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Test of Environmental Exposure to Arsenic and Hearing Changes in Exposed Children

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Arsenic determination was carried out on hair, urine, and blood samples taken from groups of 10-year-old boys, each numbering 20 to 25 individuals, residing in a region polluted by arsenic. In all the examined materials considerably elevated concentrations of arsenic were found. The relation of the observed levels of arsenic to the distance of the place of residence up to a distance of more than 30 km from the source of the emissions was studied. On the basis of the results obtained, the most advantageous material for estimation of nonoccupational exposure to arsenic seems to be hair, in spite of some problems with the decontamination procedure involved. Considerable variability among individual arsenic values in the hair makes group examination a necessity. Hearing changes were analyzed in a group of 56 10-year old children residing near a power plant burning local coal of high arsenic content. The results of both audiometric and clinical examination were compared with those of control group numbering 51 children of the same age living outside the polluted area. The highly standardized audiometric and clinical examination were completed with a questionnaire analysis concerning the personal medical histories of the children. The obtained data were elaborated statistically by means of the χ^2– test.

In the case of air conduction, important hearing losses were found at frequencies of 125, 250 and 8000 Hz, especially at the lowest frequency range. Significant degrees of hearing loss were found in bone conduction as well as in the corresponding ranges of frequencies. The high statistical significance of the hearing impairments found points to very low probability of their being only an "accidental" finding. The possibility of toxic damage to the ear cannot yet be excluded.

The air pollution caused by fly ash and sulfur oxides released from coal burning is complicated in some places by the presence of excessive quantities of toxic elements in the emissions of power plants. We have studied the hygienic-toxicologic problems of environmental pollution by arsenic in the vicinity of a power plant which burns local coal with an arsenic content of 900 to 1500 g/ton of dry substance in the form of sulfides. Despite the use of electrostatic eliminators, about half a ton of arsenic is emitted daily in the smoke, according to rather conservation calculations. Most of the arsenic is in the form of arsenic trioxide contained in the solid phase of the emissions. The first indication of arsenic pollution was the mass extinction or severe depletion of colonies of bees at the distance of 30 km from the power plant in the direction of prevailing winds. Several epidemiological studies have been performed to determine the environmental impact on health in the area excessively polluted with arsenic-containing emissions. The analysis of medical statistics gave evidence of a high rate of occurrence of skin or gastrointestinal diseases in the population living in the area (*1*). Clinical and hematological examinations on groups of 10-year-old children reveal warning changes in blood profiles and hemoglobin values which frequently approach the extremes of normal physiological limits (*2*). The morbidity rate in children, from birth to the age of 15, has been semilongitudinally studied and the results compared with those obtained in suitably selected control area. The incidence rate of respiratory diseases as well as those of the eye, skin, and subcutaneous tissues was markedly higher in exposed children (*3*). We have found elevated concentrations of arsenic in the blood, urine, and hair of 10-year-old children in the arsenic-polluted area (*4–6*). During the collection of samples for the mentioned epidemiological study, the teachers of music education at a school about 1 km from the above

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mentioned power plant, voiced complaints about the singing ability of their pupils. This led to our undertaking the detailed study of the problem (*7, 8*).

Methods

As in our previous studies (*4–6*) the samples of the nonoccupationally exposed population consist of groups of 10-year-old boys, each numbering 20 to 25 individuals. Like authors of other epidemiological studies of arsenic pollution (*9–11*) we analyzed hair and urine samples. Blood samples were analyzed only in a limited quantity for technical reasons, using activation analysis. The details of the analytical procedure for blood have been published recently (*12*). Arsenic in hair and urine was determined by a colorimetric method, using silver diethyldithiocarbamate, which allows quantitative determination of 1 μg and possibly, when modified, of 0.5 μg of arsenic. In order to achieve the most reliable determination possible, we processed 3g and more of samples of hair and 200 ml of urine. Details of hair analysis, including decontamination and several other steps in the procedure, have been published earlier (*5*). With the exception of the decontamination procedure, samples of urine were processed in the same manner.

The 24-hr samples were collected into 2-liter Teflon bottles that had been pretreated before use as follows. For 24 hr 1% merthiolate solution was left in the bottles to remove possible arsenic contamination. Then the bottles were carefully rinsed several times with distilled water. From those bottles 200 ml mean urine samples were taken for analysis after collection.

Our audiometric examination of two groups of children was open to possible errors arising from the subjective character of the method. For this reason the examination was standardized as follows. All examinations were performed by one thoroughly trained person, using the same apparatus (portable performance Kamplex TA 51), during the same time of day, 8:30–11:30 AM, in silent audiologic chambers. Both groups of children were approximately of the same size (exposed group 56, control 51 children), and the ratio of boys and girls was almost the same. The age of the children averaged about 10 years, with limit of 9.5–11 years. From the above-mentioned, it follows that efforts made not only to standardize technique of measurement, but to examine, as far as possible, homogenous groups of individuals.

Thresholds of hearing were examined at frequencies of 125–8000 Hz for air conduction and 125–4000 Hz for bone conduction. Children with symptoms of acute nasopharyngitis were examined only after they had recovered from the condition. Both groups of children were carefully examined for symptoms of otorhinolaryngological disease throughout the course of audiometric testing. Because clinical examination is itself to some extent subjective, both groups of children were examined by two experienced clinicians (Prof. V. Chládek, M.D., Sc.D., and Assoc. Prof. J. Pihrt, M.D., Sc.D.); each of them did his own examinations in both groups.

Audiometric and clinical examination were complemented with a questionnaire analysis. Questionnaires were filled out by the parents of the children. The objective of this part of our work was to detect any possible congenital causes of hearing impairment, maternal influenza, or rubella during pregnancy, delivery trauma, hereditary factors, and to identify children with somatic parameters not corresponding to their age. We did not at any rate find any such case. The remaining questions were in connection with possible hearing damage due to infectious diseases, as well as present status of tonsils (removed or not), presence of middle ear inflammation, discharge from the ears, or frequent rhinitis in the personal medical histories of the children.

Results

In Table 1, which summarizes our results from the part of this study concerned with arsenic in hair, is an added group, designated group P, which consists of individuals living in an industrial city outside the area of the power plant, and which we used as a control. Table 2 presents the results of urine analysis. Samples were taken from eight communities only.

We terminated this work for technical reasons: determination including mineralization procedure is much more laborious, and the collection and transport of material more complicated. Apart from these technical difficulties, the findings are not as conclusive or strikingly demonstrative as were those from hair analysis.

For a more detailed analysis we determined the proportions of values of arsenic content in the hair up to 1, from 1.01 to 3, and above 3 μg/g. We used those figures because values up to 1 μg/g may be considered normal; concentrations up to 3 μg/g reflect some exposure; and concentrations above 3 μg/g (after cautious external decontamination) can be considered to be sign of excessive exposure in cases of group examinations (*4, 13*). The results shown in Figure 1 demonstrate a much higher number of high and medium values in the exposed groups in the vicinity of the power plant, while on the outskirts of this region, "normal" values up to 1 μg/g predominate. The scatter of the arsenic values

Table 1. Arsenic concentrations in the hair of groups of 10-year-old boys residing at different distances from the emission source in an arsenic polluted area and of a control group (P).

Community[a]	Distance from the source of emission, km	Number of children examined	Arsenic in hair, μg/g		
			Mean	Min/max value	Standard deviation
A	16	32	0.878	0.01-2.15	1.911
B	11	25	1.057	0.21-4.30	1.533
C	10	20	1.196	0.18-3.33	0.960
C_1	10	25	1.176	0.24-2.87	0.779
D	75	21	3.562	1.40-8.90	2.045
E	4	26	2.621	0.60-7.61	1.911
F	1.5	25	3.186	1.18-7.94	2.060
F_1	1.5	23	3.793	0.86-10.33	2.321
G	4	23	3.261	0.63-8.08	2.431
H	8	24	1.822	0.30-5.14	1.130
I	10	27	1.021	0.24-3.76	0.798
J	12	25	1.854	0.80-4.60	0.908
K	15	22	2.054	0.25-4.50	1.112
L	21	26	1.337	0.16-6.12	1.400
M	23	20	0.794	0.16-3.16	0.882
N	30	22	0.657	0.18-1.28	0.779
O	36	23	0.295	0.00-0.90	0.413
P	—	44	0.152	0.00-1.13	0.279

[a]The subscript 1 following community code means results of repeated examination of group of children after a 5-yr period.

Table 2. Arsenic concentration in the urine of groups of 10-year-old boys residing at different distances from the emission source in an arsenic polluted area and of a control group (P).

Community	Distance from the source of arsenic emissions, km	Number of examined children	Arsenic in urine mg/l.		
			Mean	Max/min	Standard deviation
A	16	24	0.0078	0.0363-0.000	0.011
B	11	23	0.0201	0.0685-0.001	0.020
D	7.5	22	0.0201	0.0770-0.000	0.021
E	4	24	0.0241	0.0925-0.001	0.023
F	1.5	20	0.0189	0.0515-0.001	0.012
G	4	25	0.0253	0.1050-0.001	0.025
O	36	20	0.0082	0.0360-0.000	0.014
P	—	24	0.0109	0.0440-0.001	0.016

in hair determined in the groups did not give a normal frequency distribution, as is evident from the relatively high standard deviations. Hence the mean values of arsenic content in the separate groups are of only relative significance, and statistical evaluation had to be performed by means of the nonparametric U-test.

The level of statistical significance is seen to be markedly higher for the hair than for the urine, even if the relatively not "advantageous" control (group B) is taken into account (Table 3). We shall analyze this finding in our discussion. Three groups of children were analyzed for blood arsenic content. Results are presented in Table 4 along with the urine and hair levels of these groups. The values are not fully comparable, since the different types of samples were not taken simultaneously. Nevertheless our repeated examinations of hair arsenic levels in two groups of children (C and F; see Table 1) performed after a 5-yr period have shown that contamination is indeed of a permanent character, and that great fluctuations could not be expected as long

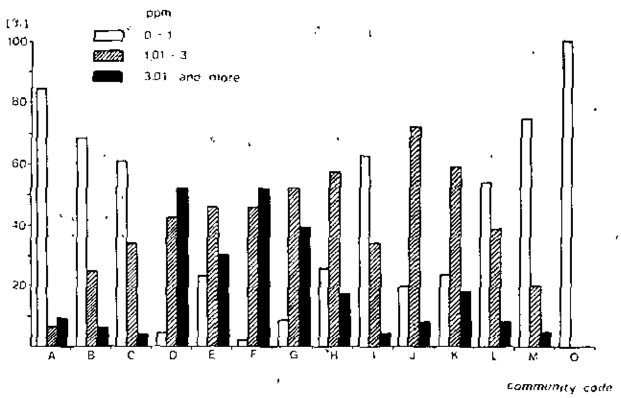

FIGURE 1. Distribution of values of arsenic content in the hair samples taken from children living in communities A to O. The source of arsenic emissions is located between communities E and F.

Table 3. Statistical significance of urine and hair arsenic values of the exposed groups of children compared to the control group (P) and difference in concentration of arsenic in hair in communities, D, E, F, G, I, and J compared to B[a].

Community	Urine	Hair	Hair
A	0.0761[b]	0.022	—
B	0.1740[b]	0.000003	—
C	—	2×10^{-9}	—
D	0.1041[b]	"	0.001
E	0.0189	"	0.001
F	0.0172	"	0.001
G	0.0190	"	0.001
H	—	"	—
I	—	"	0.015
J	—	"	0.015
K	—	"	—
L	—	"	—
M	—	0.0112	—
N	—	0.0199	—
O	0.0998[b]	0.0248	—
Control	P	P	B

[a]The nonparametrical test was used (0.01 = 1% level of significance).
[b]Not significant.

Table 4. Comparison among arsenic values in blood, urine, and hair of 10-year-old boys residing in polluted communities G and O and in control community P.

			As, ppb		
	Community[a]	No samples	Mean value	Standard deviation	Limit values max/min
Blood	G	10	4.53	14.9	8.2-2.5
	O	10	1.45	23.1	3.2-0.5
	P	10	1.88	21.7	3.8-0.5
Urine	G	25	25.3	25.0	105-1.
	O	20	8.2	13.7	36-0
	P	24	10.9	15.6	44-1
Hair	G	23	3261	2431	8080-630
	O	23	295	413	900-0
	P	44	152	279	1130-0

[a]G = the most heavily polluted part of the area; O = group residing 36 km from the source of emission; P = control group residing outside of the polluted area in a large industrial city.

as the electric power plant continues to operate at its present level. The counteract the possible seasonal fluctuations due to each season's prevailing wind direction, samples were always taken in March-April.

The results of the audiometric examinations are presented in Tables 5 and 6. Differences between exposed and control group were tested by the χ^2 test, but the groups were not tested as "sets of ears." We also forego the use of "mean value" of the hearing threshold, which is only of a statistical value. The hearing ability of each individual was evaluated at each frequency by putting measured

Table 5. Hearing threshold (mean levels) at examined frequencies in control group (51 children) for air as well as bone conduction. Values are given in dB.

		Hearing threshold, dB		
	Frequency, Hz	Boys (26), left/right ear	Girls (25), left/right ear	Mean
Air conduction	125	6.54/7.69	8.20/8.80	7.80
	250	4.42/5.00	5.00/6.00	5.10
	500	5.38/6.73	5.00/7.20	6.08
	1000	3.27/3.85	2.60/6.40	4.02
	2000	5.96/3.27	5.80/3.40	4.61
	4000	7.31/6.15	4.80/4.20	5.64
	8000	14.42/12.69	10.40/13.20	12.70
Bone conduction	125	10.77/15.19	11.80/15.00	13.19
	250	5.19/7.50	6.20/6.00	6.37
	500	2.12/2.50	3.80/2.80	2.80
	1000	3.85/3.46	3.20/5.20	3.92
	2000	2.69/2.88	2.20/3.00	2.70
	4000	−7.31/−8.27	−9.00/−8.00	−7.79

Table 6. Hearing threshold (mean levels) at examined frequencies in exposed group (56 children) for air and bone conduction.

		Hearing threshold, dB		
	Frequency, Hz	Boys (30), left/right ear	Girls (26), left/right ear	Mean
Air conduction	125	11.00/11.00	11.35/10.58	10.99
	250	8.50/8.00	8.46/8.08	8.26
	500	9.17/8.67	7.88/7.88	8.44
	1000	6.17/6.50	4.42/4.81	5.27
	2000	6.83/4.17	8.27/5.58	6.16
	4000	9.67/9.33	7.31/7.50	8.53
	8000	19.33/15.33	18.08/16.15	17.23
Bone conduction	125	16.17/17.00	19.04/20.00	17.95
	250	8.83/13.83	8.65/11.15	10.67
	500	4.67/7.67	7.31/6.35	6.48
	1000	5.00/6.50	4.62/5.77	5.49
	2000	1.17/3.17	3.27/3.08	2.64
	4000	−0.50/2.67	−1.15/−2.12	−1.61

values for the right and left ear on the abscissa and ordinate, respectively (see Fig. 2). The plane of the graphs was divided into parts, representing ranges of intensity, in 5 dB intervals. According to their presence in different ranges, persons (at each frequency separately) divided into classes were ex-

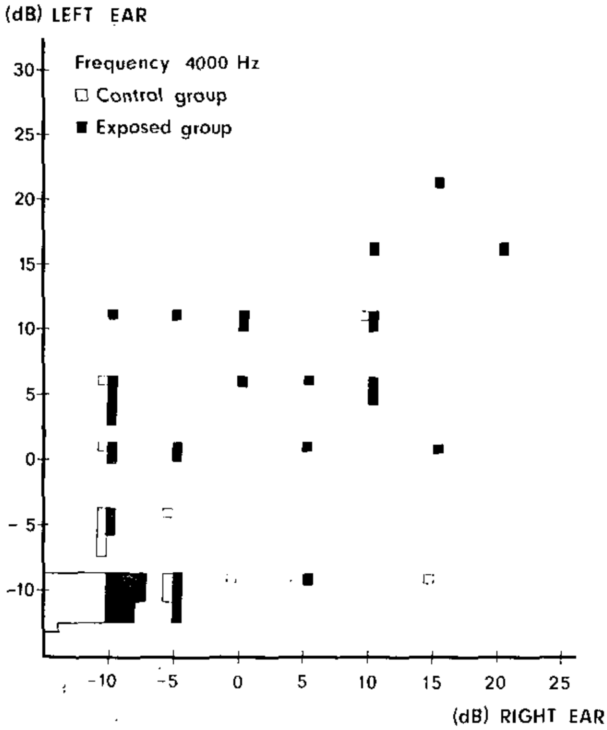

FIGURE 2. Hearing thresholds of examined children (exposed as well as control) at frequency 4000 Hz bone conduction. Each square represents one child.

amined, and the scores of members of both exposed and control groups in the mentioned classes were used as a test criterion for statistical evaluation. This procedure for group diagnosis is fully acceptable from the standpoint of statistics.

The scores of both groups for the examined frequencies are presented, and the results of statistical examination are given in Table 7. We found the same degree of importance as in our pilot study (7) at frequencies of 125, 250, and 8000 Hz in the case of air conduction.

Significant degrees of hearing loss were found in bone conduction as well. Findings of great significance were at low frequencies ($p < 0.01$); at high frequency, a level of significance of 5% was found only at 400 Hz. Analysis of the questionnaires showed that the most marked difference is in the higher occurrence of scarlet fever and middle ear inflammations in the control group which, according to clinical experience, might be a handicap for this group, especially for hearing at low frequency ranges. In the exposed group, we find a higher incidence of repeated rhinitis (colds) which correlates quite well with results of semilongitudinal epidemiological studies in this area (3).

Discussion

Experts in forensic and occupational medicine use the accumulation of arsenic in hair as evidence of criminal and occupational poisoning. On the

Table 7. Rate of hearing thresholds in different ranges of intensity (5, 10, 15, and 20 dB) at the examined frequency ranges.[a]

		No. of cases								
	Frequency	>5 dB		10 dB		15 dB		<20 dB		
	Hz	A	B	A	B	A	B	A	B	p
Air conduction	125	15	3	27	24	9	25	0	4	0.0005
	250	33	14	13	32	5	8	0	2	0.001
	500	26	14	16	30	7	9	2	3	0.05
	1000	38	32	6	19	6	3	2	1	0.05
	2000	30	24	12	22	5	8	4	2	—
	4000	21	16	22	19	5	15	3	6	—
	8000	7	0	25	9	11	11	18	36	0.01
Bone conduction	125	2	0	14	5	20	11	15	40	0.0005
	250	23	6	23	24	5	19	0	7	0.001
	500	39	26	10	19	2	10	0	1	0.01
	1000	32	28	14	17	4	10	1	1	—
	2000	34	38	10	13	6	5	1	0	—
	4000	49	42	1	10	1	2	0	2	0.05

[a]A = control group; B = exposed group of 10 year old children. Statistical significance of the differences found between the hearing thresholds of the control and exposed children at examined range of frequencies is shown as p.

basis of hair analysis, it was suspected that Napoleon and King Erik XIV of Sweden were poisoned by arsenic (*14, 15*). We attempted to use the concentration of arsenic in hair to assess the extent of nonoccupational exposure in previous studies (*4–6*). Because the main excretory route for arsenic is via urine, we investigated whether the urinary arsenic level would reflect the pollution in this area. Because of the fluctuation in the limits of "normalcy" (due to different analytical methods, local dietary habits, and so on) the choosing of suitable control groups must be given high priority in such studies.

Considerable variability of arsenic values in the hair in the individual groups, in controls and, more especially, in the exposed individuals, makes the practice of group examination essential. It seems that the lower limit for the number of members in a group should be approximately 20. By our method of hair analysis, despite careful decontamination, it is impossible to differentiate between arsenic residue, originating from external direct contamination with possible direct binding to SH-groups of scleroprotein keratin, inhaled arsenic, and ingestion, which is not negligible in polluted areas. In spite of these facts, we believe that determination of arsenic in the hair in the case of an environmental exposure of a nonoccupational character can be applied as a test of the exposure of a population to arsenic.

Compared to urine levels of arsenic, which reflect the actual concentration of arsenic in the blood, the hair level represents something like a chart in which every exposure is marked down, and for this reason represents the degree of exposure for the period of its growth. On the other hand, levels found in urine reflect the quantities of arsenic inhaled or ingested after their absorption into the blood and give a more realistic picture of possible daily intake. Exposure to arsenic is usually verified by analysis of urine after mobilization of accumulated arsenic by dimercaptopropanol or some chelating agent. We cannot use this otherwise very tempting possibility for ethical reasons. This study was performed on children and we cannot accept even the slightest risk of complications, however minor.

There exists no uniform rule on age limits for the use of subjective methods for measuring hearing threshold. Generally, an inverted relation is stated between low age and reliability of obtained results. Different authors give age limits ranging from 3 to 7 years (*16–18*). According to clinical experience, the use of audiometry for evaluation of both air and bone conductive hearing in children aged 10 (such as composed our groups) is fully acceptable. Due to transmission difficulties which we expected after the first series of examinations, the examined person responds with less accuracy. For this reason we not only standardized the examination procedure (see above), but performed the audiometric examinations with the least possible stress to the children. There was no rushing about, and more than 25 min was allotted for the examination of each child. According to some authors, there is a diurnal fluctuation of hearing sensitivity (*19*), though others deny this. To avoid any objection along this line we performed all examinations between 8:30 and 11:30 AM.

Hearing losses could be explained as a result of scarring caused by mitigated infections surrounding the Eustachian tubes and compromising their ventilatory function. In exposed children, the rhinophonia clausa can be expected, resulting in defects in singing, but repeatedly proved hearing losses in the high frequency ranges as well as losses of bone conduction left no grounds for the previous simple interpretation. As is clear from Figures 3 and 4, the control group more frequently suffered from inflammation of the middle ear (note that there had been an epidemic of scarlet fever in this school several years before). While an assumed influence of the anamnestic presence of middle ear inflammation is fully documented in the control group, this does not appear to be the case in the exposed group.

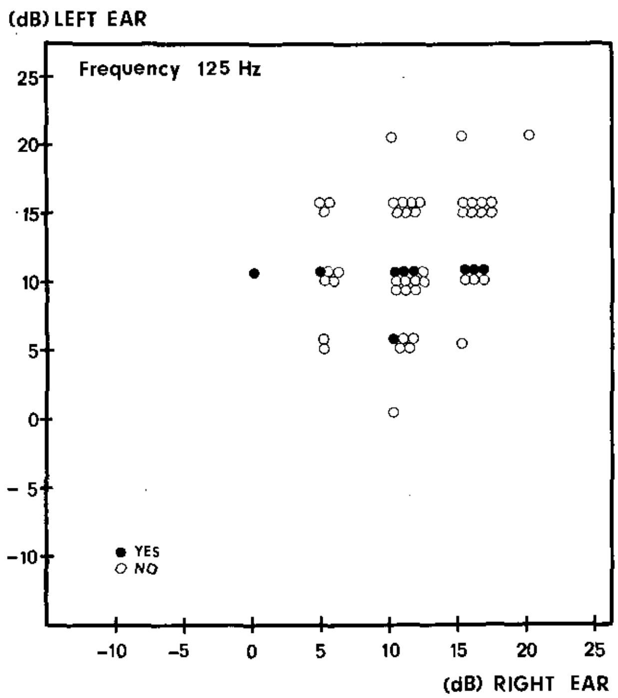

FIGURE 3. Results of audiometric examination (air conduction) in exposed group of children with inflammation of middle ear in personal history. Hearing threshold in dB.

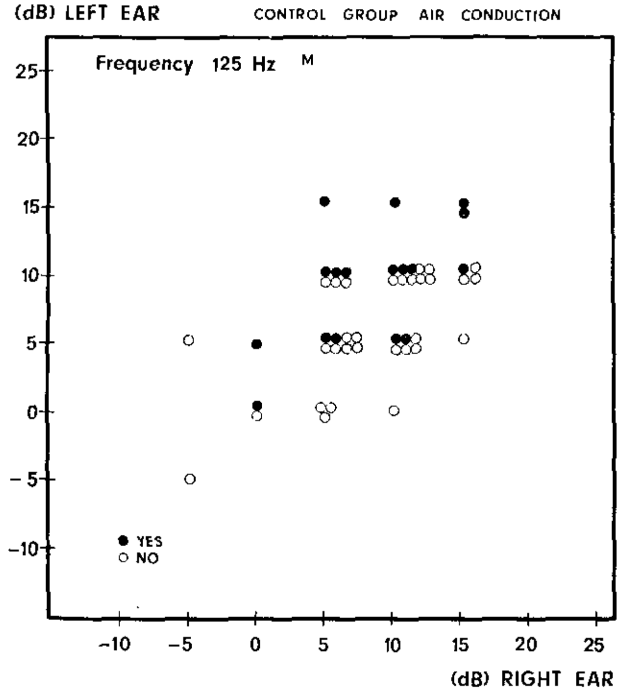

FIGURE 4. Results of audiometric examination of hearing thresholds of children with otitis media in personal history.

The character of the losses encountered suggests possible toxic damage to the hearing analyzer. The high statistical significance of the hearing impairments found points to the very low probability of their being only an "accidental" finding. Arsenic poisoning is known to produce polyneuritis in sensitive structures, and the hearing analyzer is the most sensitive analyzer in the human body. There are known cases of auditory damage, including total deafness, caused by arsenic poisoning in clinical experience (*21*); damage to the auditory analyzer has been proved in animal experiments as well (*22*). The possibility of toxic damage to the ear cannot yet be excluded.

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REFERENCES

1. Cmarko, V. Hygienic problems of arsenic exhalations of ENO plant. Čs. Hyg. 8: 359 (1963).
2. Kapalín, V. Reflect of the influence of some external conditions on the organism of school children. Čs. Hyg. 11: 468 (1966).
3. Medvedová, H., and Cmarko, H. Some results of the observation of morbidity of 0-15 years old children in the area polluted by industrial exhalations. Čs. Hyg. 19: 142 (1974).
4. Bencko, V. Arsenic in hair of a not occupationally exposed population. In: Health Effect of Air Pollution on Children. Public Health Service, Vol. 3. 1969, 948.
5. Bencko, V., Dobišová, A., and Máčaj, M. Arsenic in the hair of nonoccupationally exposed population. Atm. Environ. 5: 275 (1971).
6. Bencko, V., and Symon, K. Health aspects of burning coal with a high arsenic content. Part 1. Arsenic in hair, urine and blood in children residing in a polluted area. Environ. Res., in press.
7. Symon, K. and Bencko, V. Health aspects of air pollution by arsenic. Proc. 3rd Internat. Clean Air Congress, Düsseldorf, A. 53 (1973).
8. Bencko, V., et al. Health aspects of burning coal with a high arsenic content. Part 2. Hearing changes in exposed children. Environ. Res., in press.
9. Hammer, D. I. Hair trace metal levels and environmental exposure. Paper presented to Epidemiology Section, 98th Annual Am. Publ. Health Assoc. Meeting, Houston, Texas, Oct. 26, 1970.
10. Milham, S., Jr. and Strong, T. Human arsenic exposure in relation to copper smelter. Environment. Res. 7: 176 (1974).
11. Corridan, J. P. Head hair samples as indicators of environmental pollution. Environ. Res. 8: 12 (1974).
12. Šimková, M. Use of phosphoantimonic acid for arsenic determination by activation analysis. Isotopenpraxis 6: 207 (1975).
13. Smith, H. Estimation of arsenic in biological tissues by activation analysis. J. Forensic Med. 8: 165 (1961).
14. Forshfvud, S., Smith, H., and Wassen, A. Arsenic content of Napoleon I hair probably taken immediately after his death. Nature 192: 102 (1961).
15. Christell, R., and Sjöstrand, B. A simplified method for the determination of arsenic by means of activation analysis. Acta Chem. Scand. 16: 2129 (1962).
16. Portmann, M., and Portmann, C. Précis d'Audiometric Clinique. Masson, Paris, 1959.
17. Myklebust, H. R. Auditory Disorders in Children. 3rd ed., Grune-Stratton, New York, 1960.
18. Eagles, E. L., Wishik, S. M., and Doefler, L. G. Hearing sensitivity and related factors. Laryngoscope, Pittsburgh, 1963.
19. Taniewski, J. On the changes of auditory sensitivity during a 24 hour period. Otol. Polska 18: 341 (1964).
20. Ward, W. D. Diurnal variability of auditory threshold. Acta Otol. (Stockholm) 58: 139 (1964).
21. Beck, K. Handbuch der HNO Heilkunde. Die Krankheiten des Gehörorgans. Springer, Berlin, 1926, p. 829.
22. Westerhagen, B. Histochemisch nachweisbare Stoffwechsel-veränderung am Innerohr des Meerschweinchens nach chronischer Arsenvergiftung. Arch. Klin. Exp. Ohr. Nas. Kehlk. Heil. 197: 7 (1970).