al. (7) determined Mn in various areas of several normal human brains, and obtained concentrations comparable with ours. On the other hand, the results of Yamada et al. (43), who recently determined Mn levels in 21 regions of 4 human brains, are about 80% higher than our values.

Iron. Research on children has revealed an association between iron deficiency and delayed psychomotor development, higher irritability and deficits in attention and alertness (44-48). Yehuda et al. (49) reported a reduced learning capacity in Fe deficient rats. Youdim et al. (50, 51) suggested that the influence of Fe on behavior is possibly due to an association of this element with the dopaminergic pathways of the brain. Neurological damage due to Fe overload, on the other hand, can occur in individuals suffering from the Hallervorden-Spatz syndrome and can also result from hemolysis of red blood cells after head trauma (52).

Raised Fe levels are found in the nucleus caudatus (no. 6), the putamen (no. 7), the globus pallidus (no. 8) and the substantia nigra (no. 22). All these regions are involved in the inhibition or facilitation of movement, suggesting that Fe is, in one way or another, involved in the motoric function of the brain (5). The reduced motor activity in Fe deficient rats (51) seems to support this idea. The Fe concentration is also particularly high in the plexus choroideus (no. 16) and in blood vessels (no. 50). The dorsum pontis (no. 40), nucleus olivaris inferior (no. 41), dorsum medullae (no. 43), tractus corticospinalis (no. 39) and the white matter regions (nos. 11, 3, 5, 14, 25, 37, 26, 42, and 48) show the lowest

Fe concentrations. The increase of brain Fe with age, as suggested by Figure 7, has also been indicated in some other studies (5, 28, 53).

When comparing our Fe concentrations with the results reported by other authors, a fairly good agreement is found with the whole brain data of Hamilton et al. (29) and Ehmann et al. (30) (292 μg/g and 336 μg/g, respectively, after conversion). Höck et al. (5), Völkl et al. (53) and Harrison et al. (54), who analyzed respectively 33, 13 and 10 different regions from various brains, also confirm our results. The Fe data of Henke et al. (55), on the contrary, are for most of the regions they analyzed lower than ours.

Copper. The requirement of copper by the nervous system was discovered in 1937 by Bennetts and Chapman (56), who demonstrated that neonatal ataxia (swayback) of lambs is caused by maternal Cu deficiency. The disorder is characterized by an amyelination of the cerebrum and a progressive demyelination of the spinal cord (57). More recently, the susceptibility of the developing brain to Cu deficiency has also been shown in experiments with other animals, such as guinea pigs (58) and rats (59, 60). It has been proposed that neonatal ataxia is basically due to a deficiency in cytochrome oxidase in the motor neurons (1). Another possibility centers on the influence of Cu on the norepinephrine levels in the brain (52). In humans, Menkes' kinky hair syndrome (a genetic disease, characterized by a failure to absorb Cu) is associated with myelin paucity and neuronal death (57). On the other hand, excess Cu also can result in brain damage. Wilson's disease (a genetic disorder linked with a Cu accumulation, mainly in the liver but also in the brain) involves progressive neurological symptoms (61).

Although the Cu level in the gray matter areas of the brain is fairly constant, Cu is somewhat more concentrated in the cortex of the cerebellum (nos. 45-47) than in the cortex of the cerebrum (nos. 1, 2, 4, 9, 10, 18, 23, 24, and 38). As to the white matter regions, the cerebellum (no. 48) also shows a higher Cu concentration than the cerebrum (nos. 21, 11, 3, 5, 14, 25, 37, 26, and 42). Of the 50 analyzed brain regions, the substantia nigra (no. 22) has by far the highest Cu level. Szerdahelyi and Kása (62), who used a staining procedure to locate Cu in rat brains, reported that in this brain region the element was exclusively present in the glial cells. They suggested that Cu may play an important role in normal physiological functioning of the glial cells, and, via glia-neuron interactions, in neuronal processes. Lowest Cu levels are found in the chiasma opticum (no. 11) and in the blood vessels (no. 50).

Our Cu values for the whole brain and the basal ganglia agree with the results of Hamilton et al. (29)(29 μg/g and 31 μg/g respectively, after conversion). Our data also generally confirm the results of Völkl et al. (53), Harrison et al. (54) and Smeyers-Verbeke et al. (63). Bonilla et al. (8) and Henke et al. (55), on the contrary, found substantially lower Cu levels for most of the structures they analyzed.

Zinc. Zinc is another element known to be essential for the brain. Fetal and/or neonatal Zn deficiency causes impaired growth and maturation of the nervous system of experimental animals, and alters the brain neurotransmittor levels (64). The brain of suckling rats fed Zn deficient diet contains less lipids, myelin and synaptosomes (65). Maternal Zn deficiency also results in inferior learning ability and behavioral abnormalities of the offspring (66, 67). In humans, acute severe Zn deficiency causes neuropsychological impairment (68). Lower than normal plasma Zn levels have been measured in alcoholics, epileptics, and in certain types of schizophrenia and dementia (69). The importance of Zn for the brain development is probably related to its effect on DNA synthesis, chromatin structure and cell division (57). It is also required for effective digestion, absorption, and utilization of other nutrients and it is a cofactor in numerous enzymes (57).

Zn is mainly associated with the aqueous phase of brain tissue (see Table VI). The highest Zn levels are found in the corpus amygdaloideum (no. 12), plexus choroideus (no. 16), uncus hippocampi (no. 17), corpus pineale (no. 36) and in blood vessels (no. 50). The mean concentrations are lowest in the genu corporis callosi (no. 3), chiasma opticum (no. 11), capsula interna (no. 25), pedunculus cerebri (no. 26), and radiatio optica (no. 37). A close look at the individual Zn and Ca data for the plexus choroideus and the corpus pineale indicates that both elements are highly correlated (linear correlation coefficients for the 2 structures of 0.91 and 1.00, respectively), suggesting that calcification is attended with Zn enrichment. This correlation has also been reported by Nathan et al. (35) for the corpus pineale. Smeyers-Verbeke et al. (70) and Michotte et al. (38) suggested that in the lattice of calcifications Zn can take the place of Ca due to a surface ion exchange. The high Zn concentration in the hippocampal formation has already been noticed by several other authors (5, 71, 72). The importance of the element for the working of this structure, believed essential for working memory, has been proved in a number of experiments (69, 73, 74).

Hamilton et al. (29) and Ehmann et al. (30) reported Zn concentrations for the whole brain of resp. 64.5 μg/g and 68.7 μg/g (after conversion). These values are in good agreement with our results. The data of Harrison et al. (54), Smeyers-Verbeke et al. (63), Völkl et al.

(53), Greiner et al. (25) and Höck et al. (5) are also comparable with ours for the majority of the brain regions they analyzed. Henke et al. (55) generally confirm our Zn concentrations. However, their results for the hippocampus, the nucleus caudatus and the corpora mamillaria are about twice our values.

Selenium. Selenium is an essential constituent of glutathione peroxidase, a protein believed to play a role in the protection of the cells against lipid peroxidation (1). However, DeMarchena et al. (75) found in the brain of a number of animals only a very low glutathione peroxidase activity. They concluded that brain tissue does not contain enough glutathione peroxidase activity to protect it against peroxidase damage. However, it is possible that these authors have underestimated the glutathione peroxidase activity (76). A second biological role of selenium is its protective action against toxicity from heavy metals (76).

When converted to wet weight, the Se level remains fairly constant throughout the whole brain. This has also been observed by Larsen et al. (6). However, the relatively high Se concentrations in the putamen (no. 7), globus pallidus (no. 8) and substantia nigra (no. 22) cannot entirely be explained by a high water content of these brain regions. Even when converted to wet weight, the Se levels in the genu corporis callosi (no. 3), septum pellucidum (no. 5), chiasma opticum (no. 11), and corpus fornicis (no. 14) are lower than for the other brain structures. As Se is a constituent of glutathione peroxidase, the Se distribution possibly parallels the activity of this enzyme. Brannan et al. (77) measured the glutathione peroxidase activity in 10 different regions of the rat brain. They found the highest activity in the caudate-putamen and substantia nigra. Cortical areas and several nuclear areas had somewhat lower activity, and it was the lowest in the corpus callosum. When comparing our wet weight Se concentrations with these activity values, a good correlation between both data was noticed (correlation coefficient of 0.76; $P < 0.05$, one-sided test).

Figure 8 suggests that the Se content of the human brain increases with increasing age. This finding contrasts with that of Markesbery et al. (28), who reported that the Se level in the human brain remains relatively steady throughout adult life. However, these authors did not analyze brains in the 2-19 years age range.

Our Se results are comparable with those of Ehmann et al. (30) for the whole brain (0.965 μg/g, after conversion to dry weight) and with the data of Ward and Mason (31) for the cerebral cortex (0.997 μg/g). Also, the Se concentrations of Larsen et al. (6) and Höck et al. (5) are very similar to ours. Hamilton et al. (29), on the contrary, reported lower Se levels for the whole brain and the basal ganglia (0.46 μg/g and 0.25 μg/g respectively, after conversion).

Rubidium. Although rubidium is not considered to be an essential trace element, it has received some attention in biochemical and clinical research due to its close relationship to K (1, 4). In psychiatry, it has been experimentally used as antidepressant, and its effects in schizophrenia have been studied (4).

The relation between Rb and K in the brain is also reflected by their similar distribution among the various brain areas, and their linear correlation coefficient of 0.88. Highest Rb concentrations are found in the nucleus caudatus (no. 6) and putamen (no. 7). Lowest values are present in the chiasma opticum (no. 11), the pyramid (no. 42), and the nucleus dentatus (no. 47).

Our whole brain Rb concentrations confirm the results of Ehmann et al. (30) (11.8 μg/g, after conversion). Hamilton et al. (29), however, reported Rb data for the whole brain and for the basal ganglia that are about 70% higher than ours, while the Rb value for the cerebral cortex of Ward and Mason (31) amounts to only 8.10 μg/g. Also, Henke et al. (55) reported Rb concentrations for various brain regions that are some 40% lower than our data for the same structures. Höck et al. (5), on the contrary, usually confirm our data. For the corpus pineale (no. 36), however, these authors reported a considerably higher Rb level of 97.5 μg/g. Our Rb value for this structure is more comparable to the Rb concentration of 12.3 μg/g obtained by Nathan et al. (35).

CONCLUSIONS

This study shows that the 8 elements measured are heterogeneously distributed in the human brain, and that their concentration profile is not at all accidental. For most elements (i.e. K, Ca, Zn, Se and Rb), the variation in concentration in the various brain areas can, to a large extent, be attributed to differences in water content. However, it is obvious that for the 8 elements numerous brain structures exhibit concentrations that are not expected on the basis of their water content alone. High trace element levels in some brain areas can sometimes be related to the function of these structures. It can further be noticed that brain regions with a similar function often exhibit a similar trace element profile. The elemental concentration does not always remain constant during life. Some elements like Fe and Se seem to exhibit an increase in the brain with age, while K seems to decrease.

It is our intention to extend our research on the trace elemental levels in the human brain to pathological cases,

and to use the data presented in this paper as reference values.

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