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A STUDY ON BEHAVIORAL, NEUROTOXICOLOGICAL, AND IMMUNOTOXICOLOGICAL EFFECTS OF SUBCHRONIC ARSENIC TREATMENT IN RATS

H. Schulz, L. Nagymajtényi, L. Institoris, A. Papp, O. Siroki

Department of Public Health, University of Szeged, Albert Szent-Györgyi Medical Center, Szeged, Hungary

Male Wistar rats were treated for 4, 8, and 12 wk with 3.33, 6.66, 13.3, or 26.6 mg/kg of inorganic arsenic ($NaAsO_2$) per os by gavage. Changes in behavioral and electrophysiological parameters (spontaneous open-field exploration; electrocorticogram mean frequency and power spectrum; latency and duration of somatosensory, visual, and auditory evoked potentials; conduction velocity; and relative and absolute refractory period of a peripheral nerve) were determined. Treated rats exhibited hypoactivity of horizontal ambulation in the open field and showed depressed rates of grooming. The electrophysiological data, recorded from anesthetized rats, did not show any significant dose- and time-dependent changes. Changes in humoral immune response, tested after 4 wk of treatment, were not marked. The weight of organs responsible for immune response (thymus, spleen, adrenals), was significantly reduced, as were delayed-type hypersensitivity (DTH) reaction and mean cell volume (MCV) of red blood cells a hematological parameter. Plaque-forming cell (PFC) assay proved to be insensitive in this short-time exposure. These results suggest that subchronic low-level exposure to arsenic can affect immune responses and/or spontaneous behavior of rats.

Arsenic is an element naturally occurring in the earth's crust, ubiquitously present in the rocks and soil and especially in groundwaters. It is very widely distributed in the environment so that all humans are exposed to low levels of arsenic. The anthropogenic contribution to environmental arsenic originates mostly from mining, smelting, and refining of certain ores and also from burning of coal (Polissar et al., 1990; Van der Sloot et al., 1992; Zhou, 1993). Another source is the extensive use of pesticides containing organic or inorganic arsenic such as white arsenic as rodenticide, cacodylic acid as herbicide, sodium arsenite or hexaflurate as insecticides, or (methylarsenidyl) bisdimethyldithiocarbamate as fungicide (Martin & Worthing, 1977), and arsenic pesticides for tick control (Ng et al., 1998). The principal source of nonoccupational arsenic intake (at 25 to 50 μg/d) is food, with drinking water and air being mostly minor sources (Schwarz et al., 1991; Larsen et al., 1992; Boppel, 1995). However, in some regions of the United States (Engel & Smith, 1994) as well as in Taiwan (Brown & Chen, 1995),

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Address correspondence to Dr. László Institoris, Department of Public Health, University of Szeged, Dóm tér 10, H-6720 Szeged, Hungary. E-mail: ist@puhe.szote.u-szeged.hu

India (Das et al., 1995) and southeast Hungary (Börzsönyi et al., 1992), arsenic levels in drinking water exceed the U.S. Environmental Protection Agency (EPA) limit of 50 µg/L. Up to 600 µg/L was found in the United States and up to 3700 in India, which can result in approximately 16 and 75 µg/kg/d of exposure, respectively.

Inorganic arsenic has long been recognized as a human poison. Being carcinogenic for skin, bladder, liver, and kidney (Tsai et al., 1998; WHO, 1971), arsenic is positioned among the top hazardous substances by the Agency for Toxic Substances and Disease Registry (ATSDR) and the U.S. EPA (ATSDR, 1999). Beyond that, it causes reproductive dysfunction (Golub et al., 1998) and can damage the respiratory, gastrointestinal, cardiovascular, and hemopoietic systems (WHO, 1971; Engel & Smith, 1994; Chen et al., 1995, 1996; Winski & Carter, 1998) and the skin (Lin et al., 1998; Bernstam & Nriagu, 2000).

Arsenic affects the central and peripheral nervous system of humans (Liu et al., 1994; Mazumder et al., 1998; Bernstam & Nriagu, 2000), producing, for example, abnormal electromyography and altered nerve conduction velocity. Arsenic-induced neuropathy was reported in epidemiological studies (Ramirez-Campos et al., 1998). In Japanese infants exposed to inorganic arsenic, delayed neurological disturbances (e.g., severe hearing loss) in combination with electroencephalographic (EEG) abnormalities were found 15 yr after exposure (Liu et al., 1994). Children living near an arsenic-emitting coal-fired power plant exhibited moderate hearing loss (Bencko et al., 1988). Exposure to elevated levels of arsenic in drinking water increases the risk of cerebrovascular disease and cerebral infarction (Chiou et al., 1997). In animal experiments, arsenic was found to affect the nervous system at different levels. In rats treated chronically with inorganic arsenic altered transmitter levels, abnormal behavior (Nagaraja & Desiraju, 1993, 1994) as well as electrophysiological and motility changes (Dési et al., 1998b) were observed.

There are not many reports on immune effects of arsenic exposure in humans. Bencko et al. (1988) found lower immunoglobulin G (IgG) levels in individuals exposed to airborne arsenic. However, the effect on the hemopoietic system (ATSDR, 1999; Winski & Carter, 1998) suggests a suppressive effect on the cell mediated immune defense. The immunotoxic potential of low-dose arsenic was demonstrated in vitro in human and animal cultures (McCabe et al., 1983; Burns et al., 1991) and also in vivo in mice (Blakley et al., 1980; Gainer & Pry, 1972) and rats (Savabieasfahani et al., 1998). Due to the paucity of data on humans, the relevance of these findings remains to be determined in terms of human correlates and possible clinical consequences.

Our aim in this study was to investigate the simultaneous adverse behavioral, neurophysiological, and immunotoxicological potential of inorganic arsenic in rats. The dose range applied, $NaAsO_2$ at 3.33–26.6 mg/kg/d (equal to 1.92–15.4 mg As/kg/d), is definitely higher than the average human exposure level. All the same, the lower part of a dose-response curve based on the range chosen may be relevant to the exposure of human populations with unusually high exposures from drinking water (described earlier) or

from workplace settings. Rats are also much less sensitive to inorganic arsenic exposure than humans are, due to differences in kinetics and metabolism. In rats, on one hand, a significantly higher fraction of inorganic arsenic will be bound to the erythrocytes resulting in lower free arsenic level in plasma and body fluids (ATSDR, 1999). On the other hand, the methylation rate of arsenic in rat liver is over 10 times higher than in human liver, with methylation being a detoxifying step itself and facilitating the excretion by urine (ATSDR, 1999; Styblo et al., 1999).

METHODS

Animals and Treatment

Male Wistar rats were obtained from the specific-pathogen-free (SPF) breed of the Research Institute of Laboratory Animals, Gödöllő, Hungary. Most of the chemicals used were purchased at REANAL Factory of Laboratory Chemicals, Budapest, Hungary; exceptions were the lyophilized complement, Freund's complete adjuvant (FCA), and sheep red blood cells (SRBC), from HUMAN Serum Production and Medicine Manufacturing Co. Ltd., Budapest, Hungary; RPMI-1640, from Sigma, USA; and keyhole limpet hemocyanin (KLH), from Calbiochem, USA.

Rats at the age of 4 wk (weighing 110–130 g) were used in immunotoxicity investigation and rats of 10 wk (weighing 240–260 g) were used for behavioral and neurotoxicological experiments. The rats were kept under conventional conditions (temperature 20–22°C, humidity 60–70%, 12-h light–dark cycle with light on from 06:00 to 18:00) and fed with standard rodent feed. Food and water were available ad libitum. Treatment of rats used in behavioral and electrophysiological experiments (10 rats/dose) was done by gavage with 6.6, 13.2, or 26.4 mg/kg body weight of inorganic arsenic ($NaAsO_2$; dissolved in distilled water to 1 ml/kg body weight administration volume) for 4, 8, or 12 wk on a 5-d/wk schedule. Controls received distilled water only. For immunotoxicology, 3 × 10 rats/dose were treated for 28 d as described earlier with 3.33, 6.66, 13.3, or 26.6 mg/kg $NaAsO_2$. The animals were observed daily for symptoms of intoxication; body weight was recorded weekly.

Behavioral Investigation

The behavioral test was conducted on the day following the last arsenic administration. Investigation of motility and spontaneous exploration was performed in an automatic open field (OF) apparatus (40 × 40 × 40 cm size; Actiframe, Gerb Electronic, Berlin, Germany) (Schulz et al., 1997; Nagymajtényi et al., 1997; Dési et al., 1998a). Infrared (IR) sensors at a distance of 1.11 cm at 2 different levels of the OF detected horizontal as well as vertical movements. Rats were individually placed into the OF for a 10-min session between 08:00 and 12:00 (that is, in the first part of the light phase). Illumination at the floor of the OF was 10 ± 2 lux; background white noise was about 30 dB. The movement signals (IR beam interruptions) were stored and processed by computer.

Neurophysiological Investigation

This investigation was done 1–3 d after the behavioral one. The rats were anesthetized with urethane (1g/kg ip) (Bowman & Rand, 1980) and placed in a stereotaxic frame. The skull was opened and silver electrodes were placed on the primary somatosensory, visual, and auditory centers (Par1, Oc1B, Te1 areas described by Zilles, 1982). After recovery from the surgery (30 min), an electrocorticogram (ECoG) was simultaneously recorded from these areas for 5 min. The analyzed ECoG parameters were mean amplitude, mean frequency, and power spectrum. Cortical sensory evoked potentials were subsequently recorded with the same electrodes. Somatosensory electric stimulation was performed by electrodes pricked into the whiskery part of the nasal skin. The parameters of the rectangular stimuli were 1 Hz, 3–4 V, and 0.2 ms. The visual stimuli were flashes (1 Hz, 60 lux) provided by a flashbulb device and conducted via an optical-fiber conductor to the contralateral eye. Acoustic stimulation was performed by clicks (1 Hz, 40 dB), produced by a small earphone put into the contralateral ear of the rat. Fifty evoked potentials of each modality were recorded and later averaged. On the averaged potentials, latency and duration were measured offline. Conduction velocity of the tail nerve of the rats was measured as described by Miyoshi and Goto (1973) at room temperature (21–22°C). Relative and absolute refractory periods were calculated according to Anda et al. (1984). All neurophysiological recording and data analysis were performed using Neurosys software (Experimetria Ltd, UK). Finally, the rats were sacrificed with an overdose of urethane. The weights of brain, liver, heart, lung, kidneys, thymus, and adrenal glands were recorded and relative organ weights were calculated.

Immunotoxicological Investigation

General Toxicology and Hematology Parameters The animals (10/dose, described earlier) were sacrificed on d 29 and weights of brain, thymus, lung, heart, liver, spleen, kidneys, adrenals, testes, and popliteal lymph nodes were recorded. Blood from the abdominal aorta was taken for hematological studies. White blood cell (WBC) count, red blood cell (RBC) count, hematocrit (Ht), mean cell volume of RBCs (MCV), and the cell content of the femoral bone marrow were determined with a PS-5 blood cell counter (Medicor, Budapest, Hungary). The cellularity of the bone marrow was determined from one of the femurs as described earlier (Institóris et al., 1995).

IgM-PFC Assay Another 10 animals/dose were immunized ip with 2×10^9 SRBC in 0.2 ml phosphate-buffered saline (PBS) at a pH 7.2 on d 25 of treatment. Four days later, the spleen was removed and the IgM-type plaque-forming cell (PFC) number (calculated for 10^6 cells and for the whole spleen) was determined (Institóris et al., 1995).

Delayed-Type Hypersensitivity Assay Animals in a separate set of 10 animals/dose were immunized on d 14 of treatment by sc administration into the base of tail of 1 mg KLH in 0.4 ml antigen preparation (KLH dissolved in sterile PBS plus an equal volume of FCA). The delayed-type hypersensitivity (DTH) reaction was challenged on d 29 of treatment by injecting

17.5 μg KLH in 50 μl PBS into the left hind footpad. Footpad thickness was measured just before and 24 and 48 hours after challenge by means of a Microstat micrometer. The specific footpad swelling (D%) was calculated as described (Institóris et al., 1995).

Statistics

Data were checked for normality by means of the Kolmogorov–Smirnov test. Effects of the treatment on behavioral outcomes were tested in a 3 × 4 (treatment schedule × doses) design following square-root transformation of the data and equal cell content, using two-way analysis of variance (ANOVA; dose × treatment time) following Bartlett's test or, in the case of nonnormality, Kruskal–Wallis ANOVA. Group differences were checked post hoc by subsequent Dunnett's test. The probability level was set at $p < .05$. The other groups of data (general toxicology, electrophysiology, immunology) were analyzed by univariate ANOVA. Post hoc analysis of group differences was performed by a subsequent least significant difference (LSD) test.

RESULTS

General Toxic Effects

In the animals treated for 4 wk from their wk 4 of age, there was a clear dose-dependent reduction in the body weight gain, which was significant versus control in the group receiving 26.6 mg/kg $NaAsO_2$ (Figure 1). In the groups treated for 4, 8, or 12 wk from their wk 10 of age on, arsenic pro-

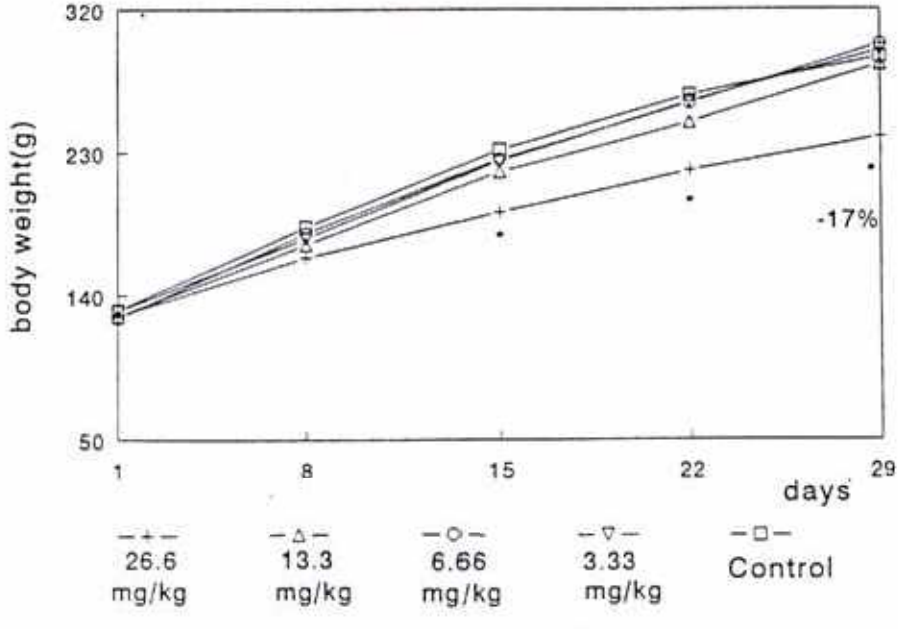

FIGURE 1. Effect of 4 wk of treatment with $NaAsO_2$ on the body weight gain of male Wistar rats (4–5 wk old at the beginning of treatment). Values are given as mean ± standard error of mean of 10 rats per group. Asterisk indicates significant difference from control ($p < .05$)

TABLE 1. Organ Weights Following 28 d of Arsenic Treatment (Immunotoxicity Study)

Doses (mg/kg)	Body weight (g)	Brain weight (g)	Organ to 100 g body weight		
			Thymus	Liver	Spleen
26.6	240 ± 7.32[b]	1.69 ± 0.04	0.199 ± 0.01[a]	5.52 ± 0.30	0.266 ± 0.02
13.3	285 ± 6.05	1.73 ± 0.03	0.241 ± 0.02	4.60 ± 0.04	0.227 ± 0.01
6.66	298 ± 7.07	1.80 ± 0.04	0.257 ± 0.02	4.44 ± 0.05	0.237 ± 0.01
3.33	294 ± 8.10	1.80 ± 0.03	0.282 ± 0.02	4.29 ± 0.06	0.212 ± 0.01
Control	290 ± 5.46	1.86 ± 0.02	0.263 ± 0.01	4.05 ± 0.10	0.210 ± 0.01

Note. Data are means ± SEM of $n = 10$ rats per group.
[a]Adrenal weight is expressed as mg/100 g body weight or mg/g brain weight.
[b]Significant at $p < .05$ compared to control.

duced no significant effect on body weight gain (see Table 2). Relative organ weights were altered by As treatment in both treatment schemes (Table 1 and 2), but the significance and in some cases the direction of change were dissimilar. In the rats treated for 4 wk from wk 4 of age, the relative weights of thymus, liver, spleen, adrenals, and testes increased significantly, while thymus weight decreased. When the same treatment duration started from wk 10 of age, the relative weight of thymus and liver decreased and that of the lungs increased significantly. By the end of 8 wk of exposure, the weights of thymus, liver, and kidney decreased, whereas following 12 wk of treatment the weights of the liver, kidney, and lungs were changed significantly.

TABLE 2. Organ Weights Following 4–12 wk of Arsenic Treatment (Behavior and Neurotoxicity Studies)

Doses (mg/kg)	Weeks of treatment	Body weight (g)	Brain weight (g)	Organ to 100 g body weight	
				Thymus	Liver
26.6	4	313 ± 8.0	1.74 ± 0.04	0.13 ± 0.01[a]	2.81 ± 0.05[a]
13.3		308 ± 9.1	1.73 ± 0.05	0.15 ± 0.01	2.98 ± 0.09[a]
6.66		342 ± 13	1.81 ± 0.03	0.13 ± 0.01[a]	3.07 ± 0.07[a]
Control		320 ± 3.0	1.81 ± 0.03	0.17 ± 0.01	3.42 ± 0.10
26.6	8	366 ± 13	1.77 ± 0.03	0.12 ± 0.01[a]	2.82 ± 0.10[a]
13.3		395 ± 7.7	1.89 ± 0.04	0.10 ± 0.01[a]	2.81 ± 0.07[a]
6.66		383 ± 12	1.79 ± 0.03	0.11 ± 0.01[a]	2.89 ± 0.08[a]
Control		366 ± 10	1.85 ± 0.03	0.14 ± 0.01	3.51 ± 0.13
26.6	12	432 ± 18	1.80 ± 0.06	0.08 ± 0.01	2.35 ± 0.08
13.3		450 ± 13	1.85 ± 0.04	0.09 ± 0.01	2.70 ± 0.06
6.66		414 ± 13	1.81 ± 0.04	0.10 ± 0.01	2.57 ± 0.07
Control		455 ± 16	1.96 ± 0.03	0.09 ± 0.01	2.61 ± 0.18

Note. Data are means ± SEM of $n = 10$ rats per group.
[a]Significant at $p < .05$ compared to control.

(g/100 g)		Organ to brain weight			
Adrenals[a]	Testes	Thymus	Liver	Kidneys	Adrenals[a]
22.0 ± 0.83	1.27 ± 0.04[b]	0.281 ± 0.02[b]	7.85 ± 0.47[b]	1.09 ± 0.03[b]	31.3 ± 1.47
17.4 ± 1.10	1.12 ± 0.04	0.394 ± 0.02	7.55 ± 0.15[b]	1.24 ± 0.03	28.6 ± 1.82
17.8 ± 0.68	1.16 ± 0.04	0.425 ± 0.03	7.35 ± 0.14	1.24 ± 0.04	29.4 ± 0.87
17.7 ± 1.08	1.08 ± 0.03	0.456 ± 0.02	7.00 ± 0.17	1.19 ± 0.02	29.0 ± 1.85
18.3 ± 0.91	1.16 ± 0.02	0.412 ± 0.02	6.32 ± 0.17	1.20 ± 0.03	28.5 ± 1.44

Behavioral and Neurophysiological Effects

Treated rats exhibited some dose- and treatment time-dependent alterations in their open field behavior. Decreased horizontal ambulation (hypoactivity) and decrease in grooming activity were observed (Figure 2).

Hematological and Immunotoxic Effects

Among the hematological parameters (Table 3), MCV showed a significant decrease. Immunotoxicological effects evoked by the arsenic treatment are represented in Table 4. The maximum intensity and the duration of DTH reaction, however, were significantly diminished by the highest dose of arsenic.

(g/100 g)		Organ to brain weight			
Kidneys	Lung	Thymus	Liver	Kidneys	Lung
0.66 ± 0.07	0.50 ± 0.02[a]	0.22 ± 0.01[a]	5.10 ± 0.22[a]	1.35 ± 0.05	0.90± 0.04
0.78 ± 0.04	0.51 ± 0.03[a]	0.25 ± 0.02	5.23 ± 0.17[a]	1.25 ± 0.10	0.91 ± 0.05
0.72 ± 0.02	0.45 ± 0.02	0.24 ± 0.02[a]	5.84 ± 0.33	1.36 ± 0.04	0.85 ± 0.04
0.66 ± 0.01	0.40 ± 0.01	0.29 ± 0.01	6.06 ± 0.17	1.17 ± 0.03	0.71 ± 0.02
0.67 ± 0.01	0.46 ± 0.03	0.23 ± 0.01	5.84 ± 0.34	1.38 ± 0.03[a]	0.95 ± 0.06
0.67 ± 0.04	0.46 ± 0.02	0.21 ± 0.02	5.88 ± 0.26	1.39 ± 0.09[a]	0.97 ± 0.04
0.69 ± 0.01	0.45 ± 0.01	0.22 ± 0.02	6.17 ± 0.27	1.45 ± 0.04[a]	0.96 ± 0.03
0.68 ± 0.02	0.37 ± 0.01	0.22 ± 0.02	6.46 ± 0.49	1.65 ± 0.06	1.09 ± 0.04
0.61 ± 0.02	0.42 ± 0.01	0.20 ± 0.01	5.64 ± 0.24[a]	1.45 ± 0.02[a]	1.01 ± 0.04[a]
0.61 ± 0.02	0.46 ± 0.01	0.22 ± 0.03	6.58 ± 0.20	1.42 ± 0.05[a]	1.12 ± 0.04[a]
0.64 ± 0.02	0.48 ± 0.03	0.25 ± 0.02	5.93 ± 0.33	1.45 ± 0.06[a]	1.08 ± 0.07[a]
0.68 ± 0.04	0.45 ± 0.02	0.25 ± 0.02	6.53 ± 0.18	1.26 ± 0.02	0.69 ± 0.02

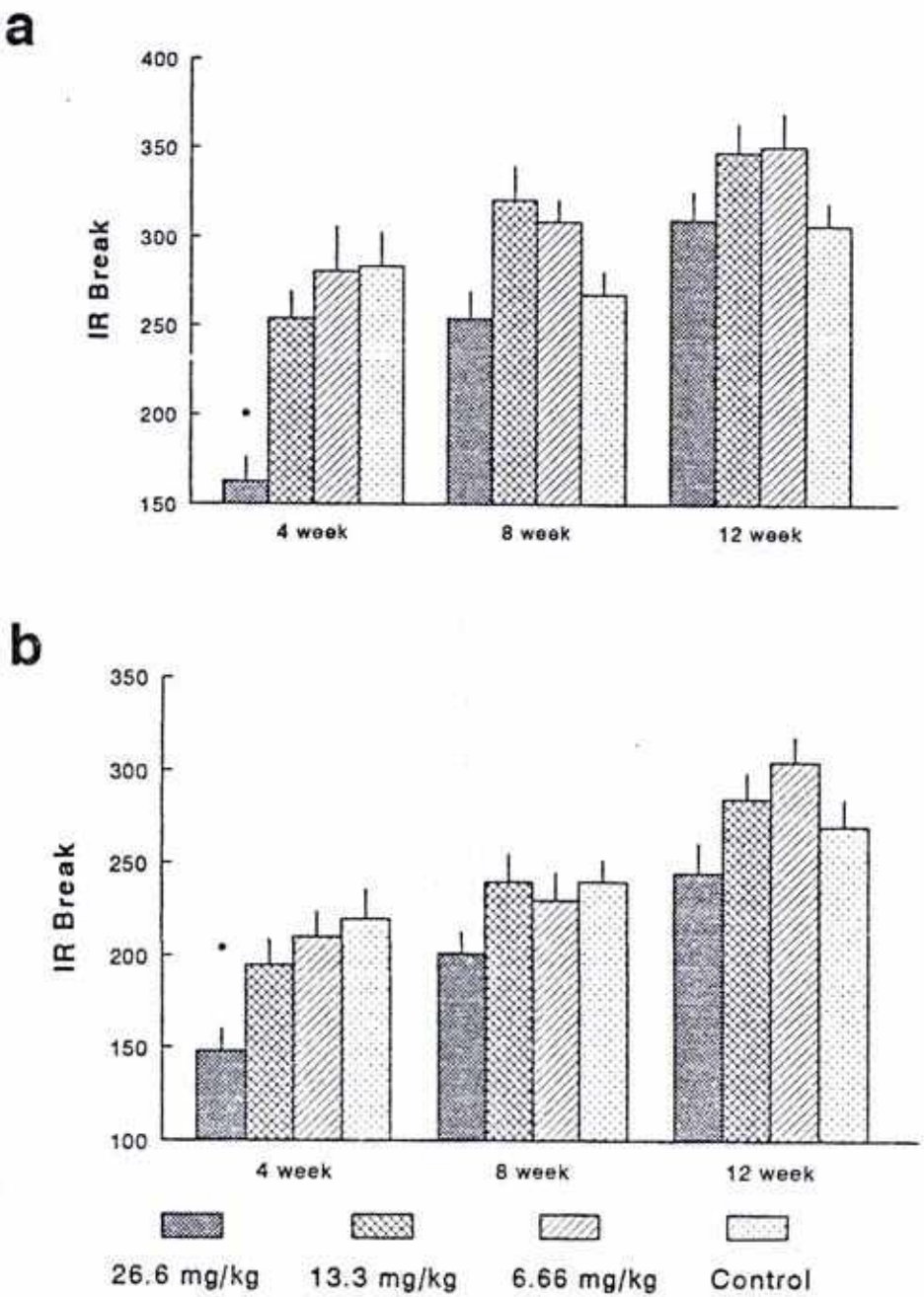

FIGURE 2. The effect of subchronic arsenic treatment of different doses (x axis: 1 = controls, 2 = 6.66, 3 = 13.3, 4 = 26.6 mg/kg body weight) on (a) total horizontal open field ambulation and on (b) stationary movements (grooming) in male Wistar rats at different time intervals (4, 8, and 12 wk) of treatment. y Axis: breaks of infrared sensors. Values are given as mean ± standard error of mean of 10 rats per group. Asterisk indicates significantly different from control ($p < .05$).

TABLE 3. Effect of 28 d of Arsenic Treatment on Certain Hematological Parameters

Dose (mg/kg)	Hematology			
	RBC ($\times 10^6$/ml)	Ht (%)	MCV (fl)	Cell/femur ($\times 10^8$)
26.60	5.85 ± 0.16	33.8 ± 1.02	57.8 ± 0.49*	1.25 ± 0.11
13.30	6.34 ± 0.15	38.0 ± 1.01	59.8 ± 0.46	1.32 ± 0.18
6.66	5.90 ± 0.25	35.1 ± 1.62	59.3 ± 0.39*	1.43 ± 0.12
3.33	6.39 ± 0.10	38.5 ± 0.56	60.2 ± 0.37	1.20 ± 0.11
Control	5.98 ± 0.25	36.6 ± 1.66	60.7 ± 0.33	1.44 ± 0.13

Note. Data are means ± SEM of $n = 10$ rats per group.
*Significant at $p < .05$ compared to control.

DISCUSSION

In this study, several toxicological parameters were found to be altered by arsenic exposure at the two higher doses (in one case also at the 6.66-mg/kg dose). Significantly reduced body weight gain was observed in the 4-wk-old (juvenile) rats treated with 26.6 mg/kg arsenic for 28 d from d 15 on. This finding is in line with the results of Mahaffey et al. (1981), who exposed young adult Sprague-Dawley rats with 50 ppm arsenic in the diet for 10 wk and found a 20% retardation of body weight gain accompanied by decreased food consumption. Decreased body weight gain, however, did not appear in rats 10–22 wk old (young adults), which indicates an age-related sensitivity to arsenic exposure. Sprague-Dawley rats seem, irrespective of differences in the doses and treatment schedule, to be more sensitive to arsenic exposure than Wistar rats as regards body weight gain.

In the 4-wk-old rats, the organ weight of thymus (related to weight or brain weight) decreased (at 26.6 mg/kg arsenic), while weights of the adrenals and liver increased (at 26.6 mg/kg and at 26.6 and 13.3 mg/kg, respectively). The increasing adrenal and decreasing thymus weight, together

TABLE 4. Effect of 28 d of Arsenic Treatment on the Immune Function Parameters Investigated

Dose (mg/kg)	PFC assay			DTH reaction, specific footpad swelling (%)	
	Spleen cells ($\times 10^6$)	PFC/10^6 cells ($\times 10^3$)	PFC/spleen ($\times 10^6$)	24 h	48 h
26.64	8.87 ± 0.67	1.21 ± .013	1.07 ± 0.14	12.1 ± 1.3*	10.5 ± 3.1*
13.32	8.78 ± 0.36	1.42 ± 0.12	1.26 ± 0.14	20.1 ± 2.5	15.7 ± 2.5
6.66	8.90 ± 0.60	1.44 ± 0.50	1.29 ± 0.11	25.9 ± 3.4	18.7 ± 2.6
3.33	9.10 ± 0.96	1.31 ± 0.13	1.21 ± 0.19	23.5 ± 1.4	13.8 ± 1.7
Control	9.56 ± 1.09	1.33 ± 0.71	1.28 ± 0.16	23.4 ± 2.2	13.8 ± 1.8

Note. Data are means ± SEM of $n = 10$ rats per group.
*Significant at $p < .05$ compared to control.

with the depressed DTH reaction, may be due to increased glucocorticoid production of the adrenals, suggesting that arsenic acts as a stressor at the 26.6-mg/kg dose. In the 10-wk-old rats (in which body weight gain effects were negligible), reduced thymus and liver weights (at 13.3 and 26.6 mg/kg arsenic) were seen but the relative adrenal weight was not affected. This points to an age dependence of arsenic action, also seen in the effect on body weight gain (described earlier) and the stressorlike activity of the 26.6-mg/kg dose.

Arsenic penetrates the blood–brain barrier and accumulates in the brain (Zheng et al., 1991), which can explain its central nervous system (CNS) effects seen in humans and animals. Several of the neurotransmitter systems involved in central functions were found affected in rats (Nagaraja & Desiraju, 1994). The hypoactivity exhibited by the treated rats in our experiments may be the result of an interaction with dopaminergic neurotransmission (Svensson et al., 1994). The effect on spontaneous and evoked cortical activity also seen in our previous investigations (Dési et al., 1988b) may be due to an arsenic effect on muscarinic cholinergic receptors (Fonseca et al., 1991) involved in the ascending cholinergic modulation of cortical activity (Metherate et al., 1992; Donoghue & Caroll, 1987).

The immunosuppressive effect of arsenic is well documented in the literature. In studies in vitro, $NaAsO_2$ produced a decreased PFC number (Burns et al., 1991; Yoshida et al., 1987), and reduced the phytohemagglutinin (PHA)-induced lymphoproliferation in human and cattle peripheral blood lymphocytes at 2.5–3 μM and 6–10 μM concentration in the culture medium, respectively (McCabe et al., 1983). Oral arsenic exposure of mice resulted in decreased PFC response and antibody titre (Blakley et al., 1980) and in decreased host resistance against pseudo-rabies and encephalomyocarditis viruses (Gainer & Pry, 1972), while pulmonary exposure reduced the antibacterial activity in the lungs (Aranyi et al., 1985). In wild cotton rats treated with 5 and 10 ppm $NaAsO_2$ in drinking water for 6 wk, decreased food intake and depressed PHA hypersensitivity reaction were found, but the lymphoproliferative capacity of T and B cells, the tumoricidal activity of LAK cells, and the phagocytic activity of macrophages were unchanged (Savabieasfahani et al., 1998). In our experiment with Wistar rats, the PFC assay failed to detect an immunosuppressive effect after 4 wk of arsenic exposure at the 3.33–26.6 mg/kg dose range. With the highest dose, however, a decrease in the maximum DTH reaction and time course of DTH reaction was observed. These results support the notion that the immune system of rats may be less sensitive to arsenic exposure than that of mice.

In large and densely populated regions, millions of people are exposed to arsenic, especially through food and drinking water (Engel & Smith, 1994; Brown & Chen, 1995; Das et al., 1995). Beyond the well-known symptoms of chronic arsenic intoxication (anemia), effects on the nervous and immune systems seem to be important components of chronic intoxication. The majority of the changes in the parameters investigated in our study—except for

body weight, certain organ weights, and DTH—were not significant. Hence one cannot speculate that life-long exposure to low doses of arsenic may result in alterations in CNS function and immune defense.

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