NEUTRON ACTIVATION ANALYSIS OF ALUMINUM IN POSTMORTEM HUMAN LUNG TISSUE[1, 2]

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ABSTRACT

Neutron activation analysis was used to assay trace quantities of aluminum in postmortem human lung tissue samples. Dried accurately weighed lung tissue samples were activated in a 3.3 x 10^{13} neutrons/cm^2/sec thermal neutron flux and analyzed using a lithium-drifted germanium detector and 4096 channel gamma spectrophotometer. The 1778 kev gamma photon emitted by Al^{28} was quantified using the comparator technique with highly purified aluminum cyclohexane butyrate as the Al standard.

The aluminum content of human lung tissue was shown not to be related to occupational exposure to industrial fiberglass. Residents of Akron, Ohio, were shown to have significantly higher concentrations of aluminum in their lungs than do residents of Newark, Ohio. A set of normal concentrations for aluminum in postmortem human lung tissue was developed, based on neutron activation analysis. The relative simplicity, accuracy, and precision of this technique suggest its use in further studies of this type.

INTRODUCTION

The possibility that a hazardous health condition may exist in a Newark, Ohio, fiberglass manufacturing plant was suggested by postmortem examinations of former factory employees. A quantitative analysis of postmortem human lung tissue was undertaken to compare the trace-element constituents present in the lungs of former fiberglass workers and of individuals with no known exposure to industrial fiberglass. Because extreme precision and sensitivity were required to assay elements in the microgram range, neutron activation analysis was selected as the analytical technique. In order to determine which element or elements should be assayed, lung tissue samples from both fiberglass workers and non-fiberglass workers were analyzed. Following activation with thermal neutrons, the entire gamma emission spectrum was searched for prominent photopeaks. Lung tissue samples from fiberglass workers contained a relatively higher proportion of aluminum than did those from nonfiberglass workers, and this parameter was selected for further study.

Some investigators have concluded that the inhalation of aluminum dust does not damage the lungs. Denny, Robson, and Irwin (1939) successfully used the inhalation of grease-free metallic aluminum powder as an antidote to the action of free silica. In studies on rabbits they found that aluminum, on conversion to hydrated alumina, reduces the toxicity of quartz in tissues by adsorbing silica from solution and by flocculation, but chiefly by coating the quartz particles with an insoluble and impermeable film. The inhalation of aluminum dust in large quantities showed no effect on the general health of the experimental rabbits and no evidence of toxicity to rabbit tissues. Englebrecht and Bester (1969) also concluded that a *small* amount of inhaled aluminum suppressed the fibrogenicity of quartz dust. Hunter *et al.* (1944) studied the effect of aluminum on the lungs of grinders of Duralumin for aeroplane propellers. Though aluminum in postmortem specimens ranged from 2,700 to 12,000 μg/g of dried lung, these investigators were unable to find any evidence that the inhalation of aluminum dust had caused fibrosis of the lung.

Many investigators have concluded, however, that inhalation of aluminum dust does cause severe fibrosis of the lungs. Goralewski (1947) has reported

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that German workers exposed to high concentrations of aluminum powder experienced dry cough with pain on breathing, shortness of breath, and loss of appetite. Radiographic studies showed a rapidly progressive and extensive fibrosis of the lung. Many of these patients developed spontaneous pneumothorax, and several subsequently died. The development of the illness is rapid and appears to bear no relation to the length of exposure to aluminum. Shaver (1948) in Canada reported 35 cases of a similar disease in men making the abrasive Alundum. Mitchell *et al.* (1961) reported fibrosis in the lungs of 6 out of 27 workers (with two fatal cases) who had been exposed to finely divided aluminum dust. King *et al.* (1955) obtained massive fibrosis in the lungs of rats by use of large amounts of intratracheally injected alumina. With metallic aluminum, alone and mixed with a little quartz, a severe fibrosis was produced. Englebrecht *et al.* (1959) demonstrated that the conflicting reports concerning the effects of alumina in animals were due to a species difference between rats and rabbits in the fibrogenic reaction of the tissues to intratracheally injected dusts. Englebrecht and Bester (1969) demonstrated that aluminum by itself produced fibrous changes in lung tissue when administered in a relatively high dose. They found that aluminum in combination with dusts other than silica increased the fibrogenicity of these dusts. Mitchell (1959) reported a fatal case of progressive pulmonary fibrosis in a young man industrially exposed to a heavy concentration of aluminum dust. Clinically, radiologically, and pathologically this case was indistinguishable from cases of aluminum fibrosis of the lung described by Shaver (1948) in Canada.

Previous investigations relied upon spectrophotometric or chemical methods to analyze aluminum in microgram quantities. Mitchell *et al.* (1961), by chemical analysis of diseased lung tissue, found aluminum in wet postmortem tissue as follows: 12 ppm at the root, 15 ppm at the apex, 8.5 ppm at the base. McLaughlin *et al.* (1962) found 430 ppm in the upper lobe and 340 ppm in the lower lobe when investigating similar postmortem tissue by spectrographic analysis. The discrepancy in reported concentrations of trace aluminum suggests the need for a uniform precise method of analysis.

The objectives of the present study were twofold. (1) The relationship between occupational exposure to industrial fiberglass and aluminum content of the lungs of fiberglass workers was investigated. (2) Accurate aluminum concentrations in normal human lungs were determined using a sensitive and precise technique: neutron activation analysis.

METHODS

Postmortem samples of frozen lung tissue were collected from hospitals in Newark and Akron, Ohio, between June 1971 and June 1972. The samples were grouped into three categories: (I) Newark residents (fiberglass workers), (II) Newark residents (nonfiberglass workers), and (III) Akron residents (nonfiberglass workers). Group I was studied to determine the quantity of aluminum in the lungs of workers in a fiberglass manufacturing plant. Group II represents a control population from the same city. Group III provides a control population for comparing the residents of Newark and Akron.

All samples were minced and ashed at 550° for 24 hours. The ashed samples were then ground with a mortar and pestle, accurately weighed on a Mettler balance, and encapsulated in polyethylene activation vials. Sample weights ranged from 0.03 to 0.40 g, depending on the amount of lung tissue available for study. Meticulous care was taken not to contaminate the samples by direct handling or by contact with unclean utensils. Activation vials containing samples were stored in larger plastic vials to prevent contamination prior to activation.

Approximately 0.5 g of highly pure aluminum cyclohexane butyrate [$(C_6H_{11}(CH_2)_3COO)_2AlOH$] was desiccated over fresh phosphorous pentoxide for 2 hours.

Aluminum is the only element in this compound that is readily activated in the neutron flux used in this study—organic elements require much more neutron bombardment for activation. Aluminum cyclohexane butyrate is thus well suited as an activation standard. Exactly 0.32171 g of aluminum cyclohexane butyrate was dissolved in 250 ml of a 1:1 mixture of highly pure toluene and pentylamine. The resulting solution contained 90.773 g aluminum per ml. Aliquots containing 0.50 ml, 0.25 ml, and 0.05 ml of this standard solution were then pipetted into activation vials, evaporated with a heat lamp, and stored for activation.

All activations and analyses were performed at the Oak Ridge Research Reactor, Oak Ridge National Laboratories, Oak Ridge, Tennessee. This facility is equipped with a pneumatic transfer system for loading samples into the core of the reactor. All samples were irradiated for 10 seconds in a thermal neutron flux of 3.3×10^{13} neutrons/cm^2/sec and counted for 60 seconds using a 20-cm^3 coaxial lithium-drifted germanium Ge(Li) detector. The detector was connected to a Nuclear Data 4096 channel analyzer which was interfaced with a PDP–15 "mini"-computer. The memory was divided into two groups of 2048 channels, allowing two spectra to be stored for each sample—one immediately following activation and one after a short period of decay. The spectra obtained were printed in tabular form by a teletypewriter.

Quantitative analysis of all samples was performed using the comparator technique. The isotope Al^{28} decays with a 2.28-minute half-life and emits a 1778 kev gamma photon. Because the analyzer was calibrated at 1 kev/channel, the 1778 kev gamma photopeak, characteristic of the decay of Al^{28}, was found in channel 1778. Several standards were activated for each group of samples to determine the ratio of Al^{28} counts to weight of aluminum present. This ratio was in close agreement for all samples, indicating their reliability. Count rates for the samples were extrapolated to T_0 (time of departure from the core of the reactor) and the weight of aluminum present was determined from the above ratio.

RESULTS AND DISCUSSION

The Newark fiberglass workers (Group I) and Newark control group (Group II) were compared first. The mean aluminum concentration for the Newark fiberglass workers was 275.8 micrograms Al/g dried lung tissue, standard deviation 231.5 (tables 1, 2). The Newark control sample demonstrated a mean of 195.7 micrograms Al/g dried lung tissue, standard deviation 100.8. The difference in these means based on t-distribution was found not to be significant ($d.f.=24$, $t=1.4563$, $p>0.10$). To further explore this comparison, the males from the Newark

TABLE 1

Aluminum concentrations in human lung tissue

Sample no.	Age	Sex	Occupation	Aluminum concentration (μg/g)
			Group I: Newark Fiberglass Workers	
12	—	M	fiberglass worker	224.8
18	—	M	fiberglass worker	222.9
28	—	M	fiberglass worker	44.4
34	—	M	fiberglass worker	419.4
35	—	M	fiberglass worker	268.7
73	78	M	fiberglass worker	586.1
165	57	M	fiberglass worker	164.0
mean				275.8
standard deviation				177.2

TABLE 1—(*Continued*)

Sample no.	Age	Sex	Occupation	Aluminum concentration (μg/g)
			Group II: Newark Residents (Nonfiberglass Workers)	
58	62	F	housewife	102.1
59	78	F	housewife	241.5
60	64	M	factory expediter	333.9
61	88	F	housewife	122.6
62	74	M	electrician	188.2
63	64	F	housewife	184.0
64	84	F	housewife	142.6
65	59	M	coal miner	224.9
66	68	M	lumber shop worker	48.6
74	—	M	air force member	71.9
76	72	M	planing mill owner	388.8
88	60	M	unknown	74.3
90	87	F	housewife (farm)	326.6
92	—	F	housewife	122.6
160	80	M	unknown	110.6
161	87	F	school teacher	242.6
162	74	M	aluminum processor	297.0
163	87	F	housewife	307.1
75	84	M	highway worker	187.9
mean				195.7
standard deviation				100.8
			Group III: Akron Residents (Nonfiberglass workers)	
168	85	F	housewife	186.8
169	74	F	housewife	280.6
170	60	M	university professor	704.6
171	76	M	unknown	806.8
172	52	M	dairy worker	134.6
173	57	M	tire factory worker	235.9
174	65	M	unknown	154.0
175	60	F	housewife	416.4
176	87	F	housewife	887.7
177	78	M	rubber company worker	662.2
178	81	F	housewife	225.2
179	72	F	housewife	96.9
180	60	M	rubber factory worker	1,027.4
181	56	M	chemical company worker	203.8
182	78	M	rubber factory worker	335.6
mean				423.9
standard deviation				308.6

control group were compared with the all-male Newark fiberglass worker sample. Mean aluminum concentration for the males of Group II was 192.6 μg Al/g dried lung tissue, standard deviation 118.5 (table 2). This mean concentration was not significantly different from the mean for the Newark fiberglass workers ($d.f. = 15$, $t = 1.1645$, $P > 0.20$).

The pooled Newark sample (Groups I and II) was then compared with the Akron sample. The mean aluminum concentration for the pooled Newark sample was 217.2 μg Al/g dried lung tissue, standard deviation 127.1 (table 2). The mean aluminum concentration for the Akron sample was 423.9 μg Al/g dried lung tissue, standard deviation 308.6. Lung tissue from the Akron sample contained

significantly higher levels of aluminum ($d.f.=39$, $t<3.0205$, $P-0.005$) than did the pooled Newark sample.

The mean concentration of aluminum in dried postmortem lung tissue for all samples (Groups I, II, and III) was 217.2 μg Al/g dried lung tissue, standard deviation 118.5 (table 2).

TABLE 2

Summary of aluminum concentrations in ashed postmortem human lung tissue in various stratifications of the sample population.

Sample group	No. in sample	Mean (μg/g)	Standard deviation	Degrees of freedom
Newark fiberglass workers (I)	7	275.8	177.2	6
Newark residents—nonfiberglass workers (II)	19	195.7	100.8	18
Akron residents—nonfiberglass workers (III)	15	423.9	308.6	14
Total sample (I+II+III)	41	292.9	231.5	40
Pooled Newark residents (I+II)	26	217.2	127.1	25
Newark males	10	192.6	118.5	9

SUMMARY

On the basis of a limited number of lung samples from former fiberglass workers, the wide variation in aluminum content of human lung tissue has been shown not to be related to occupational exposure to industrial fiberglass. Study of additional lung tissue from former fiberglass workers would be helpful in establishing the statistical validity of this conclusion. Residents of Akron, Ohio, have been shown to have significantly higher concentrations of aluminum in their lungs than do residents of Newark, Ohio. A set of normal values for aluminum concentration in human lung tissue has been developed, including the mean and standard deviation of the sample.

The factors contributing to the accumulation of aluminum in human lungs remain to be determined. Further study is suggested, including obtaining of additional personal information about the individuals whose lungs are being studied: smoking history, factory employment, hobbies, etc. Additional evidence is needed concerning the pathological effects of aluminum on human lung tissue, as indicated by contradictions in the literature concerning this subject. These examinations should be conducted using human tissue because the fibrotic response to aluminum has been shown to be species specific. The present study based on neutron activation analysis has developed normal levels for aluminum in human lung tissue. The relative simplicity, precision, and accuracy of this technique suggest its use in further studies of this type.

REFERENCES CITED

Denny, J. J., W. D. Robson, and **Dudley Irwin.** 1939. The prevention of silicosis by metallic aluminum. Can. Med. Assn. J. 40: 222–227.

Englebrecht, F. M., and **J. C. P. Bester.** 1969. The mechanism of aluminum in the prevention of pneumoconiosis. S. Afr. Med. J. 43: 203–205.

Englebrecht, F. M., P. D. Beyers, B. D. Stacy, C. V. Harrison, and **E. J. King.** 1959. Tissue reactions to injected aluminum and alumina in the lungs of mice, rats, guinea pigs, and rabbits. J. Path. Bact. 77: 407–416.

Goralewski, G. 1947. Die Aluminiumlunge—eine neue gewerburkrankung. Z. Ges. Inn. Med. 2: 665–672.

Hunter, Donald, R. Milton, Kenneth M. A. Perry, and **D. R. Thompson.** 1944. Effect of aluminum and alumina on the lung of duralumin aeroplane propeller workers. Brit. J. Indus. Med. 1: 159–164.

King, E. J., C. V. Harrison, G. P. Mohanty, and **G. Nagelschmidt.** 1955. Effect of various forms of alumina on lungs of rats. J. Path. Bact. 69: 81–93.
McLaughlin, A. I. G., G. Kazantis, E. King, Donald Teare, R. J. Porter, and **R. Owen.** 1962. Pulmonary fibrosis and encephalopathy associated with the inhalation of aluminum dust. Brit. J. Indus. Med. 19: 253–263.
Mitchell, J. 1959. Pulmonary fibrosis in an aluminum worker. Brit. J. Indus. Med. 16: 123–125.
Mitchell, J., G. Manning, M. Molyneux, and **F. E. Lane.** 1962. Pulmonary fibrosis in workers exposed to finely divided aluminum. Brit. J. Indus. Med. 18: 10–23.
Shaver, C. G. 1948. Pulmonary changes encountered in employees engaged in manufacture of alumina abrasives; clinical and roentgenological aspects. Occup. Med. 5: 718–728.

GORDIUS SP., A NEW HOST RECORD.[1] Three specimens of *Gordius* sp. were recovered from the body cavities of three millipedes, *Narceus* sp., during late July 1972 in a single locality in Ohio. These specimens represent the first record of gordioid worms utilizing millipedes as hosts for the development of the juvenile stage in North America. All specimens were taken from a single pool of Indian Creek, a 1st-order tributary of Clear Creek, within the property boundaries of the Barneby Center, School of Natural Resources, The Ohio State University, in Fairfield County.

Several dead millipedes were observed in a pool of Indian Creek by the second author on July 29, 1972. Two millipedes were somewhat broken apart, and the coils of the juvenile gordioids were observed within the hosts. Subsequent dissections of the remaining millipedes in the pool revealed a third infection. The specimens measure 151 mm, 325 mm, and 344 mm in length.

Leidy (1851) described *Gordius robustus* from a free-living female specimen. *Gordius robustus* Leidy, 1851, is the only species of this genus reported from North America. Leidy provided no information concerning the hosts utilized by the parasitic juvenile stage. May (1919) collected this species extensively in Illinois and found only members of the grasshopper family Tettigonidae serving as hosts in the field and during the course of experimental infections. Dorier (1965) lists *Glomeris marginata* Villers and *Iulus* sp. as millipede hosts of gordioid worms in Europe.

Two specimens are deposited in the USNM Helminthological Collection, USNM No. 72627. The remaining specimen is in the collection of the first author. The authors wish to acknowledge Dr. John E. Zapotosky of The Ohio State University for the benefit of his insight into gordioid biology.—C. LAWRENCE COOPER and TED W. STORCK, *Department of Zoology, The Ohio State University, Columbus, Ohio 43210.*

LITERATURE CITED

Dorier, A. 1965. Classe des Gordiaceš, p. 1202–1222. *In* P. P. Grassé [ed.]. Traité de Zoologie. Tome IV, Troisième Fascicule. Paris, Masson et Cie Editeurs.
Leidy, J. 1851. Gordiaceae. Proc. Acad. Nat. Sci. Phila. 5: 262–263.
May, H. G. 1919. Contributions to the life histories of *Gordius robustus* Leidy and *Paragordius varius* (Leidy). Illinois Biol. Monogr. 5: 123–240.

[1]Manuscript received April 9, 1973.