Systemic Effects of Occupational Exposure to Arsenic
with Special Reference to Peripheral Circulation and Nerve Function

by

BIRGITTA JSON LAGERKVIST

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BIRGITTA JSON LAGERKVIST

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Systemic Effects of Occupational Exposure to Arsenic with Special Reference to Peripheral Circulation and Nerve Function

Birgitta Json Lagerkvist. Dept. of Environmental Medicine and Dept. of Clinical Physiology, Umeå University, S-901 87 Umeå, SWEDEN.

ABSTRACT

Smelter workers who were exposed to air-borne arsenic for a mean of 23 years, and age-matched referents, were examined with clinical, physiological, and neurophysiological methods. Exposure to arsenic in workroom air was estimated to have been around the Swedish occupational limits, which were 500 μg/m³ before 1975 and 50 μg/m³ thereafter.

An increased prevalence of Raynaud's phenomenon and a reduced finger systolic blood pressure (FSP) during local and general cooling were found in the smelter workers. Slight, but significant sub-clinical neuropathy, in the form of slightly reduced nerve conduction velocity (NCV) in two or more peripheral nerves, was more common among the arsenic workers than among the referents. There were positive correlations between cumulative exposure to arsenic, reduced NCV in three peripheral motor nerves, and decrease in FSP during cooling. Arsenic levels in urine were 1 μmole/1 (75 μg/1) in the arsenic workers and 0.1 μmole/1 in the referents.

In 21 arsenic workers with no or very low exposure to vibrating hand tools, the FSP during cooling had increased significantly after 3 years with the lower arsenic exposure. There was no change in FSP during the summer vacation, whereas urinary levels of arsenic decreased to normal values. Thus there seems to be a slow improvement of finger blood circulation which is independent of short-term fluctuations in the exposure to arsenic. No seasonal variation was found in FSP during cooling with the standardized method used.

When the NCV-measurements were repeated five years later the difference between arsenic workers and referents had increased, despite the fact that 14 of the 47 arsenic workers had had no exposure to arsenic during the last 1-5 years. These observations indicate, that in subjects with long term exposure to arsenic, sub-clinical neuropathy is not reversible.

Ten milligrams of Ketanserin, a serotonin receptor antagonist, was given intravenously to five arsenic workers with cold-induced vasospasm. Skin temperature and FSP during cooling increased significantly with Ketanserin as compared with saline solution. After oral treatment, 2 x 40 mg/day for four weeks, no significant increase of FSP during cooling or rise in skin temperature was found in six arsenic workers and eleven patients with Raynaud's phenomenon. The decrease of vasospastic tendency after intravenous injection of Ketanserin indicated that similar mechanisms might operate in arsenic-induced and other types of Raynaud's phenomenon.

A general conclusion from the five studies in this dissertation is that long-term occupational exposure to arsenic has had adverse effects on the peripheral circulation and nerve conduction. The tendency to vasospasm, but not the sub-clinical neuropathy, seemed to be reversible with decreasing exposure.

Key words: Arsenic toxicity, Cold-induced vasospasm, Finger systolic pressure, Nerve conduction velocity, Occupational exposure, Raynaud's phenomenon, Serotonin antagonist.
This thesis is based on the following papers, which will be referred to in the text by their Roman numerals, I-V.

I Vasospastic tendency and Raynaud's Phenomenon in smelter workers exposed to arsenic. 
Lagerkvist B, Linderholm H, and Nordberg G F. 

II Arsenic and Raynaud's Phenomenon - Vasospastic tendency and excretion of arsenic in smelter workers before and after the summer vacation. 
Lagerkvist B E A, Linderholm H, and Nordberg G F. 

III Cold hands after exposure to arsenic or vibrating tools: Effects of Ketanserin on finger blood pressure and skin temperature. 
Lagerkvist B E and Linderholm H. 

IV Arsenic exposure to smelter workers. Clinical and neurophysiological studies. 
Blom S, Lagerkvist B, and Linderholm H. 

V Exposure of smelter workers to arsenic. A five-year follow-up study on clinical and neurophysiological effects. 
Lagerkvist B and Zetterlund B. 
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Ten milligrams of Ketanserin, a serotonin receptor antagonist, was given intravenously to five arsenic workers with cold-induced vasospasm. Skin temperature and FSP during cooling increased significantly with Ketanserin as compared with saline solution. After oral treatment, 2 × 40 mg/day for four weeks, no significant increase of FSP during cooling or rise in skin temperature was found in six arsenic workers and eleven patients with Raynaud's phenomenon. The decrease of vasospastic tendency after intravenous injection of Ketanserin indicated that similar mechanisms might operate in arsenic-induced and other types of Raynaud's phenomenon.

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1. INTRODUCTION

Arsenic (As) is a metalloid, number 33, next to selenium and in the same group (V) as phosphorus in the Periodic Table. It is a constituent of more than 200 different minerals, though in small amounts, and is found most frequently in association with sulphur. The mean concentration of inorganic As in the continental crust is about 1.5-2.0 ppm (Wolson, 1983).

In certain minerals As is present in higher concentrations. The average in sedimentary iron ores is 400 ppm of As. Certain coals are also rich in arsenic. Through human activities, e.g. coal burning, mining, smelting, and the use of As-containing pesticides, inorganic As, in the form of arsenious oxide, $\text{As}_2\text{O}_3$, is released to the environment.

The environmental levels of arsenic around As-emitting plants are generally increased. It has been suggested that air levels of As are about 10 to 100 times higher in smelter areas than in urban and rural environments (Wolson, 1983). Soil levels are reported to vary from 0.3-10 ppm in cropland areas and 0.6-15.7 ppm in rural areas (values from the National Soils Monitoring Program, Wolson, 1983). In the 1970's the maximum level of As in soil near the copper smelter of this study, the Rönnskär smelter, was reported to be 30 ppm (Lindau, 1977).

The background concentration of As in food and drinking water is generally low (Lindau, 1977; Wolson, 1983). In certain areas of the world As concentrations in drinking water have been high with serious adverse health effects after prolonged exposure.

In fish and other seafood organic As compounds, e.g., arszeno- betaine, $(\text{CH}_3)_3\text{AsCH}_2\text{COOH}$, may be present in relatively large amounts. At present these are not considered to be toxic to humans. Different authors have found no indication of biotransformation in humans after ingestion of lobster, crab
meat, or flounder containing as much as about 2 mg of organic As in one meal (Crecelius, 1977; Cannon et al., 1979; Charbonneau, et al., 1980; Vahter, 1983, review).

Substantial exposure to inorganic As with risk for adverse health effects is reported after medical treatment and occupational exposure. See Chapter 3 in this thesis.

The present studies deal with the effects of long-term occupational exposure to airborne arsenic on smelter workers. At the Rönnskär smelter the As contents of the ore have been very high and thus the exposure levels have been considerable, at least in the past.

2. AIMS OF THE STUDY

Before this study there were some reports on neuropathy caused by comparatively high occupational As-exposure, e.g., Feldman et al., 1979. Adverse effects caused by inorganic arsenic on peripheral circulation were reported in populations with a high intake of arsenic in drinking water, and in vintagers and vintners exposed to As in pesticides and wine (Butzengeiger, 1940; Astrup, 1968; Grobe, 1976; Tseng, 1977; Borgoño et al., 1977). There were, however, no reports of adverse effects on peripheral circulation by occupational low-dose exposure to airborne arsenic. Other systemic effects were reported in many different studies (For reviews see Pershagen and Vahter, 1979; WHO, 1981; Pershagen, 1983; Hindmarsh and McCurdy, 1986). In this study special interest was focused on peripheral circulation and peripheral nerve function.

The aims of the different investigations were:

- to examine whether abnormalities in finger blood circulation relative to referents could be detected in smelter workers by means of finger systolic blood pressure (FSP) measurements during local and general cooling (I).
to elucidate the effects of a break in arsenic exposure on FSP during cooling and on the excretion of arsenic in the urine (II).

to elucidate the effects of "Ketanserin", a serotonergic antagonist, on FSP in As-exposed persons with vasospasm, and to compare the effect of Ketanserin on the cold-induced vasospasm in arsenic workers and other patients with Raynaud's phenomenon (III).

to study possible clinical and subclinical effects of arsenic exposure on peripheral nerve function by use of neurophysiological methods (IV).

to reexamine the workers after 5 years in order to detect any changes in the health status or the neurophysiological parameters during continuous low-dose exposure to arsenic (V).

3. HEALTH EFFECTS OF ARSENIC

Historical background

Inorganic arsenic has been used since ancient times in medical treatment and as a poisoning agent. Hippocrates (460 - 370 B.C.) used arsenic tetrasulfide (As$_4$S$_4$) in a paste for ulcer treatment (Buchanan, 1962; Squibb and Fowler, 1983).

In 1786 Fowler created his solution, which contained 1% As$_2$O$_3$. This solution has then been used for treatment of such different diseases as leukaemia, psoriasis and bronchial asthma. During the years and decades to follow after Fowler's discovery many reports on side effects of this medication have been published (For a review on different arsenic preparations in drugs see Pershagen, 1983). As late as in 1959 As-trioxide was on the List of Drugs in Sweden (Pharmacopea Svecica, 1958).
Arsenic may still be a health hazard in homeopathic medicines (Kerr and Saryan, 1986).

During the Middle Ages white arsenic (As₂O₃) was commonly used as a suicidal and homicidal agent (Buchanan, 1962). A well known case in Sweden is King Erik XIV, the eldest son of Gustav Vasa, who died in 1577 after a week with pains in his chest and stomach. It was probably his brother Johan III, who had ordered the murder (Casparsson et al., 1962; Kock, 1963).

Health effects

Adverse health effects after exposure to inorganic arsenic compounds, both in the general- and occupational environment, are described in a great number of reports and reviews. Both acute, subacute and chronic effects are reported. Almost every organ system may be affected (reviews: Jenkins, 1966; Chhuttani and Chopra, 1979; WHO, 1981; Pershagen, 1983; Hindmarsh and McCurdy, 1986). Dermatitis, chronic bronchitis, liver cirrhosis, peripheral neuropathy, CNS disturbances, and liver, lung, stomach and skin cancer are considered to be associated with exposure to inorganic arsenic. A more detailed discussion on health effects in different organs is found in the separate studies of this thesis.

Health effect studies at the Rönnskär smelter

Since the 1940's a great number of health effect studies have been undertaken at the Rönnskär smelter (Reviews in: Holmqvist, 1951; Beckman, 1978; Wall, 1980; Pershagen, 1983; Gerhardsson, 1986; Wall, 1988). Dominating diseases were dermatitis and upper and lower respiratory airway affection. Nasal septum perforation and bronchitis were common among the arsenic workers during the 1940's (Lundgren et al., 1951).

In 1947 Sjöstrand published a study on 180 workers from different working stations who had been employed for more than 8 years. Forty of them had respiratory problems which had
"chiefly been caused by the gases and dust present at the rough smelting process". Further examinations of 20 workers with the most pronounced symptoms revealed "chronical naso-pharyngo-laryngo-tracheo-bronchitis" and a reduced physical working capacity in the majority of them (Sjöstrand, 1947). This study was considered as preliminary, and in 1951 Lundgren et al., published the results from a large medical survey on 1500 smelter workers and 700 referents. The findings from the 1947 study were confirmed. The authors called the chronic bronchitis, the "Rönnskär disease" and concluded that the etiologic agent apparently was "arsenic trioxide liberated during smelting of the ore, possibly in combination with sulphur dioxide" (Lundgren et al., 1951).

In 1978 Axelsson et al., reported an increased mortality due to lung cancer and cardio-vascular diseases among the smelter workers. The authors found a positive dose-response relationship between arsenic exposure and mortality. In 1980 Wall et al., reported an excess mortality in lung cancer and circulatory diseases among Rönnskär smelter workers as compared with the general population. Pershagen in 1982 reported an increased mortality of lung cancer for arsenic workers, with a multiplicative effect with smoking. In 1988 Järup et al., reported a positive dose-response relationship between cumulative exposure to arsenic and lung cancer. No positive dose-response relationship was found between cumulative exposure to sulphur dioxide ($SO_2$) and lung cancer risk, though the risk was increased in all $SO_2$ exposure groups. Järup et al. also found "an almost significant elevation of the total relative risk" for death from ischemic heart or cerebro-vascular disease. In a recent study there was still an excess incidence of total cancer, mainly due to the lung cancer cases, of about 30% (Sandström et al., 1989). However, since 1976 lung cancer incidence has been decreasing. Increased frequencies of chromosomal aberrations in arsenic and lead workers at the smelter have been reported (Nordenson et al., 1977; Nordenson et al., 1978).
An antagonistic (protective) effect of selenium against arsenic, lead, or sulphur dioxide (SO\textsubscript{2}) in human lymphocyte cultures was shown by Beckman and Nordenson in 1986. No synergistic effects were found, and the interactions between arsenic, lead and SO\textsubscript{2} were mainly antagonistic. The authors concluded that these unexpected findings indicated that mixed exposure, where arsenic, lead and SO\textsubscript{2} are involved, may cause less genetic damage than expected (Beckman and Nordenson, 1986).

Toxicity of inorganic arsenic

The main mechanism behind the toxicity of As(III) compounds (arsenite) is their interaction with sulfhydryl-(SH)-groups in tissues. Since the beginning of the 1900's many in vitro studies on enzyme inhibition by arsenic have been published (Squibb and Fowler, 1983, review). Other mechanisms of enzyme interactions, e.g., competitive inhibition of substrate binding due to structural similarities, may also play a role.

In vivo toxicity of inorganic arsenic is dependent on many different factors, such as the animal species, the chemical form and the solubility of the As compound. As(III) compounds are more toxic than the As(V) salts (arsenate). Pentavalent arsenicals, due to their structural similarity with phosphate, uncouple oxidative phosphorylation. In addition, part of the arsenate administered is reduced to arsenite in vivo (Hindmarsh and McCurdy, 1986).

In humans, as in many animal species, arsenic is methylated and excreted in the urine as monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA). The metabolism of As is fairly rapid and the biological halftime in humans is about 2-3 days (Crecelius, 1977; Pomroy, et al., 1980; Vahter, 1983, review). In a study by the present author urinary As-levels had decreased to normal values after four weeks (Study II).
Different studies on the distribution of inorganic arsenic in vivo show the highest concentrations in skin, gastro-intestinal mucosa, liver and hair (Vahter, 1983). The mucosa, the skin and the scalp are all organs rich in capillaries.

4. THE RÖNNSKÄR SMELTER AND THE WORKPLACE OF THE REFERENTS

The Rönnskär smelter

The Rönnskär smelter is situated on a peninsula on the east coast in northern Sweden. It was specially built during the late 1920's to process the copper ore from the Boliden mines, 70 kilometers inland. During the first years of mining about 80,000 tons of copper ore had been shipped to the Takoma smelter in the state of Washington, U.S.A. As the ore was very complex with many impurities, e.g., 15% arsenic, it could not be refined in Sweden. When production increased a new smelting process was developed at the Rönnskär smelter (Wall et al., 1988).

In the beginning the waste arsenic was simply moulded into concrete blocks and dumped into the sea. Some years later the crude arsenic could be processed and developed into commercial products. Since then the smelter has been one of the major producers of arsenic products in the world (Lindau, 1977; Fishbein, 1981). In the 1970's 20% of the world production of arsenic came from the Rönnskär smelter.

During the 1930's no protective measures were taken in the work environment. Open, hand-rolled wagons were used to transport material within the smelter works (Nygren, 1980). No exposure data are available from this time. There were adverse effects on health, however. In the beginning of the 1930's, 6-9% of the total workforce were on sick leave for dermatitis or respiratory airway affection. In some departments, up to one third of the workforce could be on sick leave (Holmqvist, 1951; Nygren, 1980).
During the early 1940's analyses of suspended dust were made at different parts of the roaster plant. Arsenic contents varied from 0.02 to 7.1 mg/m$^3$ of air. Selenium, nickel, and lead were also analysed. The content of lead varied from 0.04 to 1.25 mg/m$^3$ of air (Holmqvist, 1951, quotation from a report issued by the National Institute of Public Health [NIPH] in 1945).

In 1945 average concentrations of arsenic in air from 0.06 to 2 mg/m$^3$ during "some hours" were reported in the converter hall, and at the copper furnaces and the roasters. The air levels of sulphur dioxide (SO$_2$) varied from 15 to 300 mg/m$^3$ (Lundgren et al., 1951; Lundgren, 1954). For shorter time periods the air levels could be "very high". At that time we had no official Swedish occupational limit, but the authors referred to the ACGIH (American Conference of Governmental Industrial Hygienists) 8-hour values, which were 0.50 mg/m$^3$ for arsenic and 10 ppm (0.26 mg/m$^3$) for SO$_2$. For arsenic, three of eleven measurements, and for SO$_2$, all but one, from different departments of the smelter were above these limit values.

During the 1960's the concentrations decreased and varied from 0.1 to 0.5 mg/m$^3$ of arsenic and 5 - 10 mg/m$^3$ of SO$_2$ at the roasters. At some workplaces the concentration of arsenic could be rather high, e.g., the packing of pure arsenic was done by inappropriate methods from an occupational health point of view up to 1962, when a new arsenic refinery was built. In the old arsenic packing store exposure levels and inhaled levels were considered to be about 0.64 mg/m$^3$ (Nygren, 1980). Meanwhile the emissions to ambient air and water have been considerable (Tables 1 and 2). From 1969 the official Swedish occupational exposure 8-h limit value was 500 µg/m$^3$, and from 1975 it was 50 µg/m$^3$. From 1987 it is 30 µg/m$^3$, and as low as 10 µg/m$^3$ in new establishments.
Table 1. Emissions to ambient water of different metals from the copper smelter, tons/year.

<table>
<thead>
<tr>
<th>Year</th>
<th>Arsenic</th>
<th>Lead</th>
<th>Copper</th>
<th>Thallium</th>
<th>Gold</th>
</tr>
</thead>
<tbody>
<tr>
<td>1973</td>
<td>1500*</td>
<td>148</td>
<td>115</td>
<td>0.56</td>
<td>0.016</td>
</tr>
<tr>
<td>1985</td>
<td>31</td>
<td>3.1</td>
<td>7.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 2200 tons in 1968 and 3200 tons in 1967


Table 2. Emissions to ambient air of different metals from the Rönnskär smelter, tons/year.

<table>
<thead>
<tr>
<th>Year</th>
<th>Arsenic</th>
<th>Lead</th>
<th>Copper</th>
<th>Cadmium</th>
<th>Mercury</th>
</tr>
</thead>
<tbody>
<tr>
<td>1967-76</td>
<td>115</td>
<td>485</td>
<td>207</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>1974-76</td>
<td>80</td>
<td>215</td>
<td>186</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>1985</td>
<td>40</td>
<td>101</td>
<td>41</td>
<td>2.7</td>
<td>0.57</td>
</tr>
</tbody>
</table>


In 1984 samples of airborne dust at the arsenic refinery were collected with a high volume sampler. The dust was sent for neutron activation and analysed for a great number (22) of metals. Arsenic was the major component comprising 19% of the dust collected. Iron comprised 11.4%, lead 6.2%, copper 4.8%, selenium 4.0%, bismuth 2.8%, zinc 1.6 and antimony 1.6% of the
dust collected (Leffler et al., 1984). Mercury constituted 1.2, tin 0.26, and cadmium $6.4 \times 10^{-4}$ percent of the dust by weight. The medium diameter of the dust collected was 5.0 μm with a geometric standard deviation of 2.1 μm.

Vahter et al., in a study on correlation between airborne arsenic and urinary excretion, reported concentrations of arsenic in the breathing zones of the workers from 1 to 194 μg arsenic/m³ (Vahter et al., 1986).

The workplace of the referents

The referents were from the Volvo Truck Corporation Plant in Umeå, Umeverken, 140 kilometers from the smelter. Today Volvo Umeverken is a large concern with more than 1,100 employees and a yearly production of driver's cabs above 38,000. About 40,000 fuel tanks, and 11,000 pneumatic tanks, brackets and steel pressings for the Volvo Group are also produced here.

The company which was to become the Volvo Plant was founded in 1929. From 1934/35 the production was focused on the manufacture of truck cabs, which were made of wood until 1948. From 1948/50 the truck cabs were made of steel and the collaboration with Volvo started.

The main occupational problems concerning the workers' health at Volvo Umeverken have been vibrating tools, noise, musculoskeletal disorders and exposure to solvents. The emissions of solvents to ambient air have been around 200 tons per year during the 1980's. I have no detailed record on workroom levels of solvents, but according to the chief industrial hygienist, they have been below the maximum allowable level according to Swedish standards.
5. SUBJECTS - SMELTER WORKERS AND REFERENTS

Studies I and IV

The selection of subjects was made in cooperation with Dr. Thorulf at the health care unit of the smelter. A group of smelter workers was collected which fulfilled the following criteria.

- exposure to arsenic for 10 years or more
- no long-term exposure in the lead production at the smelter
- no concomitant diseases which might affect peripheral circulation or nerve function, such as diabetes or neuropathy

Nine out of 62 men with long-term exposure to As declined to participate. Forty-seven men fulfilled the above criteria and made up the study population. Their mean age was 53.6 years, range 36-65 (when these studies began in 1981). They had been working in the arsenic refinery, the arsenic metal and arsonic salt works, the selenium works, the converter hall, at the roasters and at the gas purifier, for eight to 40 years, mean 23 years. Their exposure to arsenic has been calculated to have been around the Swedish occupational standard. This has probably given an under-estimation of the total absorption of arsenic, which will be discussed in Chapter 6, page 28. Three men had been working in the lead works for 5-12 years.

The referents had no known exposure to arsenic and were selected in cooperation with Assistant Professor and Company doctor, Dr. Michaelson. They were chosen by means of a questionnaire sent to a total of 262 men at Volvo Umeverken, who were of the same age as the arsenic workers. A total of 198 (76%) responded. Among them 50 referents were chosen who were the best matches to the arsenic workers with respect to age and use of tobacco and vibrating handtools. Their mean age was 52.2 years (range 36-65).
The medical records of the 64 men who had not answered the questionnaire were studied at the health care unit. These non-responders had a mean age of 52.8 years. The only difference found between them and the 198 men who had declared themselves willing to take part in the study was that a greater number (1/3) of the non-responders suffered from acute back pain.

Studies II, III and V

In Study II 27 of the arsenic workers, who had had no or low exposure to vibrating tools, participated. FSP during cooling was measured before and after the summer vacation.

In Study III, which was a clinical trial, seven arsenic workers and 13 other patients with Raynaud's phenomenon participated. Our aim was to test 10 As workers without exposure to vibration and 10 other RP patients. However, in the end only 6 As workers were willing to fulfil the whole study, which was in two parts.

In Study V the subjects were the same as those in Study IV, with the exception of a few drop-outs.

6. METHODS

Arsenic-exposed and non-exposed workers were studied by means of a questionnaire, a verbal interview, an examination by a physician, and certain physiological tests and laboratory analyses.

Questionnaire

The questionnaire contained approximately 80 questions about the occurrence of respiratory difficulties, chest pain, Raynaud's phenomenon, nerve dysfunction in arms and legs, smoking or tobacco usage, environmental conditions at workplaces, and exposure to solvents, lead, arsenic and sulphur dioxide. As a basis for our questionnaire we used WHO's questionnaire for
evaluating dyspnoea, angina pectoris and intermittent claudication in leg arteries (Rose and Blackburn, 1968). For evaluation of vessel spasm a somewhat modified form of Thulesius's Raynaud questionnaire was used (Thulesius, 1978; Taylor and Pelme, 1976). By means of the questionnaire the best matches in the referent group were chosen (c. f. the preceding section, Subjects). A Swedish version of the questionnaire used is published in Lagerkvist et al., 1983.

History and physical examination

In connection with the physical examination each subject was interviewed and the questionnaire was filled in, if that had not already been done. The questions about use of vibrating tools were analysed carefully because those data were used to group the subjects into vibration-exposed and non-exposed groups. Ten As-workers and 15 referents had indicated in the questionnaires that they had not used vibrating tools. However, during the interviews it became clear that the 10 As-workers and 15 referents in fact had used vibrating tools, either several years earlier or at a level which they thought was insignificant. Therefore those subjects were considered to have been exposed to vibrating hand-tools according to the criteria used (Study I). The interview also included questions about symptoms suggesting sciatic nerve damage, operations for slipped disk, any possible family history of neuropathy and past and present alcohol use (or abuse).

The physical examination included inspection of skin, mouth, pharynx and nasal septum, auscultation of heart and lungs, palpation of peripheral pulses (at radial artery, femoral-, popliteal-, and dorsalis pedis arteries) and palpation of abdomen.
Distal blood pressure and plethysmography.

In order to detect arterial obstruction systolic blood pressure (BP) was measured in one finger and one toe at a skin temperature of 30°C or more (Strandness and Bell, 1965). Finger and toe pulse curves were determined on a plethysmograph with a mercury strain gauge according to Strandness et al., 1961. Propagation times and inclination times were measured on the pulse wave curves according to Zetterqvist, 1978.

Finger blood pressure during cooling

Throughout the years, different cold provocation tests have been used to diagnose Raynaud's phenomenon (RP) in the laboratory (Lewis, 1929; Agate, 1947; Sigroth, 1957; Reviews: Arneklo-Nobin, 1983; Pyyköö, 1986; Ekenvall, 1987). Immersion of the hands in cold water, visual inspection, and/or recording of the recovery time of skin temperature, is uncomfortable for the patient and time consuming for the staff. Different modifications of these tests are still used. However, the intra-individual variability among the different test methods due to, e.g., diurnal variations and the body's heat balance, is large (Pardy et al., 1982; Ekenvall and Lindblad, 1979; Hack et al., 1986). An advantage is that all five fingers can be measured simultaneously.

During the late 1970's the method to measure FSP during local cooling with strain-gauge plethysmography which we have used in our studies was developed (Nielsen and Lassen, 1977; Krähenbühl et al., 1978; Nielsen, 1978; Olsen and Nielsen, 1978; Nielsen et al., 1980; Olsen et al., 1981).

Our reasons for choosing this method were that it was simpler to perform and easier to standardize than the measurement of skin temperature recovery after cooling the hands in cold water, which was then used at our laboratory. As the local cooling was performed during arrest of the blood flow, temperature gradients among the digital arteries were avoided.
(Arneklo-Nobin, 1983). In addition, this local ischaemia makes the skin temperature, sub-cutaneous temperature, and thus arterial wall temperature equal (Nielsen and Lassen, 1977).

Finger systolic pressure (FSP) during cooling was measured at a room temperature of 20-22°C. The measurements were made during standardized conditions using combined local finger cooling and a general cooling of the body with water-perfused blankets. Simultaneous FSP measurements were made in two fingers on each hand. The occluding cuff around the cooled finger (digit II) was perfused with water at preset temperatures of 30, 15 and 10°C. The cuff around the reference finger (digit IV) was inflated with air. After five minutes of occlusion the pressure was slowly reduced until an arterial inflow was registered on the plethysmograph. The pressure at that moment is defined as the reopening - or critical opening pressure (COP). Skin temperature was recorded with thermocouples placed under the finger BP-cuff.

FSP at 15 and 10°C was expressed in mm Hg and as a percentage of the pressure at 30°C, corrected for changes in blood pressure in the reference finger (FSPref) according to the following formula (Nielsen, 1978).

\[
FSP\% = \frac{FSP_{15\text{ or }10°C}}{FSP_{30°C} - (FSP_{\text{ref, }30°C} - FSP_{\text{ref, }15\text{ or }10°C})} \times 100
\]

The lowest of the FSP-values at 15°C or 10°C skin temperature from the right or the left hand was used as the FSP value for each individual. Arm blood pressure was measured immediately after each FSP measurement.
Evaluation of the FSP measurement during cooling

Calculation of the FSP%. In healthy controls Ekenvall and Lindblad found a significant linear correlation between the change in the systolic pressures in the arm and in the reference finger during the test procedure (Ekenvall and Lindblad, 1986). Between the measurements at 30 and 15°C of the cooled finger the median arm BP-change was 0 (-15 to 15) mm Hg and the FSP-change in the reference finger was -5 (-20 to 10) mm Hg. Some of their patients with vibration-induced white fingers had a large decrease of the FSP in the reference finger between 30 and 15°C. In our study both As-workers and referents showed a significant, and approximately equal, reduction of finger BP during body cooling (Table 3). The arm BP increased slightly.

Table 3. Change in systolic blood pressure (6 P) during general body cooling for 15 minutes. T-test using paired observations in 47 arsenic workers and 48 referents, mean ± SD.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>6 P (mm Hg during body cooling)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>test finger***</td>
<td>reference finger***</td>
</tr>
<tr>
<td>Arsenic workers</td>
<td>- 20±17</td>
<td>- 19±14</td>
</tr>
<tr>
<td>Referents</td>
<td>- 20±17</td>
<td>- 18±14</td>
</tr>
</tbody>
</table>

*** Statistically significant decrease in systolic finger blood pressure in both arsenic workers and referents, p<0.001. * Slight increase, p=0.04. † Slight increase, p=0.07.
According to Ekenvall and Lindblad (1986) the vasospastic reaction on general cooling makes the FSP in the reference finger less suitable to compensate for systemic arterial pressure changes during the test procedure. Instead, they suggested that the FSP in the cooled finger should be expressed in percent of the systolic arm BP measured on the same occasion. Ekenvall (1987) found no significant difference between the two ways of calculating the FSP%. I recalculated the results from the finger BP measurements in Study I, defining the FSP% as the FSP in the cooled finger in percent of the arm BP, measured almost simultaneously. There was a significant difference between the FSP% calculated by the two different methods among the As workers, but not among the referents (Table 4).

Table 4. Systolic blood pressure (mm Hg, mean ± SD) in the test finger (FSP) before and after body cooling (b.c.) at finger skin temperatures 30 and 10°C in 47 arsenic (As) workers and 48 referents. FSP%; I: FSP at 10°C in percent of FSP at 30°C in the test finger corrected for changes in the reference finger between 30 and 10°C. II: FSP at 10°C in percent of the actual arm blood pressure (BP).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>FSP 30°C mm Hg</th>
<th>FSP 10°C mm Hg</th>
<th>FSP% 10°C I**</th>
<th>FSP% 10°C II**</th>
</tr>
</thead>
<tbody>
<tr>
<td>As workers</td>
<td>139±20</td>
<td>119±19</td>
<td>64±37</td>
<td>51.6±27.5</td>
</tr>
<tr>
<td>Referents</td>
<td>134±15</td>
<td>114±17</td>
<td>77±36</td>
<td>62.3±27.0</td>
</tr>
</tbody>
</table>

** Statistically significant difference between As workers and referents, p<0.01 with the rank sum test. No significant difference between reference BP measured in one finger and in the arm, p>0.5 with the paired t-test among the referents; p<0.01 among the As workers.
It thus seems that it is questionable if one can use FSP in percent of arm BP instead of the more complicated FSP in the cooled finger in percent of FSP at 30°C corrected for FSP changes in a reference finger. When discussing these two methods one must consider that the pressure in the reference finger can be measured at exactly the same time as the measurement in the cooled finger. Arm BP must be measured immediately after one has measured the pressure in the cooled finger. Therefore it is theoretically possible that the systemic BP might change during the short period between the finger BP measurement and the arm BP measurement. Before a definite standpoint is taken, further studies should be made, including tests of the reproducibility of the two methods.

The reproducibility of the FSP measurements during local cooling has been evaluated by duplicate FSP recordings at 10°C skin temperature in 66 subjects in Study I as described in Study II. There was no significant difference between two consecutive measurements. The coefficient of variation (CV) was 9% in the test finger and 4% in the reference finger. At 15°C skin temperature Nielsen found a CV of 21.6% in repeated measurements at 3-5 day intervals (Nielsen, 1978). If the interval between the measurements increases the difference between the test results also increases. In Study III we examined 21 of the arsenic workers form Study I. If these measurements were regarded as duplicate measurements the CV was 50% at 10°C. During three years a natural improvement or impairment of the cold-induced finger vasospasm may have occurred, and this change contributes to the increase of CV with increasing interval between duplicate measurements.

The tendency to develop vasospasm even to a standardized cooling procedure varies from time to time in patients with vibration-induced white fingers (Pyykkö et al., 1986; Ekenvall, 1987). This variation is possibly due to differences in the heat-balance in the body (Ekenvall and Lindblad, 1979). Therefore it is very important to standardize the test procedure as much as possible. Water-perfused blankets are probably more
efficient in decreasing a body heat excess than cool air in the room.

Seasonal variation of the FSP during local cooling has been assumed to be a possible factor which may affect the FSP-values (Lindblad, personal communication). Therefore we subdivided the subjects in Study I into four groups: As-workers and referents examined during May - September, and As-workers and referents examined during December - February. There was no statistically significant difference between the subjects examined during the summer or the winter season, neither among the As-workers, nor the referents (Study II). If there are seasonal variations they do not appear with the standardized method used. Thus the standardized body and local cooling minimized possible seasonal effects on the FSP.

Smoking habits, use of certain drugs, e.g., β-receptor blocking agents, and use of vibrating hand tools must also be considered. In our studies the referents had the same smoking habits as the arsenic workers, and the referents and the arsenic workers were age-matched. Our aim was to match the referents to the As-workers with respect to the use of vibrating hand tools. However, the As-workers which had been exposed to vibrating hand tools had been exposed to a shorter total time than the corresponding referents. That presumably reduced the difference between the two groups.

The sensitivity of the FSP measurement, with local cooling, to detect white fingers was 70% in our arsenic workers with the criteria used (Study I). Out of ten arsenic workers with Raynaud's phenomenon, seven had vasospasm at the FSP-measurement during cooling. Different authors have reported a sensitivity of about 75% in different patient groups (Thulesius, 1982; Ekenvall and Lindblad, 1986). The sensitivity increased with general cooling and with standardized local and body cooling (Nielsen, 1978; Olsen and Nielsen, 1979; Ekenvall and Lindblad, 1982). The sensitivity of the test also increased
with increasing intensity of the symptoms according to the Taylor-Pelmear symptom scale from 57% in mild to 77% in advanced disease (Ekenvall, 1987).

Clinical and neurophysiological studies

The neurological examination of the patients included estimation of total strength in the lower legs, feet and hands, tests of reflexes in arms and legs (including Babinski reflex) and testing for sensitivity to pressure, pin prick and vibration in extremities. Sensitivity for vibration was tested with a tuning fork (129 Hz).

Electromyography (EMG) was recorded bilaterally in the lower extremities on a Medelec MS92 electromyograph. Concentric needle electrodes were used and the results were graded according to principles used in routine clinical work (Mayo Clinic, 1971).

Nerve conduction velocity (NCV) measurements were performed in all four extremities in Study IV and in the right arm and leg in Study V. Skin temperature was approximately 32°C in Study V. In Study IV the temperature may have varied more. Surface electrodes were used for both stimulation and recording. In Study IV bipolar stimulation electrodes (DISA) were used for recording of both sensory and motor NCVs. In Study V the motor responses were recorded with separate electrodes. Motor NCVs of the ulnar (elbow-wrist) and deep peroneal and tibial (knee-ankle) nerves were measured. Sensory NCVs were obtained after antidromic stimulation of the ulnar (wrist-finger) and the sural (calf-foot) nerves. All latencies were measured to first negative deflection.

Variability in NCV measurements

There are several possible sources of errors in the NCV-recording technique, such as poorly defined take-off of the
evoked response, inaccurate calibration, and imprecise surface determination of the nerve length. On repeated testing the values may vary several meters per second due to these limitations of the technique. However, the results are sufficiently reproducible if standardized techniques are used (Kimura, 1983).

A number of external factors may further modify the results in motor and sensory NCV studies. These are skin temperature, nerve length, age and ischemia. The NCV increases linearly by approximately 5% per degree when tissue temperature is increased from 29 to 38°C. There is a linear correlation between skin temperature and subcutaneous and intramuscular temperature. Therefore the limbs should be warmed if skin temperature is below 34°C (Kimura, 1983). Longer nerves generally conduct more slowly than shorter nerves, which is one explanation for the lower NCV seen in leg nerves as compared with nerves in the arm. There is a rapid increase in NCV during infancy and childhood and a slight decrease in higher age groups (Kimura, 1983).

In our Studies IV and V we were faced with the problems concerning reproducibility and variability of the NCV measurements. These are discussed in Study V. Because we compared subjects with referents, examined during the same time period, the variability in the measurements is less important than if we had followed only one group.

Analytical methods

Elevated levels of arsenic in urine reflect an environmental exposure to arsenic, as there is a significant correlation between arsenic levels in urine and air levels (Pinto et al., 1976; Smith et al., 1977; Vahter et al., 1986). Single determinations of As in urine, however, give no information on the cumulative exposure to As.
In addition, urinary levels cannot be used for direct comparison of exposure levels in different studies due to different methods of analysis. In earlier studies total arsenic, which includes organic arsenic compounds, was analysed. During the 1970's the hydride generation technique made it possible to determine inorganic arsenic and its methylated compounds with AAS, atomic absorption spectrophotometry (Braman and Foreback, 1973; Norin and Vahter, 1981).

Inorganic arsenic and its methylated metabolites, MMA and DMA, were analysed in morning samples of urine. The analyses were made by the hydride generation technique on a Perkin-Elmer atomic absorption spectrophotometer Model 503, equipped with an MHS-20 mercury/hydride system. The analyses were made by Ulla Westerlund, M.S., at the factory laboratory at the smelter. The method used was a slight modification of the "operator's manual" procedure (Study I; Norin and Vahter, 1981). To test the accuracy of these measurements duplicate samples were also tested at two different laboratories, the National Institute of Environmental Medicine (Study I) and the commercial laboratory, Analytica, in Stockholm (Study V). A very good correlation was obtained, $p < 0.001$, at both laboratories. Linear regression coefficients were 0.98, and 0.98, $n_I = 23$ and $n_V = 6$, respectively.

Absorption of arsenic

In Study I we discussed total absorption, assuming exposure levels around the Swedish occupational standard. Considering the particle size of the dust, the deposition in the airways, and the lung ventilation, we arrived at a daily absorption of arsenic of up to 300 µg from 1975 and ten times higher before that. The mean duration of exposure was 23 years which gave us a mean total absorption of about 4 g (mean daily absorption being about 100 µg after 1975 and 1000 µg before that). Maximal total absorption was calculated to be about 20 g. By estimating total exposure from the occupational limit values we
did not consider the intensity of exposure which has varied in
different work places (Holmqvist, 1951; Nygren, 1980; Järup et
al., 1988). This might have given an underestimation of the
cumulative exposure and total absorption to arsenic.

Statistical analysis

In the studies of this thesis both "conventional" statistical
methods (Colton, 1974) and a computerized multivariate data
analysis developed during the 1980's by Wold H, Wold S et al.
were used. This multivariate analysis, the partial least
squares (PLS) method, allows comparison between many and dif­
ferent biological parameters. The variables need not be inde­
pendent of each other and may be linear or non-linear e.g.,
blood pressure in mm Hg, skin temperature in degrees centi­
grade and smoking habits. With the PLS method we examined the
relation between predictor variables (X), such as age and ab­
sorption of arsenic, and response variables (Y), such as nerve
conduction velocity and finger blood pressure. The underlying
principle is that each object has a definite position in each
of two multidimensional spaces, one for X and one for Y,
determined by the values of its variables X and Y (Wold S et
determines a linear model in each space so that the coor­
dinates in the X-model predicts the coordinates in the Y-model.

7. VASOSPASTIC TENDENCY AND RAYNAUD'S PHENOMENON IN SMELTER
WORKERS EXPOSED TO ARSENIC

Results

Raynaud's phenomenon and vasospasm in smelter workers

Ten As-workers and two referents reported Raynaud's phenome­
on (RP), p<0.05. Fifteen As-workers and eleven referents had
signs of arterial obstruction in their legs, and one As-worker
in one arm at the distal BP and toe plethysmography measurements \( p > 0.2 \).

During local finger cooling to \( 10^\circ C \) there were significant differences between As-workers and referents. In Table 4 the results from the FSP measurements are given both in mm Hg and as percent of the pressure at \( 30^\circ C \), corrected for BP-changes in the reference finger, between the FSP-measurements at 30 and \( 10^\circ C \), FSP\% (see Methods). FSP at \( 10^\circ C \) in percent of the arm BP is also given. The mean decrease of FSP between the measurements at \( 30^\circ C \) and those at \( 10^\circ C \) (FSP-difference) was 55 mm Hg in the As workers and 37 mm Hg in the referents, \( p < 0.01 \). There was also a significant difference between the groups in FSP\% at \( 10^\circ C \), \( p < 0.01 \). This means that the arsenic workers reacted more strongly than the referents to local and body cooling. Thus the As-workers had a greater tendency to vaso­spasm. The FSP-difference between the measurements at 30 and \( 10^\circ C \) was significantly correlated to the estimated cumulative absorption of arsenic, \( p < 0.05 \).

The concentration of inorganic and methylated As in urine was 1 \( \mu \text{mole/l} \) (71 \( \mu \text{g/l} \); range 10-340) in the As-workers and 0.1 \( \mu \text{mole/l} \) (7 \( \mu \text{g/l} \); range \( \leq 20 \)) in the referents, \( p < 0.001 \). Two thirds of the referents' values were \( \leq 5 \mu \text{g/l} \). No statistically significant correlation was found between As in urine and vasospasm.

In Study II -- Effects of summer vacation on arsenic induced vasospasm -- we found no difference in FSP at local cooling before and after the summer vacation. The tendency to vasospasm at cooling did not change during 4-8 weeks with no exposure to arsenic. The arsenic levels in urine, however, decreased to normal values. Our conclusion was that peripheral vascular disturbances were independent of short-term fluctuations in arsenic exposure. FSP levels on cooling were significantly increased as compared to the measurements made three years earlier. We have interpreted that finding as a slowly
occurring improvement in finger blood circulation. The reason for an improvement could be that the arsenic levels were then lower than in previous years.

In Study III -- Effects of Ketanserin on arsenic induced vaso-spasm -- we showed that Ketanserin, a serotonin antagonist, reduced the cold-induced vasospasm in finger arteries when given intravenously. This was recorded as higher skin temperature and FSP after cooling with Ketanserin than with saline solution. We concluded that serotonin is involved in the mechanism behind the vasospasm of arsenic workers as it is known to be in patients with primary and vibration-induced Raynaud's phenomenon. Oral treatment with 2 x 40 mg Ketanserin daily did not give any significant improvement of the FSP or skin temperature. This might have been due to lower tissue concentrations with oral treatment than with intravenous administration.

Discussion

The increased prevalence of Raynaud's phenomenon and vasospastic tendency were consistent with earlier reports on peripheral vascular disorders after ingestion of arsenic with drinking water (Geyer, 1898; Butzengeiger, 1940; Tseng et al., 1968; Borgono et al., 1977; Tseng 1977).

Maurice Raynaud, general practitioner in Paris, first described a syndrome characterized by sequential blanching, cyanosis and redness of the hands in response to a cold stimulus (Raynaud, 1888). In 1901 Hutchinson suggested that this local asphyxia should be called Raynaud's phenomenon (RP). He pointed out that it was not a new syndrome and that the skin colour changes were either due to vasospasm or lesions on the vessel walls (Hutchinson, 1901). The typical skin colour changes in RP occur when there is a complete closure of the digital arteries.
A "milder" form of RP in which the blood vessels contract but total stoppage of blood flow does not occur is referred to as vasospastic condition or vasospastic tendency. Attacks of RP are usually provoked by cold. Other factors, such as noise or emotional stress, may also induce the vasoconstriction in sensitive persons (Allen and Brown, 1932; Fox, 1968; Freedman and Ianni, 1983).

RP occurs in two different types of subjects, and the RP is referred to as either idiopathic or secondary RP. Idiopathic RP is often referred to as Raynaud's disease (Allen and Brown, 1932; Lewis, 1938; Taylor, 1974; Coffman and Davies, 1975). Blunt and Porter suggest Raynaud syndrome (RS) instead of RP, and use the categories: Idiopathic RS and RS with an associated disease or cause, instead of idiopathic and secondary RP (Blunt and Porter, 1981). The prevalence figures for idiopathic RP, or Raynaud's disease, or idiopathic RS, vary in different studies, from 2-6% among men and 9-22% among women (Thulesius, 1981; Gemne, 1982; Olsen and Nielsen, 1978). A common finding is an increased ratio of women to men (Gifford and Hines, 1957; Spitell Jr, 1972; Taylor, 1974; Coffman and Davies, 1975).

Since Raynaud gave his criteria for RP: episodic colour changes, bilateral distribution, absence of organic arterial occlusions -- several authors have discussed and modified these criteria (Allen and Brown, 1932; Hunt, 1936; Allen et al., 1962; Blunt and Porter, 1981; Ekenvall, 1987). I have used the classification introduced by Taylor and Pelmear, which includes neurological symptoms such as numbness and paresthesia (Taylor and Pelmear, 1976; Thulesius, 1976 and 1978; Lagerkvist et al., 1983).
Pathophysiology of Raynaud's phenomenon

Five different mechanisms have been discussed as being involved in the pathogenesis of RP: increased vasoconstrictor tone (Raynaud's own hypothesis), local vascular abnormality, low blood pressure, increased viscosity of the blood, and immunological factors (Blunt and Porter, 1981; Arneklo-Nobin, 1983; Thulesius, 1985; Ekenvall 1987).

Increased vasoconstrictor tone may play a role in RP. Finger skin temperatures and blood flow are lower in patients with Raynaud's disease than in normals (Peacock, 1959; Blunt and Porter, 1981). The finger blood flow decreases in both patients and healthy subjects when the body is cooled (Jamiesson, 1971; Nielsen, 1978). The finger BP decreased significantly in both arsenic workers and controls after 15 minutes body cooling (Study I).

The increased sympathetic activity seen in response to general cooling is necessary for the temperature balance of the body but causes vasospasm or complete closure of the digital arteries in RP patients. Subjects with RP react more strongly than other subjects to general cooling. However, sympathectomy or nerve blockade does not prevent attacks of RP (Johnston 1965; Blunt and Porter 1981, review).

The receptor concept was introduced by Langley, Erlich and Sir Henry Dale in the beginning of this century (Wennmalm, 1976). In 1931 Lewis and Pickering suggested that sympathetic vasodilator nerves existed in man (Lewis and Pickering, 1931). Such nerves were not isolated, but during the last 40 years there has been intensive research in this field. Two different kinds of α- and β-receptors are now recognised. The α-receptors are involved in the contraction and the β-receptors in relaxation of peripheral vessels (Ahlquist, 1948; Lands et al., 1967; Drew and Whiting, 1979; Cohen and Coffman, 1981; Arneklo-No­bin, 1983, review; Goldberg and Robertson, 1984). Serotonin
receptors are also present in peripheral vessels, and different serotonin receptors, $S_1$ and $S_2$, have been described (Peroutka and Snyder, 1979). Like noradrenalin, serotonin is a potent vasoconstrictor (Janssen, 1985). Selective damage to the $a_1$-receptor has been discussed as a cause to secondary RP (See page 35).

Local vascular abnormality. Increased sympathetic activity as a cause of RP was first questioned by Lewis. In 1929 he introduced his theory of the "local fault" (Lewis, 1929). In the beginning he discussed arterial wall hypertrophy or arterial occlusion as causative agents of RP. In a later study (Lewis, 1938) he found no significant difference between finger arteries of patients or control subjects. Thulesius points out that the special anatomy of the digital arteries, a wall-lumen ratio of nearly 2:1, gives marked reduction in blood flow with only small changes in tone or diameter (Thulesius, 1985).

In the beginning of the 1980's Furchgott et al. found a relaxing factor in intact endothelial cells: endothelium-derived relaxing factor, EDRF (Furchgott et al., 1983). Since then a growing interest has been focused on local vasoconstrictor and dilator factors, e.g. prostacykline and nitric oxide. (Moncada et al., 1987; Palmer et al., 1987; Thulesius, 1988; Vanhoutte, 1988). Vasodilatory factors are probably released mainly on the arterial side while the vasoconstrictive factors are mainly on the venous side. Thus Lewis' almost 60-year-old theory has gained renewed attention.

Low blood pressure may play a role in RP. In a group of patients with primary RP, Thulesius found lower blood pressure than among controls of similar age (Thulesius, 1976).

Increased viscosity of the blood has been discussed as one explanation for RP. Different studies on blood viscosity have been performed but the results are not conclusive (Pringle et al., 1965; Jamieson et al., 1971, Goyle et al., 1976; Jarret et al., 1978; Jahnsen et al., 1977).
Immunological factors have been discussed in connection with secondary RP in connective tissue disorders (Kallenberg *et al.*, 1980; Masi *et al.*, 1980; Blunt and Porter, 1981; Arneklo-Nobin, 1983).

Other causes known to be associated with secondary Raynaud's phenomenon are trauma, exposure to vibration, occlusive vascular disease, collagen disease, vascular compression (thoracic outlet) syndrome, hypothyroidism, and certain drugs, e.g., ergotamins and beta blockers (Reviews: Strandness, 1969; Taylor, 1974; Coffman and Davies, 1975; Blunt and Porter, 1981; Arneklo-Nobin, 1983).

In RP secondary to vibration, vessel wall hypertrophy causing a lumen reduction, or sympathetic hyperreactivity, have been proposed as possible pathogenic mechanisms (Sivertson and Ljung, 1976; Pyykkö and Hyvärinen, 1976; Gemne, 1982, review).

During the 1980's several authors, using $\alpha_1$- and $\alpha_2$-agonists and antagonists, have studied the relationship between the $\alpha_1$- and $\alpha_2$- receptors in the vasoconstriction at exposure to cold in cutaneous vessels (Flavahan *et al.*, 1985; Vanhoutte *et al.*, 1985; Ekenvall, 1987). One mechanism behind the cold-induced vasospasm might be an increased ratio of $\alpha_2$- to $\alpha_1$-receptors in the finger vessels, e.g., through damage to the $\alpha_1$- receptors caused by vibration trauma.

Possible mechanism behind the As-induced vasospasm

As one associated and contributing group of factors to secondary RP, Taylor (1974) names "intoxication" by ergot and nicotine. The vasospasm induced by arsenic may fall into this category. Coffman and Davies, however, state that the evidence for arsenic poisoning producing RP is tenuous (Coffman and Davies, 1975).
In Study III the cold-induced vasospasm was reduced by intravenous injection of Ketanserin, a serotonin antagonist. Arneklo-Nobin and Owman showed that serotonin caused the same contractile response in isolated hand arteries as did adrenaline and noradrenaline. Ketanserin acted as a competitive antagonist to serotonin (Arneklo-Nobin and Owman, 1985). At low concentration, $10^{-8} \text{M}$, there was, however, often a slight potentiation of the serotonin effect.

The effect of Ketanserin to block vasoconstriction in peripheral vessels may be due to blockage of both serotonin- and $\alpha$-adrenoceptors. Ball and Robertson compared the effects in humans of Ketanserin with that of $\alpha_1$-adrenergic antagonists in current use. They found evidence for both $\alpha_1$-adrenergic and serotonergic antagonism during chronic administration of Ketanserin (Ball and Robertson, 1985). However, Ketanserin in concentrations of $10^{-8}$ to $10^{-5} \text{M}$ had no antagonistic effect on the noradrenaline-induced contraction in hand arteries and veins in vitro (Arneklo-Nobin and Owman, 1985).

As discussed in Study III Ketanserin may modify the contractile response to serotonin and other vasoconstricting agents in several ways. The vasodilation obtained with intravenous injection of Ketanserin indicated that similar mechanisms are involved in the cold-induced vasoconstriction in As-workers and in other patients with RP.

I have, however, no evidence of selective damage to a specific type of receptor. Arsenic is neurotoxic and affects peripheral motor and sensory nerves (Studies IV and V), and an adverse effect of As on the nerve endings or the receptors in digital blood vessels is possible.

Another hypothesis is that arsenic damages endothelial cells. In Study I we concluded that the increased vasospastic reactivity in the arsenic workers as compared with the referents might be due to functional alterations in the hand vessels.
caused by long-term exposure to inorganic airborne arsenic. Intact endothelial cells can release either vasodilatory or vasoconstrictive factors (page 34). It is possible that endothelial damage, or abnormal secretory function of endothelial cells on the arterial side, can be correlated to increased vasmotor tone such as that seen in hypertension or peripheral perfusion abnormalities (Thulesius, 1988). Both an endothelial injury and damage to the nerve ending or the receptor by arsenic could cause an imbalance between vasoconstriction and dilation. Other possible mechanisms behind the As-induced vasospasm, such as vessel wall hypertrophy, are not discussed here. Further studies are needed for definite conclusions of the underlying mechanism, before we can state as definitely as Butzengeiger: "Dass das Arsen zu Capillarschädigungen führt, ist allgemein anerkannt" (Butzengeiger 1940).

8. EFFECTS OF ARSENIC ON PERIPHERAL NERVES

Results

In Study IV physical examination, electromyography (EMG), and nerve conduction velocity (NCV) measurements in five peripheral nerves were made in 47 smelter workers and 50 referents. Forty As workers reported episodes of acute arsenical dermatitis, and 10 subjects had a perforation of the nasal septum. Otherwise the prevalence of single clinical symptoms, signs, and abnormalities at the electromyographic examination was approximately the same in arsenic workers and referents.

The As workers had lower mean NCVs than the referents in all five examined nerves, but the differences were not statistically significant for single nerves (Table 3 in Study IV). Seven As workers and two referents had NCVs below the lower limits (mean -2 SD) of the reference group in two or more nerves. They were classified as having subclinical neuropathy. Two As workers and two referents had possible explanations
other than arsenic for this finding. Even if these four men were excluded the difference between As workers and referents was statistically significant, \( p=0.03 \). A multivariate data analysis was performed with the PLS method, including data from the NCV-measurements, the FSP-measurements during cooling, certain anamnestic data, such as smoking habits, use of vibrating hand-tools, arsenic in urine, and cumulative exposure to arsenic. This analysis revealed statistically significant correlations between reduced NCV in three peripheral nerves, decrease in FSP during cooling and cumulative exposure to arsenic and age.

In Study V, which was a follow-up study five years after the 1982 study (IV), the difference in NCV between arsenic workers and referents had increased (Tables 2 and 4 in Study V). The arsenic workers had also experienced a greater number of medical problems (Table 1, Study V). These findings indicated that the environment at the smelter might be more damaging to a person's health than the milieu at the referents' workplace.

From 1982 to 1987 there were no statistically significant changes in the prevalence of subclinical neuropathy, defined as reduced NCV in two or more nerves (Table 6, V). The significant difference between the arsenic workers and referents in the mean NCVs of two peripheral nerves, found when the nerves were tested one at a time, was also defined as a sign on subclinical neuropathy. The subclinical signs of neuropathy correlated to cumulative absorption of arsenic. The neuropathy which the arsenic workers had contracted during their long-term exposure to arsenic did not appear to be reversible, even if exposure ceased. The multivariate data analysis, PLS, of the combined group of As-workers and referents revealed statistically significant correlations between NCV in four peripheral nerves, age and estimated cumulative exposure to arsenic.
The As-workers' mean levels of inorganic and methylated arsenic in urine were 71 µg/l (range, 10-340) in Study IV and 40 µg/l (range, 5-520) in Study V.

Discussion

Peripheral nerve affections, from simple neuralgia to severe paralysis, caused by inorganic arsenic have been known and discussed for centuries. Geyer, in his review on the different clinical manifestations of chronic As exposure through As-polluted drinking water cited about 200 references (Geyer, 1898). One of the oldest is from 1788, Fieliz, and has the title: Paresie des membres inferieurs.

Without stating his own opinion, Geyer refers to the discussion among his colleagues whether the arsenic neuropathy is of spinal nature or is a polyneuritis with or without involvement of the motor- and sensory end-plates.

Geyer's main objective was to describe the skin changes, such as melanosis and hyperkeratosis, caused by arsenic, but systemic effects were also discussed. Among them were the above mentioned nerve affections. "Old age gangrene" in the toes, feet, upper and lower extremities were reported to be surprisingly common.

In 1940 Butzengeiger published 15 case histories on severe disturbances of the peripheral circulation leading to gangrene in toes or fingers in six cases. These 15 patients, and 20 subjects with symptoms of RP and paresthesias were part of a group of 180 vintners with long-term exposure to As in pesticides and wine, the "Haustrunk". The daily consumption of this wine, which was made from already pressed grapes, was 1 - 2 l. Frey (1943) estimated the daily intake of As to be about 3-30 mg (Frey, 1943, dissertation in German cited in Lüchtrath, 1983).
Only three of Butzengeiger's 15 patients had "normal" urinary levels, 100 µg/l. The hair levels were "slightly elevated", <20 µg/g, in two patients, and elevated in twelve. In five of his patients Achilles tendon reflexes were missing. Thus, both Geyer and Butzengeiger describe toxic effects by high-dose exposure to arsenic through drinking water or wine on the peripheral circulation and nerve function.

Most of the peripheral nerve lesions, described in the literature are caused by comparatively high doses of arsenic (for references see Studies IV and V). There are few studies on nerve effects by occupational low-dose exposure. Hine et al., discussing the medical problems associated with As exposure in the Tacoma smelter, Washington, found no clinical neuropathy in the employees, though they "might have been heavily exposed to airborne As₂O₃ in the past" (Hine et al., 1977).

Feldman et al., in a study on 70 copper smelter workers and 41 controls, found an increased prevalence of clinical neuropathy, such as paresthesia and reflex abnormalities, and subclinical neuropathy in the high-As-exposure groups. High-As exposure meant work-room areas where the 5-year average of periodic urinary arsenic samples was above 200 ppb. Subclinical neuropathy was defined as "one or more reduced velocities, or two or more nerves with reduced amplitudes" (Feldman et al., 1979).

Singer et al., found decreased NCVs in the median and sural nerves in 127 copper smelter workers exposed to arsenic and lead for 12 years (range, 0.5-40) as compared with controls. The present levels of lead in blood (mean: 32.8 µg/dl) and arsenic (mean: 33.9 µg/l) in urine were not correlated with the NCVs (Singer et al., 1982).

Kreiss et al. studied 147 persons exposed to arsenic from well water in Alaska, with NCV and clinical neurologic examinations (Kreis et al., 1983). The median As concentration was 41 µg/l (range, 1-4781) in the drinking water and 51 µg/l in the urine.
(range, 6-4964). The calculated index of the daily ingestion of As was 14.5 μg and correlated closely with the urine As concentration. Median exposure time was 5 years. Six persons had symptoms or clinical signs of sensory neuropathy and 13 persons had one or more abnormal NCVs. However, no dose-response relationship was found between As ingestion and NCVs.

The urinary levels of As, provided that analytical methods were the same, were about the same in our studies and in the studies by Singer et al. and by Kreiss et al. but lower than in the Feldman et al. study, which was the only one that reported clinical neuropathy.

As discussed in Studies IV and V, most authors are of the opinion that As neuropathy is a sensory-motor distal axonopathy with accompanying demyelination (Reviews in Chhuttani and Chopra, 1979). The biochemical effects of arsenic at the cellular level, e.g. binding to SH-groups and inhibition of oxidative phosphorylation, are consistent with a process leading to distal axonal degeneration (Manzo 1985).

Differential diagnoses which should be considered in peripheral neuropathy with As exposure are exposure to alcohol, solvents, metals such as lead, gold and thallium, certain diseases, e.g. diabetes and thiamine deficiency (Reviews: Buchanan, 1962; Jenkins, 1966; Chhuttani and Chopra, 1979). As discussed in Studies IV and V these differential diagnoses are not considered to confound our results, with the possible exception of the referents' exposure to solvents. That might have decreased the difference in subclinical neuropathy between the As workers and referents.

In conclusion, we found decreased nerve conduction velocities in smelter workers with long-term exposure to arsenic. If we had performed only a clinical neurologic examination we would not have detected this subclinical neuropathy. With the clinical physiological and neurophysiological laboratory methods
used, I and my coworkers have been able to demonstrate sub-clinical effects by long-term low-dose, exposure to inorganic airborne arsenic on the peripheral circulation and nerve function.

9. GENERAL CONCLUSIONS

A group of smelter workers with long-term airborne exposure to inorganic arsenic of relatively low-dose were studied, and adverse effects on the peripheral blood circulation were found. These effects were an increased frequency of Raynaud's phenomenon (RP) and an increased tendency to vasospasm in finger blood vessels during local and general cooling.

These findings were in accordance with earlier reports on vascular disorders after ingestion of large amounts or arsenic with drinking water in Taiwan and in Chile. Disturbances of the peripheral circulation have not previously been reported in this kind of occupational airborne exposure. During a short break, 4-8 weeks, in the exposure to arsenic, As-levels in urine decreased to normal values, whereas there was no change in the vasospastic reaction in finger blood vessels on cooling.

However, the finger blood circulation during cooling had improved significantly as compared with the finger blood pressure measurements three years earlier. These findings indicated that there was a gradual improvement of the finger blood circulation, and that improvement might be due to a decreased exposure to arsenic.
Intravenous injection of the serotonin antagonist, Ketanserin, in a small number of As workers significantly decreased the vasospastic reaction on cooling. As similar effects had been reported in patients with other types of RP, serotonin seemed to be involved in the mechanism causing vasospasm in both types of subjects.

Subclinical effects on the peripheral nervous system, manifested as reduced nerve conduction velocity (NCV) were found. There were, however, no significant differences in clinical symptoms or electromyographic findings in the smelter workers as compared with the referents.

A statistically significant correlation was found between reduced NCV, decrease of finger blood pressure during cooling (vasospastic tendency) and total estimated absorption of arsenic.

At a follow-up study after five years, the differences in NCV between arsenic workers and referents had increased. Thus the adverse effects of arsenic on the peripheral nervous system do not seem to be reversible. This indicates that occupational exposure of arsenic should be as low as possible.
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