Arsenic and selenium in lung, liver, and kidney tissue from dead smelter workers

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ABSTRACT Concentrations of arsenic and selenium in lung, liver, and kidney tissue from dead smelter workers and from a control group have been determined with the aid of neutron activation analysis. A sevenfold increase of arsenic was found in lung tissue from the exposed workers compared with the control group. The median value of arsenic in lung tissue from workers dead from respiratory cancer was not higher than corresponding values from workers dead from other malignancies or from cardiovascular or other diseases. With increasing period of retirement the arsenic content diminished in liver tissue but not in lung tissue, indicating a long biological half life of arsenic in lung tissue. The workers dead from malignancies had a higher As/Se quotient than workers dead from other diseases, which does not contradict the protective theory of selenium. Accumulation of antimony, cadmium, lead, and lanthanum was observed in lung tissue from the exposed workers. Six of the workers died from lung cancer and the highest concentrations of any of the elements were always observed in the lung tissue from these six cases. This observation speaks in favour of a multifactorial cause behind the excess mortality from lung cancer in smelter workers.

Arsenic has been widely discussed in connection with carcinogenesis. There have been several reports of the acute and chronic toxic effects after occupational exposure to arsenic.^{1,2} An excess of cancer mortality, especially respiratory cancer, has been reported in workers engaged in producing insecticides containing arsenic,^{3,4} in workers spraying insecticides containing arsenic,^{5,6} and also in smelter workers exposed to high concentrations of inorganic arsenic.⁷⁻¹¹ Data from animal studies have shown an interaction between arsenic and selenium compounds which may lower the toxicity of arsenic compounds.¹²⁻¹⁴ A fivefold increased lung cancer mortality has been reported in workers at Rönnskärsverken, a Swedish copper smelter, and this excess mortality showed a dose-response relationship with exposure to arsenic.¹⁵ The aim of the present study was to investigate the amounts of arsenic and selenium in lung, liver, and kidney tissue from dead workers at Rönnskärsverken, to compare these values with corresponding values from corpses from a control area, and to relate the values obtained to the causes of death.

Material and methods

The material consisted of 40 dead men who had worked at Rönnskärsverken, a smelter and refinery in northern Sweden, and 11 age-matched dead men from a rural control area 50 km away from the factory. The mean age of the workers was 66.6 years and that of the control cases 67.4 years. All workers consecutively undergoing routine necropsies at the local hospital during 1977 and 1978 were included in the study. No selection was made on the basis of diagnosis. An inspection of the necropsy protocols showed that 15 of the 40 workers (37.5%) had died from different malignancies, six of them from respiratory cancer (16%); none of the control cases had died from malignant disease. Seventeen of the workers (42.5%) died from cardiovascular disease and five from cerebrovascular disease compared with seven (64%) and three controls respectively.

The period of exposure did not differ between workers dead from malignancies (mean 31 years) or other diseases (mean 32 years). Information about smoking habits and history of employment was gathered from questionnaires answered by relatives. The company gave information about occupations at the factory.

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Sample preparation

In the dissection of tissues and preparation of samples only thoroughly cleaned glass instruments were used. Specimens of about 2 g wet weight were dissected with quartz knives from the right liver lobe and 1 cm below the diaphragmatic surface and the same amount of kidney cortex was taken from the right upper pole. The samples were transferred to cleaned quartz ampoules and freeze-dried. Specimens of about 10 g of wet lung tissue were dissected from the lower part of the right upper lobe. The lung samples were deep frozen and ground to powder with a quartz pestle in a quartz mortar and about 2 g of the powder was transferred to quartz ampoules and freeze-dried. The ampoules were sealed by flame and were then ready for irradiation.

Most of the specimens were irradiated with a thermal neutron flux of $2 \cdot 10^8$ n/cm² sec for three days in the R2 reactor at Studsvik. Chemical separation was performed with an automated ion-exchange technique, and gamma-spectrometric measurements were carried out on the separated fractions as described earlier.¹⁶

Statistical methods

Assuming skew distributions of trace elements in tissues, Wilcoxon's test was used and median values are presented. For selenium the mean $\pm$ SD is also given. In the statistical calculations of age, period of retirement, and time of exposure Student's *t*-test was used.

Results

The content of arsenic and selenium was determined in lung tissue from 40 dead smelter and refinery workers and nine control cases. In 21 of the workers and eight of the control cases arsenic and selenium were also determined in the liver and kidney cortex.

The 40 workers were divided into three groups according to the main diagnosis in the necropsy protocol. Group 1 contained 15 workers dead from malignancies, group 2 17 workers dead from cardiovascular disease, and group 3 eight workers dead from other causes.

The results are presented in tables 1-4. Table 1 presents the amounts of arsenic and selenium in lung, liver, and kidney tissue from 15 workers dead from malignancies, table 2 the corresponding values from 17 workers dead from cardiovascular disease, and table 3 those from eight workers dead from other causes. Table 4 shows the values from the control cases. Age, diagnosis, smoking habit, duration of exposure, and period of retirement from work are also presented in the tables.

The median value of As in lung tissue from all workers was 50 ppb, which is seven times that of the control cases ($p < 0.001$). The workers in group 1 had as median value in lung tissue of 54 ppb, the workers in group 2 44 ppb, and the workers in group 3 49 ppb. All these three values were significantly ($p < 0.001$) higher than that in the control group, but they did not differ significantly from each other. The median value of arsenic in liver tissue from the workers, 7 ppb, did not differ significantly from the value of 4 ppb of the control group. Workers dead

Table 1 *Exposed workers with malignancies*

Age (yr)	Diagnosis	Smoking habits	Period in retirement (yr)	Duration of exposure (yr)	Lung		Liver		Kidney	
					As	Se	As	Se	As	Se
71	Lung cancer	Cigarette smoker	5	41	93	126	7	150	20	420
71	Lung cancer	Non-smoker	6.5	37	48	132	9	190	12	240
67	Lung cancer	Cigarette smoker	23	22	29	243	—	—	—	—
65	Lung cancer	Pipe smoker	1	34	40	68	6	250	11	320
72	Lung cancer	Ex-smoker (pipe)	8	27	205	201	7	261	2	570
62	Lung cancer	Ex-smoker (pipe)	1	34	130	150	3	—	2	—
58	Pancreas cancer	—	19	16	30	123	4	200	3	250
70	Pancreas cancer	—	5	38	51	75	2	150	2	310
73	Colon cancer	Non-smoker	8	39	60	158	—	—	—	—
82	Colon cancer	Ex-smoker (cigarette + pipe)	17	27	46	127	—	—	—	—
84	Rectal cancer	Ex-smoker (cigarette)	19	27	56	115	—	—	—	—
57	Cancer of the urinary bladder	Pipe smoker	0	24	41	160	15	350	2	490
61	Liver cancer	Non-smoker	1	31	78	430	15	140	16	2120
76	Chronic lymphatic leukaemia	Non-smoker	9	37	102	109	30	200	20	330
60	Myelomatosis	Pipe smoker	1	30	49	203	—	—	—	—
	Median values				54	132	7	195	5	330
68.6	Mean values		8.2 $\pm$ 7.7	30.9 $\pm$ 7.1		159 $\pm$ 89		210 $\pm$ 68		561 $\pm$ 594

Table 2 *Exposed workers with cardiovascular disease*

Age (yr)	Diagnosis	Smoking habits	Period in retirement (yr)	Duration of exposure (yr)	Lung		Liver		Kidney	
					As	Se	As	Se	As	Se
74	Myocardial infarction (MI)	Ex-smoker (cigarette + pipe)	7	39	37	128	—	—	—	—
65	MI	Non-smoker	6	31	33	103	6	160	4	480
69	MI		2	35	19	120	7	190	4	710
73	MI	Cigarette smoker	5	36	121	247	—	—	—	—
63	MI	Ex-smoker (cigarette + pipe)	5	20	110	91	6	170	5	250
37	MI		0	11	44	220	43	240	15	540
75	MI	Non-smoker	8	34	39	127	—	—	—	—
63	MI	Ex-smoker (cigarette + pipe)	4	30	67	137	—	—	—	—
54	MI		1	30	58	1560	9	170	14	500
62	MI	Cigarette smoker	0	26	23	150	11	230	9	770
67	MI		1	41	107	216	—	—	—	—
62	MI	Ex-smoker (cigarette)	0	40	29	295	—	—	—	—
66	MI	Ex-smoker (cigarette)	4	31	35	360	—	—	—	—
69	MI		4	43	880	8020	—	—	—	—
78	Cardiosclerosis	Non-smoker	15	29	24	117	—	—	—	—
64	Sudden death		1	34	85	170	8	270	10	1360
61	Sudden death		0	42	50	189	13	177	14	1520
Median values					44	170	9	184	10	625
64-8	Mean values		3.7 ± 3.9	32.5 ± 8.2	720 ± 1912		201 ± 40		766 ± 44	

from malignancies had about the same median As-value in liver tissue as the workers dead from cardiovascular disease. The median values for As in kidney cortex from the control cases, 5 ppb, was the same as that from all workers and did not differ significantly from the median values in the sub-groups of workers.

The median Se-value in lung tissue in the control group, 120 ppb, did not differ from the corresponding value in all workers, 145 ppb. The workers in group 1 showed a somewhat lower median value, 132 ppb, than those in group 2, though not significantly so. This tendency for a low Se-value in workers dead from malignancies complies with a 5% significance for the values in kidney cortex, 330 ppm v 755 ppm in control cases. In liver tissue there were no significant differences in Se content between controls and workers or between different groups of workers.

Discussion

A wide range of As-values in normal lung, liver, and kidney tissue has been reported.¹⁷ One reason for this may be due to problems of contamination during sample preparation or during the analytical procedure. In the present work great care was taken to avoid contamination during preparation of the samples, and with the neutron activation technique used there is no contamination during the analytical procedure.

Another reason for the wide range of normal values presented may be that As, as a volatile element, is lost during the preparation of samples or in the analytical process. The drying step in the preparation of samples and the analytical technique¹⁸ used in the present study has been tested earlier and found to be satisfactory.

Table 3 *Exposed workers with other diagnoses*

Age (yr)	Diagnosis	Smoking habits	Period in retirement (yr)	Duration of exposure (yr)	Lung		Liver		Kidney	
					As	Se	As	Se	As	Se
74	Cerebral haemorrhage	Non-smoker	9	36	58	196	—	—	—	—
69	Cerebral thrombosis	Ex-smoker (pipe)	15	22	85	150	2	200	52	340
78	Cerebral thrombosis	Non-smoker	13	33	59	93	—	—	—	—
75	Cerebral thrombosis	Ex-smoker (pipe)	8	26	14	140	—	—	—	—
56	Cerebral thrombosis	Non-smoker	0	30	39	130	3	170	4	630
68	Vitium organicum cordis	Non-smoker	2	44	80	100	2	230	5	530
81	Pneumonia	Non-smoker	16	28	16	137	—	—	—	—
33	Suicide		0	12	29	278	—	—	—	—
Median values					49	139				
65-4	Mean values		7.9 ± 6.6	28.8 ± 10	153 ± 60					

Table 4 Control cases

Age (yr)	Diagnosis	Smoking habits	Lung		Liver		Kidney	
			As	Se	As	Se	As	Se
68	Myocardial infarction (MI)	Non-smoker	13	122	13	200	10	700
69	MI	Non-smoker	18	110	9	450	5	1030
68	MI	Non-smoker	10	93	8	190	4	1030
42	MI		5	120	2	230	7	670
76	MI		1	92				
70	MI	Cigarette smoker	7	220	—		—	
75	Status post, MI + pulmonary embolus	Non-smoker	11	103	3	190	4	600
76	Cerebral haemorrhage	Non-smoker	1	44	3	460	2	830
66	Cerebral haemorrhage	Non-smoker	1	91				
60	Cerebral thrombosis	Non-smoker	6	120	1	140	14	370
72	Vitium organicum cordis	Non-smoker	9	700	4	150	3	810
	Median values		7	120	4	195	5	755
67-4	Mean values			165 ± 182	*	245 ± 122		755 ± 207

Several investigators of trace elements in biological material have observed that most elements are not normally distributed, and the use of the median as a central value has been regarded as more accurate than the mean.¹⁹⁻²¹ Consequently, median values are given and Wilcoxon's test has been applied in the present study. In a previous study of trace elements in normal human heart tissue, however, a few elements, among them selenium, appeared to have a normal distribution.²² Thus for selenium in the present study the mean $\pm$ SD values are given as well.

The As-values in our control group agree with those presented by Lyengar *et al*¹⁷ and Lievens *et al*²³ for normal lung, liver, and kidney tissue. No significant differences in As-content are noted between lung, liver, or kidney tissue. Our Se-values in the control group agree well with reported normal values. In the present study as well as others^{17 24 25} there is more Se in the kidneys and less in the lungs.

In several epidemiological studies of workers exposed to arsenic an excess mortality from malignancies is reported, especially respiratory cancer.³⁻¹¹ Axelson *et al*¹⁵ reported from Rönnskärnsverken, the smelter from which the present investigation is made, a fivefold increased lung cancer mortality and a twofold increased cardiovascular mortality, showing a dose-response relationship to arsenic exposure.

In the present study a high proportion of the workers died from malignancies, especially lung cancer. Fifteen per cent of the workers had respiratory cancer compared to 12% in the material of Axelson *et al*. A slight increase of malignant blood diseases is reported by Axelson *et al*¹⁵ and also by Ott *et al*.⁴ In the present study there are two cases of malignant blood diseases.

The content of arsenic in lung tissue from exposed workers shows a sevenfold increase compared with control cases in the present study. This increase could be taken as a support for As as a causative factor for the increased mortality in respiratory cancer in workers exposed to arsenic. There is not, however, more As in the lungs of the workers dead from respiratory cancer than in the lungs from workers dead from other malignancies, from cardiovascular or other diseases. On the other hand, this does not mean that As is without importance in carcinogenesis. It is necessary to consider many factors such as the amount and duration of exposure to As, biological half life in various tissues, time required to develop malignancy, concomitant exposure to other agents, smoking habits, and protecting factors. The duration of exposure was about the same among workers in group 1 and group 2, 31 and 33 years respectively. The duration of exposure in the present study is equal to the duration of employment, but this is not a good index of true exposure since working conditions in the factory are complex and have also changed much during the past 40 years.

There was a significant difference in the period of retirement from work between workers dead from cardiovascular disease (group 1) on the one hand and workers dead from malignancies (group 2) and other diagnoses (group 3), viz 3.7 years against 8.2 and 7.9 respectively. The short period of retirement from work in group 1 may imply that malignancies have not had the same possibility to develop as in the other groups.

The measured content of an element in tissues of exposed individuals depends among other things on the biological half life of the element in the particular tissue. The period between the last exposure and death varied in the present investigation between 0

and 23 years. In lung tissue there was no tendency for the As-values to diminish when the period of elapse increased, which indicates a long biological half life. In liver tissue, however, the biological half life seems to be much shorter. There were significantly higher As-values in workers when the period of retirement from work was 0 year (median 13 ppb) or 0-1 year (10 ppb) as compared to those with more than one year (median 6 ppb) of retirement ($p < 0.05$). The long biological half life for As in lung tissue indicates that As might be specially dangerous for this tissue.

Smoking is a well-known risk factor for developing respiratory cancer. There was a high proportion of smokers and ex-smokers among the workers (seven smokers, 11 ex-smokers, 12 non-smokers). Unfortunately, information about smoking habits is missing for 10 workers. Among the control cases there was only one cigarette smoker. The As median values in lung tissue for the workers did not differ among smokers (41 ppb), ex-smokers (56 ppb), and non-smokers (53 ppb). Among the six cases with respiratory cancer there were two cigarette smokers, one pipe smoker, two ex-pipe smokers, and only one non-smoker. Thus smoking may well have contributed to factors of importance for the development of respiratory cancer in the present study.

Selenium has been reported to have a protective effect against some toxic effects of arsenic in animal experiments,¹²⁻¹⁴ and similar interactions may be important in relation to lung cancer. The mean As/Se quotient in lung tissue in the control cases in the present material was 0.06 ± 0.05 , which was significantly lower than for the workers. The workers with malignancies had a mean As/Se quotient of 0.61 ± 0.42 , which was higher than that (0.34 ± 0.26) from the workers dead from other diseases ($p < 0.05$). The value for the workers dead from respiratory cancer, however, was the same (0.61 ± 0.30) as for the workers dead from other malignancies. Thus to judge from our results it is not unlikely that selenium may have some kind of protective effect. No definite conclusions can be drawn from the present study.

In addition to arsenic and selenium other elements or chemical compounds, such as sulphur dioxide, lead, cadmium, antimony and lanthanum, may become volatilised and appear as airborne dust or gases.

We have no information about sulphur dioxide in the present material. Antimony, cadmium, lead, and lanthanum, and some other elements were found to have accumulated in the lung tissue of the workers.^{26,27} Antimony was determined in all cases and the other elements in 21 of the workers.

Remarkably, the highest levels of any of these elements in lung tissue were always observed among the six cases with respiratory cancer. Thus the highest concentrations of antimony in lung tissue were observed in nos 1 and 5 among the cases with respiratory cancer, of cadmium in case no 3, of lead in case no 4, and of lanthanum in case no 6. The highest concentrations of arsenic were also observed in two of the cases with lung cancer (nos 5 and 6) if one worker with a short period of retirement employed at the As-refinery is excluded. In conclusion it is impossible to point out one particular factor or toxic element as the causative factor for the development of respiratory cancer. A multifactorial theory seems very likely. It may well be that different factors added together, such as smoking, exposure to toxic gases or metals, or both, etc produce the deleterious result. The retention of different toxic metals in the tissues during a sufficiently long period may well be of crucial importance for the development of cancer. Thus further multi-element studies in tissues, especially of toxic elements retained in tissues, are much needed.

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