

## Environmental Lead-210 and Bismuth-210 Accrue Selectively in the Brain Proteins in Alzheimer Disease and Brain Lipids in Parkinson Disease

\*B. Momčilović, †H. A. Alkhatib, ‡J. A. Duerre, §M. Cooley, ¶W. M. Long, \*\*T. R. Harris, and ††G. I. Lykken

*\*Institute for Medical Research and Occupational Health, Zagreb, Croatia; Departments of †Physics, ‡Microbiology, §Pathology, \*\*Mathematics, and ††Physics, University of North Dakota, and ¶Obi Laboratory, Grand Forks, North Dakota, U.S.A.*

**Summary.** We studied the occurrence of the environmental radon daughters,  $^{210}\text{Po}$  (alpha particles), and  $^{210}\text{Bi}$  (beta particles), in the protein and lipid fractions of cortical gray and subcortical white matter from the frontal and temporal lobes of human brains of persons with Alzheimer disease (AD), persons with Parkinson disease (PD), smokers, or persons with no previous evidence of clinical neurologic disease (controls). We found a 10-fold increase in  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  radioactivity in the protein fraction from both the cortical gray and subcortical white matter in AD and smokers, and a similar increase in the lipid fraction in PD. The pathognomonic distribution of the radon daughters to the lipids in PD and to the proteins in AD was inferred to reflect the increase of local chlorine availability to which radon daughters bound selectively. Cigarette smoking strongly increases radon daughter retention in the central nervous system. **Key Words:** Environmental radon daughters—Parkinson disease—Cigarette smoking—Brain proteins and lipids—Radiochemical injury.

Radon ( $^{222}\text{Rn}$ ) is a highly lipid-soluble radioactive noble gas that is ubiquitous in the human environment (Eichholz, 1987). When inhaled, environmental radon has a preference to accumulate in lipid tissue throughout the body with the highest concentration in the brain, bone marrow, and nervous system (Scully, 1934; Nussbaum 1957). Pohl and Pohl-Ruling (1967) found that one third of the inhaled radon decay products passed from the lungs into the bloodstream indicating that radon gas does not flow quickly in and out of the lungs but lingers in the body. Indeed, Lykken et al. (1983, 1990) have shown that inhaled radon was rapidly absorbed from the lung

into the body, where it accumulates in the cranium resulting in increased bismuth-214 ( $^{214}\text{Bi}$ ) gamma ray emissions and altered electroencephalographic signals.

Radon is chemically inert; however, it is radioactive and decays through a distinct series of radioactive daughters before it ends up as a stable lead ( $^{206}\text{Pb}$ ) (Table 1) (Hopke, 1987). When decaying, radon and its daughters release gamma radiation or high-energy alpha and beta particles strong enough to cause radiation cell injury (Scott, 1995). At the same time the alpha particle is emitted from the decaying radon and the radon daughter is born, a concomitant chemical transmutation also takes place. Indeed, all the radon daughters, including the biologically most relevant lead-210 ( $^{210}\text{Pb}$ ), bismuth-210 ( $^{210}\text{Bi}$ ), and polonium ( $^{210}\text{Po}$ ), down to the stable lead, are heavy metals that are highly neurotrophic and neurotoxic (Kostial et al., 1991). In contrast to mother radon, which is a lipid-soluble gas that can freely move into and

Received July 26, 1999. Revised April 28, 2000. Accepted May 1, 2000.

Address correspondence and reprint requests to Dr. Berislav Momčilović (c/o Prof. G. I. Lykken), Department of Physics, P.O. Box 7129, University of North Dakota, Grand Forks, ND 58202-7129, U.S.A.

**TABLE 1.** Major decay branches of <sup>222</sup>Rn decay series and their important characteristics

Radionuclide	Half-life	Major radiation emissions (MeV)		
		Alpha	Beta	Gamma
<sup>222</sup> Rn	3.823 days	5.49		
<sup>218</sup> Po	3.05 min	6.00		
<sup>214</sup> Pb	26.8 min		0.670 0.730 1.02	0.295 0.352
<sup>214</sup> Bi	19.7 min		1.0 1.51	0.609 1.12
<sup>214</sup> Po	164 μs	7.69		
<sup>210</sup> Pb	22.3 yr		0.015 0.061	0.047
<sup>210</sup> Bi	5.01 days		1.161	
<sup>210</sup> Po	138.4 days	5.305		
<sup>206</sup> Pb	Stable			

According to Cothorn CR, Smith JE Jr. Radioactive decay. In: Environmental radon. New York: Plenum Press, 1987:307-315.

out of the brain despite the blood-brain barrier, none of the transmuted heavy metal radon daughters are lipid soluble. Hence, they remain trapped in the brain where they emit additional gamma radiation and alpha and beta particles over their lifetime and thus add chemical injury to the radiation injury of the brain.

The object of this study was to analyze the pattern of distribution of naturally occurring environmental radon daughter <sup>210</sup>Po, a strong alpha particle emitter (5.3 MeV), and moderately strong <sup>210</sup>Bi beta particle emission (1.2 MeV) in the protein and lipid fractions of the cortical gray and subcortical white matter from the frontal and temporal lobes of Alzheimer disease (AD) and Parkinson disease (PD) patients and subjects who showed no signs of neurologic disease throughout their life span (controls). Because the concentration of radon daughters is high in cigarette smoke (Martell, 1987), we assigned the brains of cigarette smokers without a neurologic disease to a separate category.

**METHODS**

**Brain Samples**

Brain samples from human subjects with clinical and pathologic diagnosis of AD or PD, from smokers, and from nonsmoking control subjects with no known neurologic disease were kindly provided by the Alzheimer's Research and Treatment Center, St. Paul, MN, U.S.A. All the subjects with AD and PD were nonsmokers. The pathologic diagnosis of AD was based on the presence of an age-adjusted moderate to severe number of plaques in the neurocortex (Mirra et al., 1991). We received samples from the frontal and temporal lobes from the brains from 29 subjects—11 with AD, 6 with PD, 4

smokers, and 8 controls. Age and gender distributions were similar between the groups except for the smokers, who appear to be slightly younger (Table 2). In total, we analyzed 45 brain lobe samples, 25 from the frontal lobe and 20 from the temporal lobe, from these 29 subjects. In each group there were 4 subjects from whom we had both frontal and temporal lobe available for the analysis (see Table 2). The brain slices, which weighed approximately 80 g, had been frozen in liquid nitrogen and stored in the frozen state.

We separated cortical gray and subcortical white matter from each frontal and temporal lobe. One gram of each sample was fractionated into protein and lipid content before we assessed <sup>210</sup>Bi and <sup>210</sup>Po activity. Subcortical white matter was sampled 0.5 cm orthogonally below the sampled cortical gray matter area. Three repli-

**TABLE 2.** Protein content of the brain tissue

#	A/G	Brain lobe protein (mg/g brain tissue)					
		Temporal			Frontal		
		Gray	White	W/G	Gray	White	W/G
<b>Control</b>							
1	52M	148.3	70.7	.477	159.6	93.7	.587
2	53M	150.6	72.3	.480	181.4	74.4	.410
3	60M	105.3	97.3	.924			
4	70F	149.7	71.2	.476	170.5	89.8	.527
5	70M	150.9	69.8	.462			
6	76F	152.1	71.4	.469	138.5	86.5	.624
7	79M	217.4	71.4	.328			
8	88M	233.8	123.2	.527			
<b>Alzheimer disease</b>							
9	62F	126.8	42.7	.337			
10	66M	128.1	63.2	.493			
11	71F	189.7	68.8	.363	172.1	94.4	.548
12	73M	206.0	54.4	.264			
13	80F	213.5	48.4	.227			
14	82M	158.8	70.1	.441			
15	82F	160.4	78.4	.489	191.7	49.0	.256
16	84F	89.9	44.7	.497	126.2	59.8	.474
17	85M	233.8	102.2	.437			
18	86F	100.4	51.2	.510	108.4	53.4	.493
19	93F	120.6	59.1	.490			
<b>Parkinson disease</b>							
20	68M	148.4	107.8	.726	158.7	73.5	.463
21	71M	199.6	56.6	.283	224.0	60.9	.272
22	73F	181.4	81.4	.449			
23	77F	147.8	75.7	.512			
24	80M	366.6	138.9	.379	224.0	155.8	.695
25	81F	204.0	107.6	.527	165.2	56.4	.341
<b>Cigarette smokers</b>							
26	47F	171.7	68.7	.400	171.9	81.9	.476
27	68M	101.3	49.3	.487	131.6	68.8	.524
28	72F	102.6	54.4	.530	141.3	72.4	.512
29	79M	99.5	43.7	.439	123.7	64.7	.523

Subjects are arranged according to their age at death.

A/G, age and gender; M, male; F, female; G, cortical gray brain matter; W, subcortical white brain matter; W/G, ratio between the white and gray matter protein fractions.

cates of each lipid and protein fraction were prepared from gray and white matter from every brain lobe. All the chemicals used in this study were reagent grade and supplied by Fisher Scientific, Ithaca, IL, U.S.A., if not specified. All water used was distilled.

#### Trichloroacetic Acid-Soluble Fraction

Gray and white cortical matter were separated (A. L. Politoff, personal communication). One gram of either gray or white matter was homogenized at 4°C in a Potter-Elvehjem glass-Teflon tissue homogenizer with 6 mL of distilled water and 0.5 mL of 100% trichloroacetic acid. After centrifugation at 5000 rpm (CRU-5000 Centrifuge, Hamden Heights, MA, U.S.A.) for 10 minutes, the supernatant was poured off and saved in a 20-mL plastic tube. After vigorous mixing of the precipitate in 2 mL of water, the extraction procedure was repeated. The supernatant fluids were pooled and labeled as trichloroacetic acid-soluble fraction. This fraction contains primarily nucleotide and other low-molecular-weight compounds. They were found to be essentially free of all radon daughters.

#### Extraction of Lipids

Lipids were extracted from the trichloroacetic acid precipitate according to the method of Folch et al. (1957) by using chloroform:methanol (2:1 vol/vol). The precipitate was mixed with 3 mL methanol for 1 minute and allowed to stand 10 minutes, then 6 mL chloroform was added. After centrifugation as discussed above, the supernatant was filtered through sintered glass (medium porosity). The precipitate was rinsed with 4 mL of Folch reagent and the filtrates combined. Biphasic separation was achieved with the addition of 1 mL potassium acetate. The upper aqueous layer was removed and discarded. The organic phase was taken to dryness under a stream of nitrogen at room temperature. The total lipids were dissolved in 1 mL chloroform. Any residual proteins were removed by filtration through glass wool and the lipid fraction divided into two equal portions, one for alpha spectroscopy and other for beta scintillation spectrometry. All calculations were based on amount of total lipid extracted from 1 gram of tissue.

#### Protein Fraction

The protein precipitate that remained on the sintered glass was dissolved in 1 mL 0.1 M NaOH at 75°C overnight (Bradford, 1976). The protein fraction was divided into three equal portions, one to assess the amount of protein and one each for alpha and beta activity analyzes. The amount of protein was quantitatively assessed using

Bradford's reagent (Bio-Rad Laboratories, Hercules, CA, U.S.A.). After addition of reagent, absorbency was measured employing a Beckman spectrophotometer at 760 nm (Beckman Instruments Co., Fullerton, CA, U.S.A.). Bovine albumin (Bio-Rad Laboratories) served as a standard.

#### Low-Level Alpha Particle Counting

Alpha particle measurements were performed with an Alpha Spectrometer System (EG&G ORTEC, Oak Ridge, TN, U.S.A.) with a radionuclide library software package from the same manufacturer. This computer-controlled system consists of five separate surface barrier alpha spectrometers with alpha resolution of 6 keV, which allows for accurate discrimination of  $^{208}\text{Po}$ ,  $^{209}\text{Po}$ , and  $^{210}\text{Po}$  isotopes. Every week a 48-hour naturally occurring background was measured at the photo peak value for every detector and isotope— $^{208}\text{Po}$  (5.115 MeV),  $^{209}\text{Po}$  (4.880 MeV), and  $^{210}\text{Po}$  (5.305 MeV) (Weast and Astle, 1981–1982).

#### Silver Disk Sample Spiking and Plating

We essentially followed the method of P. O. Jackson, which was developed at Battelle Pacific Northwest Laboratories (Laul et al., 1985). Polonium has a high selective affinity for silver. Hence, 1-inch silver disks (HH Lucas-Milhaupt, Inc, Cudahy, WI, U.S.A.) were painted on one side with black Krylon spray paint (Home of Economy, Grand Forks, ND, U.S.A.); the other side was polished, rinsed, and placed in the plating apparatus described below.

To determine recovery (percentage) the samples were "spiked" with a standard stock solution of  $^{208}\text{Po}$  of known activity (0.0434  $\mu\text{Ci/ml}$ ; Isotope Products Laboratories, Burbank, CA, U.S.A.). The standard stock solution was diluted with 0.4 N HCl so that each sample was spiked with  $^{208}\text{Po}$ , yielding one disintegration per minute (dpm) per 10  $\mu\text{L}$ . The error of pipetting was  $\pm 2\%$ . The background for each polonium peak of any of the silver disks did not exceed two counts per 48 hour-period.

To destroy organic matter, the spiked samples were placed in a 250-mL beaker and digested in 10 mL of concentrated nitric acid ( $\text{HNO}_3$ ). After heating at 85°C for 30 minutes, 10 mL of 30% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and 3 mL of concentrated  $\text{HNO}_3$  were added and the solution heated to dryness (30 minutes). The procedure was repeated three times before 5 mL of 12 M HCl and 5 mL of distilled  $\text{H}_2\text{O}$  were added to the dry sample to form polonium chloride. The solution was heated at 85°C until dry and dissolved in 120 mL of distilled water

containing 10 mg of ascorbic acid. To enhance plating of the polonium on the silver discs, the pH was less than 2.

To "plate," the silver disk was placed in a Teflon holder, which was immersed into the solution of the spiked sample from either gray or white brain matter. The beaker was placed on a stirring hot plate at 85°C and the solution stirred at 2.5 Hz. The  $^{208}\text{Po}$  and  $^{209}\text{Po}$  from the spike and unknown  $^{210}\text{Po}$  from the sample were allowed to plate out over a 6- to 8-hour period, after which time the disk was removed, rinsed with distilled water and counted for alpha activity for 48 hours.

Separation of  $^{210}\text{Po}$  from  $^{210}\text{Bi}$  and  $^{210}\text{Pb}$ . We essentially followed the method of Orlandini et al., which was developed at the Argonne National Laboratory (Eckerman and Rundo, 1973; Gaffney et al., 1994). The lipid or protein fractions were passed through a polymembrane under a negative pressure gradient (6 mL/min). These membranes are a special system for absorption of  $^{210}\text{Bi}$  and  $^{210}\text{Po}$ , consisting of two negatively charged ion exchange membranes (Gelman Sciences, Ann Arbor, MI, U.S.A.).  $^{210}\text{Bi}$  and  $^{210}\text{Po}$  readily form oxichlorides, which bind to the membrane, whereas  $^{210}\text{Pb}$  is selectively filtered. The membranes were dried prior to alpha particle analysis or rinsed with 0.4N HCl and placed in a 20-mL vial for the assessment of beta emission.

#### Alpha Radioactivity

The standard  $^{208}\text{Po}$  and sample  $^{210}\text{Po}$  counts under the peaks were summed separately. The background counts were subtracted from the sample counts to obtain the net counts for each isotope.  $^{210}\text{Po}$  and  $^{208}\text{Po}$  net counts were corrected for decay, and  $^{210}\text{Po}$  counts were extrapolated to the time of the patient's death. The final activity was expressed as  $^{210}\text{Po}$  dpm per gram of brain tissue. Two replicates of each brain sample were prepared for alpha counting.

#### Correction for $^{210}\text{Pb}$ and $^{210}\text{Bi}$ Contribution to $^{210}\text{Po}$

$^{210}\text{Pb}$  decays to  $^{210}\text{Bi}$ , which in turn decays to  $^{210}\text{Po}$ ; each decays at a different rate (see Table 1). After 600 days a "secular equilibrium" is reached, meaning that the activities of the  $^{210}\text{Bi}$  and the  $^{210}\text{Po}$  in the sample are equal to the  $^{210}\text{Pb}$  activity. Therefore, from the time of brain sampling after death to the time of  $^{210}\text{Po}$  plating on Ag disks,  $^{210}\text{Po}$  formed in the brain from  $^{210}\text{Bi}$  directly and from  $^{210}\text{Pb}$  indirectly via  $^{210}\text{Bi}$ . The standard Bateman differential equation of growth and decay of radionuclides in a decay chain was used to correct for this contribution (Bateman, 1910; Cothorn and Smith, 1987).

#### Blanks and Recovery

Blanks were prepared separately by spiking silver disks with 10  $\mu\text{L}$  of  $^{208}\text{Po}$  and measuring them in the same way as brain samples. The  $^{208}\text{Po}$  recovery after plating was  $85 \pm 3\%$ . The dpm for all the brain samples fell within  $\pm 2$  SD.

#### Low-Level Beta Liquid Scintillation Counting

Ten milliliters of liquid scintillation cocktail solution was added to the sample and the activity measured in a Beckman scintillation spectrometer. As previously outlined, (vide supra)  $^{210}\text{Bi}$  beta activity in the sample is an excellent measure of the  $^{210}\text{Pb}$  activity, since by several years after the deaths of the subjects, the  $^{210}\text{Pb}$ ,  $^{210}\text{Bi}$ , and  $^{210}\text{Po}$  in each brain were in secular radioactive equilibrium. Therefore, the calculations for a three-component chain decay were done in the same way as for the alpha particle counting.

#### Blanks and Recovery

The efficiency of binding of  $^{210}\text{Po}$  and  $^{210}\text{Bi}$  to the membrane was assessed with a  $^{210}\text{Pb}$  standard (Battelle Pacific Northwest Laboratories, Richland, WA, U.S.A.) and was found to be 68% and 99%, respectively. The technique is similar to that of measuring the activity of cesium in sea water (Mann and Casso, 1984) and gave the same values as those reported by Argonne National Laboratory (Gaffney et al., 1994).

#### Statistical Analysis

Measurements of alpha and beta activities were expressed as the dpm per gram of brain tissue. This allows for direct comparison of instruments with different counting efficiency and hence for direct comparison of two different methods of analysis—alpha and beta counting. In other words, beta ( $^{210}\text{Bi}$ ) and alpha ( $^{210}\text{Po}$ ) dpm served as standards for each other. All samples were counted until the number of counts above background exceeded 400. This satisfies the accuracy criterion that the standard error (the square root of the number of counts in this situation) not exceed 5% of the measurement (Lykken, 1983). All data samples met the appropriate assumptions of equal variance and normal population distribution; therefore, parametric statistical analysis were undertaken.

Statistical analysis searched for possible dependence of radioactivity level on method of measurement (alpha or beta radiation), brain fraction (lipid or protein), brain lobe (frontal or temporal), brain matter (gray or white), and subject type (AD, PD, cigarette smokers, or control), as well as interactions of these effects. We used multi-

variate analysis of variance (MANOVA) with subject type as a between-subjects effect (factor) and all other effects as within-subject effects (contrasts of the response variables). Radioactivity level was log-transformed to approximate normality. Figures show untransformed data for better visual clarity. Pairwise comparisons were made using Fisher's protected t procedure with the significance set at  $p < 0.05$  (Snedecor and Cochran, 1980). All analyses were run on SAS, version 6.11 (SAS Institute Inc., Cary, NC, U.S.A.).

### RESULTS

The amount of protein in the frontal and temporal brain lobe ranged from 75.7 to 366.6 mg/g gray matter tissue and 42.7 to 155.8 mg/g white matter tissue. Thus, the amount of protein in the brain gray matter was approximately twice that in the white matter. The average ratio of white to gray matter protein for the 45 analyzed brain samples was (mean  $\pm$  SD)  $0.470 \pm 0.126$  and the coefficient of variation was approximately 25%, a reasonable value considering the biologic variability of the subjects and the limits of the analytical methodology involved.

Two different radioanalytical techniques showed no difference between alpha ( $^{210}\text{Po}$ ) and beta ( $^{210}\text{Bi}$ ) activity. Neither the alpha-beta main effect nor any interactions involving this factor were significant at the  $p < 0.05$  level (Table 3), so alpha and beta measurements were averaged and a second MANOVA was performed on the remaining factors. In this analysis, the subject type-lobe-gray/white and subject type-gray/white-protein/lipid interactions were significant, indicating nonadditivity of effects on a log scale (Table 4). Therefore, the eight measurements—defined by combinations of lobe, gray or white matter, and protein or lipid—were examined separately as response variables in one-way analysis of variance with subject type as the independent variable. Differences among subject types were highly significant in all eight cases ( $p = 0.001$  to  $0.006$ ). The analysis of these averaged measurements revealed some three-way interactions (see Table 4). Nevertheless, the relationships between subject type, brain fraction, and gray/white matter appear almost identical in the frontal (Fig. 1) and the temporal (Fig. 2) lobes (Table 5). One-way ANOVA revealed that the deposition of  $^{210}\text{Bi}$  and  $^{210}\text{Po}$  in the brain has a distinct, pathology-based distribution in the protein (Fig. 3) and lipid (Fig. 4) fractions. Figures show untransformed data to allow for visual clarity.

The retention of the radionuclides was the lowest in the control brains. In AD brains, in contrast, radioactivity of both radon daughters was about 10 times higher in the

**TABLE 3.** MANOVA comparison between alpha  $^{210}\text{Po}$  and beta  $^{210}\text{Bi}$  specific activities (AB) in the proteins and lipids (PL) from the cortical gray and subcortical white (GW) matter from frontal and temporal brain lobe (FT) in subjects with Alzheimer disease, Parkinson disease, smokers, and controls (CAPS)

Effect	Degrees of freedom	<i>p</i>
AB		NS
AB*FT		NS
AB*GW		NS
AB*PL		NS
AB*CAPS		NS
AB*FT*GW		NS
AB*FT*PL		NS
AB*FT*CAPS		NS
AB*GW*PL		NS
AB*GW*CAPS		NS
AB*PL*CAPS		NS
AB*FT*GW*PL		NS
AB*FT*LP*CAPS		NS
AB*GW*PL*CAPS		NS
AB*FT*GW*PL*CAPS		NS
FT		NS
FT*GW		<0.0001
FT*PL		<0.0002
FT*CAPS		NS
FT*GW*PL		NS
FT*GW*CAPS	3	<0.0001
FT*PL*CAPS	3	<0.005
FT*GW*PL*CAPS	3	NS
GW	1	<0.0017
GW*PL	1	NS
GW*CAPS	3	NS
GW*PL*CAPS	3	<0.0456
PL		<0.0001
PL*CAPS		<0.0001
CAPS		<0.0001

NS, nonsignificant.

protein fraction of both gray and white matter than it was in the control brains. Some of the AD brains also had somewhat increased radioactivity in the lipid fraction, but the increases were much smaller and statistically significant only for the frontal gray and temporal white matter ( $p < 0.05$ ). However, in PD, the radioactivity in the lipid fraction of the cortical gray and subcortical white matter of both the frontal and temporal lobes were approximately an order of magnitude higher than that of controls, whereas increases in radioactivity in the protein fraction were much smaller and significant only for the white matter of the temporal lobe. As in AD, the radioactivity was greatly increased in the protein fraction and somewhat in the lipid fraction of cigarette smokers' brains. Unlike AD, however, cigarette smokers do not show any increase of the activity in the lipid fraction of the brain gray matter (see Table 5).

TABLE 4. MANOVA for combined <sup>210</sup>Po and <sup>210</sup>Bi data

Effect	Degrees of freedom	p
FT		NS
FT*GW		<0.0001
FT*PL		<0.0005
FT*CAPS		NS
FT*GW*PL		NS
FT*GW*CAPS	3	<0.025
FT*PL*CAPS	3	NS
FT*GW*PL*CAPS	3	NS
GW	1	<0.005
GW*PL	1	NS
GW*CAPS	1	NS
GW*PL*CAPS	3	<0.01
PL	1	<0.0001
PL*CAPS		<0.0001
CAPS		<0.0001

NS, nonsignificant; FT, frontal and temporal cerebral lobe; GW, gray and white brain matter; PL, proteins and lipids; CAPS, control subjects, subjects with Alzheimer disease or Parkinson disease, and cigarette smokers.

DISCUSSION

The results of our study show that, indeed: (1) there is measurable radioactivity of radon daughters in the brain; (2) radon daughters accumulate 10 times more in the diseased AD and PD brains and even more in those of smokers than in the control brains; (3) this accumulation is not uniform but shows selective, disease-dependent distribution to the protein fraction of the cortical gray and subcortical white brain matter in AD and smokers

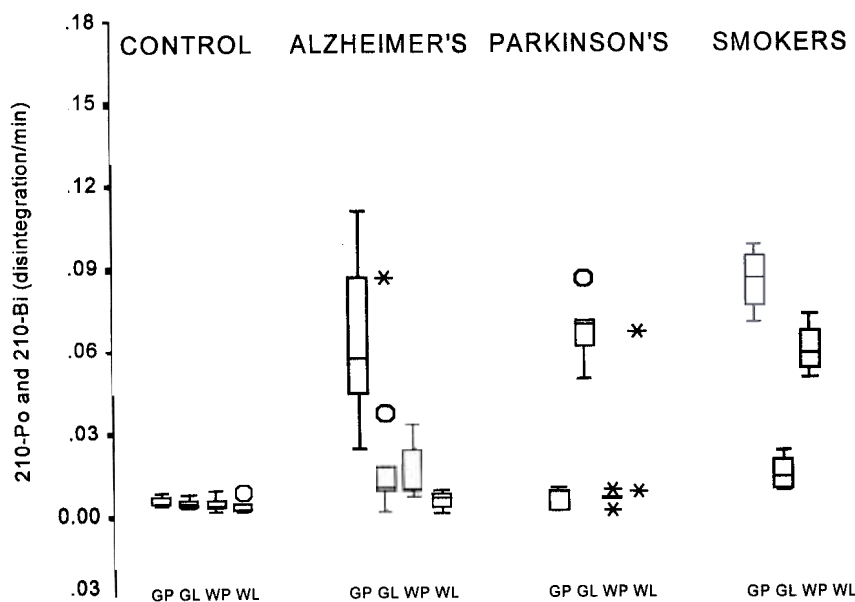
and to the lipid fraction in PD; and (4) the same results were obtained by two different radioanalytical techniques, one for alpha particles (<sup>210</sup>Po) and the other for beta particles (<sup>210</sup>Bi).

The reliability of the data and the strength of the statistical analysis are greatly enhanced because each sample was analyzed in multiple replicates and above all with the two different radioanalytical methods involving alpha and beta particles, respectively (Kateman and Pijpers, 1981). Indeed, both analytical methods yielded the same selective pattern of radon daughter distribution in the proteins and lipids of cortical gray and subcortical white brain matter within the groups of subjects who suffered from AD or PD, subjects who smoked, and controls. The radioactivity in the lipids was approximately the same for the cortical gray and subcortical white brain matter despite their presumably different fatty acid composition (Wilson and Bell, 1993). The congruency of the radioanalytical results further meant that, indeed, the samples were in secular equilibrium—yet another check of the accuracy of the radioanalytical work. Thus, environmental radon provided a naturally occurring clue that enabled us to differentiate between the biochemical changes that underlie these two neuropathologic conditions, AD and PD.

Our study demonstrated that radon has the capacity to irreversibly infest the brain with a poisonous progeny of radioactive heavy metals. Indeed, taking into account the data on environmental radon exposure that reaches the brain over the course of an average human 70-year life

FRONTAL LOBE

Fig. 1. Box and whisker plots of <sup>210</sup>Po and <sup>210</sup>Bi in the protein (P) and lipid (L) fractions from the cortical gray (G) and subcortical white (W) matter from the frontal (F) brain lobe in subjects with Alzheimer disease, subjects with Parkinson disease, smokers, and controls. The horizontal line inside the box represents the median. The lower boundary of the box is the 25th percentile, and the upper boundary is the 75th percentile. The vertical lines (whiskers) show the largest and the smallest observed values that are not outliers. Cases with values that are more than 3 box lengths from the upper or lower edge of the box are extreme values (\*). Cases with values that are between 1.5 and 3 box lengths from the upper or lower edge of the box are outliers (o). (SPSS for Windows, SPSS Inc., Chicago, IL, U.S.A., 1993.)



## TEMPORAL LOBE

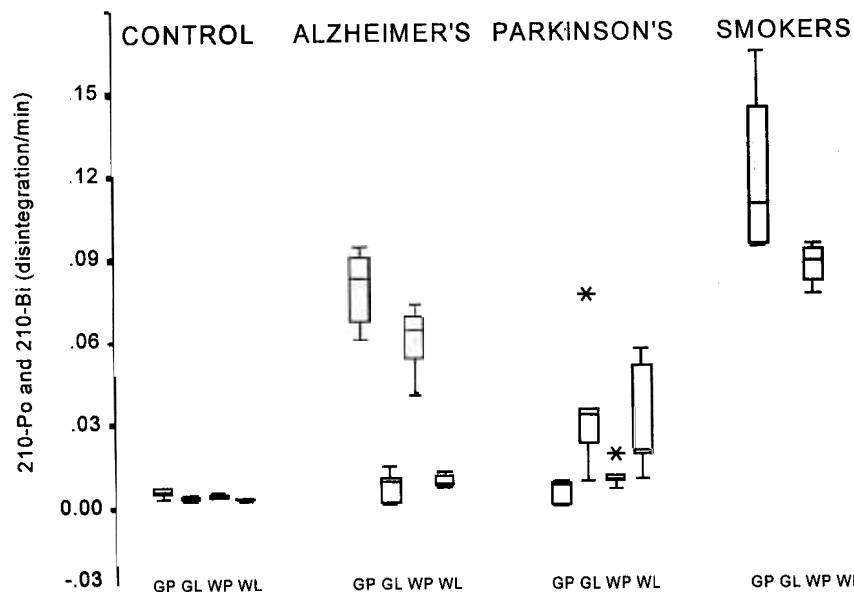


Fig. 2. Box and whisker plots of  $^{210}\text{Po}$  and  $^{210}\text{Bi}$  in the protein (P) and lipid (L) fractions from the cortical gray (G) and subcortical white (W) matter from the temporal (T) brain lobe in subjects with Alzheimer disease, subjects with Parkinson disease, cigarette smokers, and controls (see Fig. 1 legend for explanation of symbols).

span, and the number and size of the cells in a 1.5-kg brain, it can be estimated (Rossi, 1959) that there are enough high-energy alpha particles to hit and destroy every single cell of the brain more than once in a lifetime. This adds to the tremendous potential for radiation-induced microinjury, punctuate mutations, and deletions in gene expression (Bradley et al., 1995; Abrahamson, 1996). The high-energy alpha and beta particles exhibit deleterious biologic effects along their pathway and in direct proportion to their initial energy (Becker and Rosenberg, 1990; Rao et al., 1991). Along such an alpha and beta particle "death track," intensive ionization also generates a large number of free radicals (Berry, 1987), as do the heavy metals generated from transmuted radon

and radon daughters. Free radicals additionally harm the molecular biologic structures by means of protein oxidation, lipid peroxidation, and DNA intercalation and, at the cellular level, impair signal transduction, cell membrane function, and gene expression (Ohba et al., 1994; Stohs and Bagchi, 1994; Gille and Sigler, 1995; Kasprzak, 1996; Reddy and Yao, 1996).

The most likely candidate for radiation injury appears to be the glia, notably astrocytes, which are highly radiosensitive in contrast to the more radioresistant neurons, which do not divide (Campbell and Novick, 1949; Hollander, 1954). More recently, there is evidence that astrocytes may be involved in AD. Apolipoprotein E, the major apolipoprotein in the nervous system and one that is essential for neuron function, is expressed in astrocytes, and its "bad" E4 variant may interact abnormally with neuronal cytoskeletal proteins that favor microtubule degradation and the formation of neurofibrillary tangles, which occur in the brains of people with AD (Roses, 1995). Thus, the amyloid deposits and tangling observed in AD may well reflect the response to injury of the astrocyte (Selkoe, 1991). Sufficient radioactivity and free radicals accompany the presence of radon and radon daughters in the brain to act as an apogen to induce such a cascade (Corcoran et al., 1994; Selkoe, 1995).

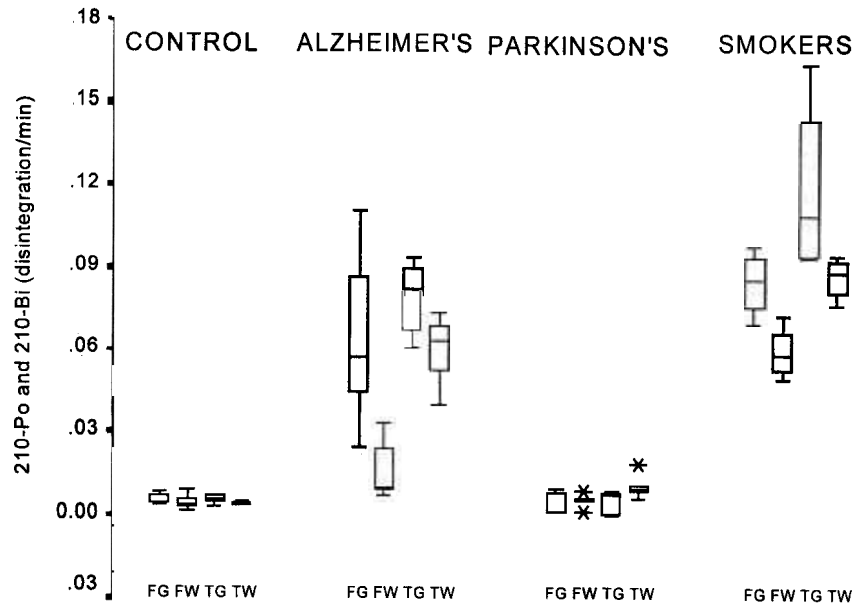
Our finding of selective, disease-specific, patterns of radon daughter accumulation in AD proteins and PD lipids was unexpected. Metal-binding affinity of neurons has long been used for their histologic visualization (Barr and Kiernan, 1993). Now it appears that different

TABLE 5. Pairwise comparisons of radon daughter concentrations in proteins (P) and lipids (L) in the cortical gray (G) and subcortical white (W) brain matter from frontal (F) and temporal (T) brain lobe in Alzheimer disease (AD), Parkinson disease (PD), cigarette smokers (S) and controls (C)

	FG	FW	TG	TW	FG	FW	TG	TW
C vs AD	*	*	*	*	*			*
C vs PD				*	*			*
C vs S			*	*	*			*
AD vs PD			*	*	*			*
AD vs S				*				
PD vs S			*	*				

\* $p < 0.05$ , Fisher's protected  $t$  test.

PROTEINS



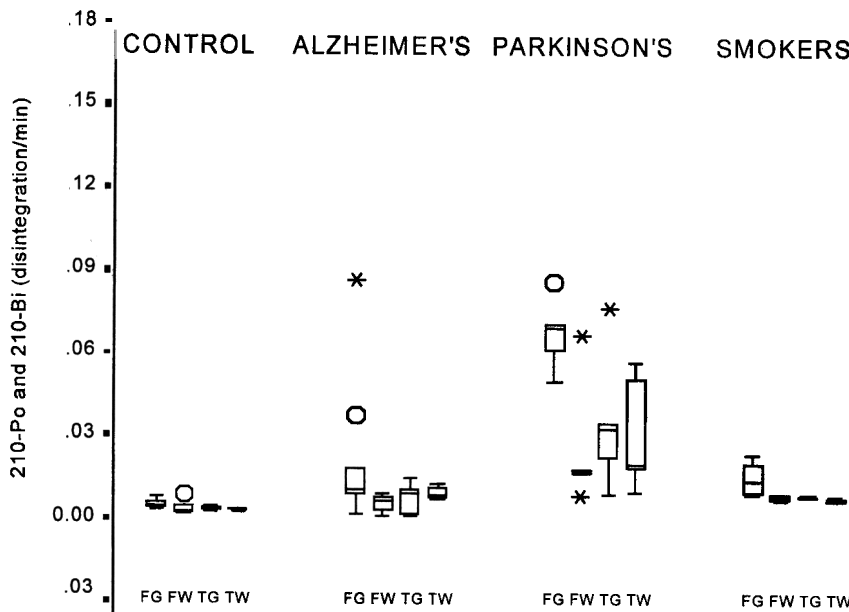
**Fig. 3.** Box and whisker plots of  $^{210}\text{Po}$  and  $^{210}\text{Bi}$  in the protein fraction (P) from the cortical gray (G) and subcortical white (W) matter from the frontal (F) and temporal (T) brain lobe in subjects with Alzheimer disease, subjects with Parkinson disease, cigarette smokers, and controls (see Fig. 1 legend for explanation of symbols).

biochemical compartments of the brain are marked with environmental radon daughters (i.e., proteins and lipids of AD and PD, respectively). Other radiochemical affinity studies (Coleman, 1965; Martell and Smith, 1997) have shown that both polonium and bismuth have practically exclusive affinity for the chloride ions to form highly reactive oxichlorides over the pH 1 to 10 range.

Hence, we concluded that the increased concentration of chloride ions in the proteins and lipids in AD and PD, respectively, would result in increased trapping of the radon daughters.

However, if transmembrane chlorine leakage indeed occurs, how does it happen that it occurs in the proteins of AD and in the lipids of PD? We inferred, in accord

LIPIDS



**Fig. 4.** Box and whisker plots of  $^{210}\text{Po}$  and  $^{210}\text{Bi}$  in the lipid fraction (L) from the cortical gray (G) and subcortical white (W) matter from the frontal (F) and temporal (T) brain lobe in subjects with Alzheimer disease, subjects with Parkinson disease, cigarette smokers, and controls (see Fig. 1 legend for explanation of symbols).

with the fluid mosaic model of the structure of cell membrane (Singer and Nicolson, 1972), that the pathognomonic distribution of the radon daughters to the lipids in PD and to the proteins in AD may reflect the disease-related changes of the brain cell membrane protein-built pores, channels, and gates in AD and that of lipid bilayer in PD, followed by the increased ionic leakage and hence the impaired membrane potential discharge and excitability.

In contrast to the peripheral nervous system (Minne- man, 1994; O'Neil and Doukas, 1994), little is known about the supposedly cholinomimetic effects of nicotine on the central nervous system (Graham-Smith and Aron- son, 1984). In our study, cigarette smokers had the high- est activity of  $^{210}\text{Po}$  and  $^{210}\text{Bi}$  in the protein fraction of the brain gray and white matter, indicating that nicotine from cigarette smoke (Williams and Weisburger, 1991) has direct access to the cortical gray and subcortical white brain matter.

**Acknowledgments.** Partly presented at (1) Experimental Biology '96: Alkhatib HA, Duerre JA, Politoff AI, et al.  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  are increased in the temporal cortex of patients with Alzheimer Disease (AD). *FASEB J* 1996;11:3907; (2) Third Annual Symposium on Alzheimer Disease: Alkhatib H, Lyk- ken GI, Duerre JA, et al. *Level of polonium-210 and lead-210 in Alzheimer's brains*. Fargo, ND, Third Annual Symposium on Alzheimer's Disease, 1995; (3) Experimental Biology '97: Alkhatib H, Duerre JA, Long WM, et al.  $^{210}\text{Po}$  is increased in the brain protein fraction in Alzheimer's disease and in the lipid fraction in Parkinson's disease. *FASEB J* 1997;12:A3638; (4) Alkhatib HA. *Environmental radon decay products in neuro- logic diseased and non-diseased brains, and brains of cigarette smokers*. Dissertation submitted to the University of North Da- kota for Ph.D., December 1996; (5) Mengen und spurenele- mente, 17. Arbeitstagung: Momcilovic B, Alkhatib HA, Duerre JA, et al. What do radon daughters tell us about Alzheimer's disease, Parkinson's disease, and the smoking habits: selective distribution of trace elements in the diseased human brain indicate the impairment of Cl-ion membrane transport. Jena, Ger- many, 1997, and Experimental Biology '98; (6) Experimental Biology '98: Momcilovic B, Alkhatib HA, Duerre JA, et al. Radon daughters in Alzheimer's disease and Parkinson's dis- ease: beyond the tangles and substantia nigra and towards the astrocytes and CL ion membrane transport.

The project was supported in part by USEPA under contract ND92-257, the Technical Training Foundation, North An- dover, MA, U.S.A., and Croatian Department of Science and Technology, Zagreb, Croatia. The generous philanthropic sup- port of the Men's Fellowship of Faith Evangelical Free Church, Grand Forks, ND, and RCS Trading Co. Ltd., Isle of Man, U.K., to Dr. Momcilovic is greatly appreciated. The previous use of the USDA, ARS, Grand Forks Human Nutrition Re- search Center, and the use of the human whole body counter and electroencephalographic equipment for preliminary assess- ment of the internal radon progeny gamma emissions and monitoring of the brain activity in human volunteers are ac- knowledged. We thank Dr. A. I. Politoff for providing expert advice on sampling the gray and white matter from the brain

lobes, Dr. K. A. Orlandini for the radioanalytical expert advise and Connie Cicha for typing the manuscript.

## REFERENCES

- Abrahamson S. 70 Years of radiation genetics: fruit flies, mice and humans. Lauriston Taylor Lecture. *Health Phys* 1996;71:624-633.
- Barr ML, Kiernan JA. *The human nervous system*. 6th ed. Philadelphia: JB Lippincott, 1993:278-292.
- Bateman H. The solution of a system of differential equations occurring in the theory of radioactive transformations. *Proc Cambridge Phi- losoph Soc* 1910;15:423-427.
- Becker CE, Rosenberg J. Clinical toxicology. In LaDou J, ed. *Occu- pational medicine*. Norwalk, CT: Appleton & Lange, 1990:131-139.
- Berry RJ. Radiation. In: Weatherall DJ, Ledingham JGG, Warrell DA, eds. *Oxford textbook of medicine*. Oxford, UK: Oxford Medical Pub- lications, 1987;6:30-135.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:34-38.
- Bradley J, Johnson D, Rubenstein D. *Lecture notes on molecular medi- cine*. Oxford, U.K.: Blackwell Science, 1995:110-142.
- Campbell B, Novick R. Effects of beta rays on central nervous tissue. *Proc Soc Exp Biol Med* 1949;72:34-38.
- Coleman GH. *Radiochemistry of plutonium*. US Atomic Energy Com- mission, NAS-NS 3058, Lawrence Livermore Radiation Laboratory, Lawrence, CA, 1965:92.
- Corcoran GB, Fix L, Jones DP, et al. Apoptosis: molecular control point in toxicity. *Toxicol Appl Pharmacol* 1994;128:169-181.
- Cothorn CR, Smith JE Jr. Radioactive decay. In: Cothorn CR, Smith JE, eds. *Environmental radon*. New York: Plenum Press, 1987:307-315.
- Eckerman KF, Rundo J. Measurement of  $^{210}\text{Pb}$  and  $^{210}\text{Po}/^{226}\text{Ra}$  ratios in human bone in vitro. In: *Argonne National Laboratory Radiology Environmental Research Division annual report ANL- 7960 (part II)*. 1973:186-196
- Eichholz GG. Human exposure. In: Cothorn CR, Smith JE Jr, eds. *Environmental radon*. New York: Plenum Press, 1987:131-213
- Folch T, Lees MT, Sloan-Stanley GH. A simple method for the isola- tion and purification of total lipids from animal tissues. *J Biol Chem* 1957;226:497-509.
- Gaffney JS, Orlandini KA, Marley NA. Measurements of  $^7\text{Be}$  and  $^{210}\text{Pb}$  in rain, snow, and hail. *J Appl Meteorol* 1994;33:869-873.
- Gille G, Sigler K. Oxidative stress and living cells. *Folia Microbiol* 1995;40:131-152.
- Graham-Smith DG, Aronson JK. Drug dependence and abuse. In: *The Oxford textbook of clinical pharmacology and drug therapy*. Oxford, U.K.: Oxford University Press, 1984:554-563.
- Hollander A. High energy radiation. In: Hollander A, ed. *Radiation biology: part 2*. New York: McGraw-Hill, 1954:9-18, 994-995.
- Hopke PK. The indoor radon problem explained for the layman. In: Hopke PK, ed. *Radon and its decay products*. Washington, DC: American Chemical Society, 1987:572-586.
- Kasprzak KS. Oxidative DNA damage in metal-induced carcinogene- sis. In: Chang LW, ed. *Toxicology of metals*. Boca Raton, FL: CRC Lewis, 1996:299-320.
- Kateman G, Pijpers FW. *Quality control in analytical chemistry*. New York: John Wiley & Sons, 1981:70-133.
- Kostial K, Blanuša M, Maljković T, et al. Age and sex influ- ence the metabolism and toxicity of metals. In: Momcilovic B, ed. *Trace element metabolism in man and animals: 7*. Zagreb, Croatia: Institute for Medical Research and Occupational Health, 1991;7: 11.1-11.9.
- Laul JC, Smith MR, Thomas CW, et al. *Analysis of natural radionu- clides from uranium and thorium series in brine ground: PNL-SA- 12851*. Richland, WA: Pacific Northwest Laboratory, 1985.
- Lykken GI. A whole body counting technique using ultralow doses of  $^{59}\text{Fe}$  and  $^{65}\text{Zn}$  in absorption and retention studies in humans. *Am J Clin Nutr* 1983;37:652-662.
- Lykken GI, Lukaski HC, Bolonchuk WW, Sandstead HH. Potential

- errors in body composition as estimated by whole body scintillation counting. *J Lab Clin Med* 1983;101:651-658.
- Lykken GI, Ong HS, Penland JG. Radon in humans: more dynamic than we thought? *Health Phys* 1990;58:S31.
- Mann DR, Casso CA. In-situ chemical absorptivity of radiocesium in sea water. *Marine Chem* 1984;14:307-318.
- Martell EA. Critique of current lung dosimetry models for random progeny exposure In: Hopke PK, ed. *Radon and its decay products*. Washington, DC: American Chemical Society, 1987:44-461.
- Martell EA, Smith RM. *NIST standard reference database 46, version 3.0: NIST critically selected stability constants of metal complexes*. Gaithersburg, MD: NIST Standard Reference Data, 1997.
- Minneman KP. Pharmacological organization of the CNS. In: Brody TM, Larner J, Minneman KP, Neu HC, eds. *Human pharmacology: molecular to clinical*. St Louis, Mosby, 1994:293-320.
- Mirra SS, Heyman A, McKeel D, et al. The consortium to establish a registry for Alzheimer's disease (CERAD). II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 1991;41:479-486.
- Nussbaum E. Radon solubility in body tissues and in fatty acids. In: *Research and development reports UR503*. Rochester, NY: University of Rochester, 1957.
- Ohba S, Hiramatsu M, Edmatsu R, et al. Metal ions affect neuronal membrane fluidity of rat cerebral cortex. *Neurochem Res* 1994;19:237-241.
- O'Neil JJ, Doukas PH. Drugs affecting the parasympathetic nervous system and autonomous ganglia. In: Brody TM, Larner J, Minneman KP, Neu HC, eds. *Human pharmacology: molecular to clinical*. St Louis, Mosby, 1994:97-113.
- Pohl E, Pohl-Ruling J. The radiation dose received by inhalation of air containing Ra222, Ra220 Pb212 (ThB) and their decay products. *Ann Acad Brasil Cienc* 1967;39:393-404.
- Rao DV, Narra VR, Howell RW, et al. Induction of sperm head abnormalities by incorporated radionuclides: dependence on subcellular distribution, type of radiation, dose rate, and presence of radioprotectors. *Radiat Res* 1991;125:89-97.
- Reddy RD, Yao JK. Free radical pathology in schizophrenia: a review. *Prostaglandins Leukot Essent Fatty Acids* 1996;55:33-43.
- Roses AD. Alzheimer's disease as a model of molecular gerontology. *J NIH Res* 1995;7:51-57.
- Rossi HH. Specification of radiation quality. *Radiat Res* 1959;10:522-531.
- Scott BR. A genetic model for estimating the risk of deterministic effects of partial organ irradiation by hot particles. *Health Phys* 1995;69:909-916.
- Scully FJ. The role of radioactivity of natural spring waters as a therapeutic agent. *J Arkansas Med School* 1934;30:206-217.
- Selkoe DJ. Deciphering Alzheimer's disease: molecular genetics and cell biology yield major clues. *J NIH Res* 1995;7:57-64.
- Selkoe DJ. The molecular pathology of Alzheimer's disease. *Neuron* 1991;6:487-502.
- Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. *Science* 1972;720-730.
- Snedecor GW, Cochran WG. *Statistical methods*. 7th ed. Ames: Iowa State University Press, 1980:234.
- Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. *Free Radic Biol Med* 1995;18:321-336.
- Weast RC, Astle MJ, eds. *CRC handbook of chemistry and physics*. Boca Raton, FL: CRC Press, 1981-1982:B-318.
- Williams GM, Weisburger JH. Chemical carcinogenesis. In: Amdur MO, Doull J, Klaassen CD, eds. *Casarett and Doull's toxicology*. New York: Pergamon Press, 1991:127-200.
- Wilson R, Bell MV. Molecular species composition of glycerolphospholipids from white matter human brain. *Lipids* 1993;28:13-17.