Epidemic outbreak of arsenic neuropathy in south-western Extremadura in 1978. Federico González Dorrego (1946-2015): pioneer of Extremaduran neurology

J. M. Ramírez-Moreno[1,2,3]

[1]Department of Neurology. Hospital Universitario de Badajoz, Badajoz, Spain.
[2]Department of Biomedical Sciences. Universidad de Extremadura, Badajoz, Spain.
[3]Instituto Universitario de Investigación Biosanitaria de Extremadura (INUBE).

ABSTRACT

Arsenic is a xenobiotic metalloid with several toxic mechanisms, affecting practically all tissues and organs, including the nervous system. In 1978, an outbreak of arsenic poisoning in south-western Extremadura due to adulterated wine affected over 300 people, 11 of whom died. The incident offered an opportunity for the clinical, pathophysiological, and neuropathological analysis of an exceptional series of 94 patients with arsenic neuropathy at Residencia Sanitaria de Badajoz. This study was reported in a doctoral thesis displaying commendable technical ability and great scientific rigour, but did not lead to the publication of additional works to further disseminate the findings. In terms of sample size and homogeneity, the patient series studied was one of the best in the scientific literature of the time, and contributed a substantial improvement on the existing knowledge of the disease. This historical review aims to raise awareness of the work of Prof González Dorrego, with a brief summary of the exciting history of arsenic, and the recent literature on its metabolism and the specific mechanisms by which it alters metabolic and cellular function, which are essential to understanding its neurotoxic effects.

KEYWORDS

Arsenic, food poisoning, neurotoxicity, peripheral neuropathy, neurophysiology, neuropathology

"Dosis sola facit venenum"
(The dose alone makes the poison)
Paracelsus (1493-1541)

Introduction

Arsenic (As) is a metalloid element with both metallic and non-metallic properties. It is also classified as xenobiotic, as it has no known physiological function but does exert toxic effects in humans.[1] Exposure usually occurs by ingestion of contaminated food or water, although the substance is also found in wood preservatives, tobacco smoke, the air, and cosmetics.[2,3]

It has toxic effects on practically all organs and tissues. Its harmful effects include skin lesions and cardiovascular, respiratory, and gastrointestinal disorders. In the nervous system, it can cause peripheral neuropathy, encephalopathy, and behavioural alterations; an association with neurodegeneration is also reported.[2] The human body contains approximately 0.02-0.08 mg of arsenic per kg body weight, mainly concentrated in the liver, kidneys, lungs, bones, and hair. Presence of arsenic in the environment is associated with severe health issues worldwide, with excessive or prolonged exposure having been associated with a wide variety of diseases, including cancer, and possibly death.[2,3]

The most common neurological complication of arsenic poisoning is symmetrical sensorimotor polyneuropathy with distal involvement.[4] The most frequent electrophysiological finding is severe sensory nerve conduction deficit with moderate motor nerve conduction deficit.[5,6] In addition to the classical chronic axonal presentation, cases have been described of demyelinating polyneuropathy resembling Guillain-Barré syndrome; data from the neurophysiological study and, occasionally, cerebrospinal fluid analysis may also support the diagnosis.[7]

Despite an abundance of toxicological and epidemiological studies demonstrating the neurotoxicity of arsenic,[8] our understanding of the substance is in constant development.[9-13]

This review provides information on an outbreak of arsenic poisoning due to adulterated wine in south-western Extremadura (Spain) in the 1970s,[14] which enabled the study of an exceptional series of patients with arsenic neuropathy. The event was reported in the doctoral thesis of the neurologist González Dorrego, supervised by Prof Pérez Miranda, who at the time was chair of pathology and clinical medicine at the medical faculty of Badajoz.[15] Unfortunately, no additional studies were published that may have further disseminated González Dorrego's results in the medical community, and particularly among neurologists. This study aims to raise awareness of Prof González Dorrego and an important part of the history of Extremaduran neurology. We briefly summarise the exciting history of arsenic, and the recent literature on its metabolism and the specific mechanisms by which it alters metabolic and cellular function, which are essential to understanding its neurotoxic effects.

Development

A brief history of arsenic

1. Arsenic and its therapeutic properties

The use of arsenicals (compounds containing arsenic) has a long history, with both benevolent and malicious intentions.[16] The word arsenic is derived from the Persian *zarnikh*, meaning "yellow orpiment," a compound of arsenic and sulphur. *Zarnikh* was translated into Greek as *arsenikon*, related to *arsenikos*, meaning "masculine" or "potent." By 2000 BC, arsenic trioxide, a by-product of copper smelting, was used as a drug and as a poison.[17]

Hippocrates (460-377 BC) used yellow orpiment and realgar (red arsenic) to treat skin ulcers.[18] Aristotle (384-

322 BC) and Pliny the Elder (23-79 AD) wrote about the medicinal properties of arsenicals obtained through the calcination of orpiment, but also underscored their potential toxic effects.[19] Galen (130-200 AD) recommended a paste of arsenic sulfide to treat ulcers.[20] Paracelsus (1493-1541) extensively used elemental arsenic. His famous quote, "All substances are poisons […]. The right dose differentiates a poison from a remedy," is completely accurate with respect to arsenic.

In 1786, Thomas Fowler reported the use of a flavoured solution of arsenic trioxide to treat fever and headache. Fowler, together with the apothecary Hughes, had identified arsenic as the main component of the "fever drops" patented by Thomas Wilson (London, 1781).[21] Fowler solution was used to treat such other conditions as psoriasis, eczematous eruptions, dermatitis herpetiformis, asthma, cholera, and syphilis. Charles Darwin is known to have used the solution to treat skin problems in adolescence. In 1865, Fowler solution was the first chemotherapy agent used to treat leukaemia.[22] In the 19th century, arsenicals were ingested, inhaled as vapours, injected intravenously or intramuscularly, or administered in enemas, to treat a wide range of disorders.[21]

In the early 20th century, physicians also used arsenicals to treat pellagra and malaria, just as Avicenna (980-1037) had done nearly a millennium before. In 1912, arsenic was recognised as the best agent in the pharmacopoeia. Almost at the same time, the experiments of Paul Ehrlich (1854-1915) produced arsphenamine (Salvarsan), which was the pillar of syphilis treatment until penicillin became widespread in 1943.[20,23]

Until recently, such arsenicals as melarsoprol and melarsonyl were used to treat African trypanosomiasis, despite their known toxicity and ability to cause severe encephalopathy and even death.[24]

During the global COVID-19 pandemic, the homeopathic medicine Arsenicum album 30C was popularised in some countries as an effective prophylactic against coronavirus infection.[25]

Arsenic has no known nutritional value in humans; however, for a long time, it has been considered to have tonic properties at low doses. "Arsenic eaters" in the mountains of Tyrol (Austria) consumed progressively larger amounts of the element, believing it improved their endurance, appearance, and well-being.[26] Some Indian women use drugs containing arsenic during preg-

Figure 1. Locusta, trusted poisoner at the service of Agrippina, the mother of Nero, tests on a slave the poison prepared for Britannicus. *Locusta testing in Nero's presence the poison prepared for Britannicus*, by Joseph-Nöel Sylvestre, 1870-1880. Public domain.

nancy, seeking to increase the likelihood of their child being a boy. Ayurveda, the traditional system of medicine followed by a large part of the Indian population, includes a branch named *Rasa-shastra*, in which herbs are combined with metals and minerals, including arsenic.[27] Since the 1970s, cases have been reported of poisoning related to its use in India; in recent years, cases have also been reported in the West due to the assimilation of Ayurvedic medicine.[28,29]

2. A poison par excellence

Aristotle and Socrates were familiar with the inorganic poison arsenic trioxide, which over the course of the following centuries became the king of poisons. Arsenic was widely used in classical Rome, where its toxicity was explored; one of the first documented cases of arsenic poisoning was the murder of Britannicus on the orders of Nero to secure his ascent to the throne in 54 AD (Figure 1).[30] The *Lex Cornelia de sicariis et veneficis* (Cornelian law against murderers and poisoners) enacted in 82 BC by Lucius Cornelius (138-78 BC) is thought to be the first regulation punishing the use of poisons.

The poisoner became an integral part of social and political life in the early Medieval period, and arsenic was the favoured poison. The records of the city halls of Florence and Venice contain extensive testimony on the political use of poisons. Contracts were registered, naming both the victim and the price paid for the poisoning; after the deed was done, the note "factum" was added for posterity in the city archives.[32]

Pope Alexander VI and his son Cesare Borja had a certain notoriety as poisoners, as did such sinister figures as Giulia Tofana (Giulia Mangiardi), who in the period 1633-1651 confessed to having killed at her discretion using an arsenic solution known as "aqua tofana." Arsenic gained such a terrible reputation that it earned the nickname "inheritance powder." The legend of Tofana and her poison lived on far beyond her death. In 1791, when Mozart became severely ill, he was convinced that he had been poisoned: "My end will not be long in coming: for sure, someone has poisoned me! I cannot rid my mind of this thought. […] Someone has given me acqua toffana and calculated the precise time of my death."[33]

Catherine Deshayes advertised her services as a poisoner, which grew to unprecedented proportions, earning her the title "la Voisin." Louis XIV appointed a tribunal known as the Chambre Ardente (fiery court) to investigate the facts. Deshayes was found guilty of a large number of poisonings, including those of numerous children. She was burned at the stake in February 1680.[34]

According to the records of the first forensic toxicologists, the use of arsenic as a poison was also widespread in 19th-century France. However, its popularity decreased dramatically in the second half of the 19th century after James Marsh developed a highly reliable, sensitive test for detecting it in urine (1836). This technique played a decisive role in the trial of an infamous poisoner, Marie Lafarge, who was found guilty of murdering her husband in 1842.[35]

Another noteworthy case was that of Charles Francis Hall, an American businessman who at the age of 37 years felt a great calling to explore the North Pole, and became one of the greatest Arctic explorers. In 1871, he led an expedition on the ship *Polaris*, which sailed to the northernmost region of Greenland before running aground near Etah. The crew was hostile and fearful, but Hall ignored them in order to achieve his aspiration. One dark, bitterly cold night he drank a cup of coffee, then rapidly fell severely ill; he died and was buried in the permafrost. Nearly a century later, a group of researchers were authorised to exhume Hall's body; tissue samples from the bones, nails, and hair showed that he died due to arsenic poisoning.[36]

There has also been debate in the literature regarding the explanation for the high concentrations of arsenic in the preserved remains of Napoleon Bonaparte's scalp. The hypotheses proposed include assassination, chronic voluntary ingestion of arsenic in the paranoid belief that this would protect him against attempts to poison him, and treatment of various diseases. However, the most widely accepted theory is the use of green copper arsenite pigments (Scheele's green, Vienna green, or Paris green), which were introduced around 1780 and were widely used in paints and wallpapers. In damp conditions, many moulds can metabolise arsenic compounds into the volatile, toxic compound trimethylarsine, which would be released into the air. Throughout the 19th century, many people working with these pigments became sick or even died in this way. Analysis of an original sample of wallpaper from Napoleon's residence on Saint Helena revealed arsenic in sufficient quantities to be harmful.[37] Paris green was also employed in oil painting, and its use was widespread among Impressionist painters including Cézanne, Manet, and Van Gogh (Figure 2).[38]

Abundant references are made to poisoning and suicide with arsenic in literature and film. Gustave Flaubert gives a wonderful clinical description in the novel *Madame Bovary* (1857), in which Emma, crippled by debt and tortured by her romantic failures, decides to end her life by swallowing arsenic, and dies an agonising death.[39] In *Queen Margot*, Alexandre Dumas recounts how Catherine de' Medici and her poisoner René ended the lives of all those who opposed their evil plans, and did not prevent the most renowned physician of the day, Ambroise Paré, from performing clinical autopsies, in the knowledge that the poisons could not be detected by medical tests at the time.[40] It may be thanks to Agatha Christie that many people know the amount of arsenic needed to kill a person. Poisoning is used as a plot device in several of her novels, such as *The mysterious affair at Styles* (1920), *Evil under the sun* (1941), *They do it with mirrors* (1952), and *4:50 from Paddington* (1957). Through her work at a hospital pharmacy in Torquay, she was familiar with the world of pharmacology; this may have been the origin of her passion for potentially toxic substances.[41]

In film, we may mention two comedies: *Arsenic and old lace* (Frank Capra, 1944), in which two angelical, charitable old ladies compassionately end the lives of old bachelors they take in by putting arsenic in their wine (Figure 3)[42]; and *The last supper* (Stacy Title, 1995), in which a group of liberal students invite a fanatical conservative to dinner in order to murder him using wine spiked with arsenic.[43]

Epidemic outbreaks related to the production of wine and other alcoholic drinks

Several food-borne illness outbreaks of arsenic poisoning have occurred in recent years, serving as a reminder of the dangers inherent to the use of the substance in any form. It should be noted that, due to the solubility of arsenic salts, contamination is easy and generally insidious, and the accident is rarely detected until several sufficiently severe or fatal cases of poisoning have occurred.

In 1888, 15 individuals died in an outbreak of 500 cases of arsenic poisoning due to contaminated wine in the towns of Hyères and Toulon. Several years later, in Liverpool, contaminated beer caused approximately 6000 cas-

Figure 2. *Les Vessenots en Auvers*, by Vincent van Gogh (1890). Oil on canvas, 55 × 66 cm. ©Museo Nacional Thyssen-Bornemisza (Madrid).

es of arsenic poisoning, 70 of whom died (the outbreak is known as the Staffordshire beer epidemic).[44] The glucose used for fermentation was extracted in the hydrolysis of sulphuric acid prepared with iron pyrites containing high concentrations of arsenic. This was addressed in an excellent clinical review published in 1901 by Kelynack and Kirkby, entitled *Arsenical poisoning in beer drinkers*.[45] The study included several interesting and illuminating photographs. Among their observations, the authors note that neuritis was the most marked nervous system disorder, and that at least in the early stage, sensory fibres were more easily affected than motor fibres; involvement of the latter caused loss of strength, marked atrophy, and poor coordination.[45]

A particularly relevant case from a scientific perspective was the poisoning of the crew of a French ship in 1932, which provided ideal conditions for scientific study. The outbreak was caused by consumption of adulterated wine. Due to its magnitude, with over 300 individuals affected, it is considered a reference case in the history of toxicology.[46] The news appeared in such outlets as the *New York Times* and the *Singapore Mercantile Advertiser*, as well as various French and Spanish newspapers of the day. The crew's daily wine ration was one litre per man, and the wine was found to contain up to 12 mg of arsenic per litre. The typical symptoms of poisoning began to appear around a month after the first ration. The entire crew was affected. The *Journal of the American Medical Association* proposed four possible causes of the poisoning: fumigation of the grapevines with copper sulphate to protect against insects; post-vintage addition of sulphurous acid to the vines as a pesticide; the addition of

Figure 3. Promotional image from the Spanish release of the film *Arsenic and old lace* (Frank Capra, 1944). Public domain.

arsenic-contaminated sugar to the must to aid alcohol fermentation; and the use of sulphuric acid to clean the casks in which the wine was transported.[47]

In the postwar period in Spain, in mid-1946, an episode of collective arsenic intoxication affected around 200 individuals, with one death and five cases of severe poisoning.[48] A shipment of wine from Tricio (La Rioja), accidentally adulterated with lead arsenate, was distributed in Pradoluengo (Burgos) and some nearby villages. The psychiatrist Ignacio López Saiz, who had considerable neurological expertise, attended some of the patients, publishing his clinical observations.[49] Mild cases presented predominantly gastrointestinal symptoms, which subsided within days and were followed by "nervous" alterations (tingling, weakness, cramps, and instability), which gradually resolved. However, two particularly severe cases were reported. The first was a local couple who became ill after drinking around a litre of the wine, with nausea, vomiting, abdominal pain, diarrhoea, and severe thirst. The husband tried to quench his thirst with more wine, whereas his wife drank water. The husband's condition progressively worsened, with blurred vision, vertigo, cramps, loss of fine motor skills in the hands, hypotonia, marked muscle atrophy in the legs, and abolished tendon reflexes. The other very severe case was one of the distributors of the wine that caused the poisoning: when he discovered that the wine he had delivered had been blamed for the disease outbreak in Pradoluengo, he attempted to demonstrate the absurdity of this supposition by drinking a large amount of the wine. He first showed the typical digestive symptoms, followed by florid neurological manifestations, leaving him disabled.[48,49]

Since then, no other outbreak related to wine consumption was reported in the literature until 1978, although this continues to be a cause of concern. In 2014, a class action suit was filed in California against some of the largest wine producers in the United States for distributing wines with high arsenic content; the suit was eventually dismissed by the Supreme Court of California. A study at the University of Washington analysed arsenic content in 65 samples of wine from the main wine-producing states in the United States, finding a mean of 0.024 parts per

million. This demonstrates that most wines produced in the country contained high levels of arsenic, although this may be due to geographical reasons.[50]

According to the World Health Organization, arsenic is one of the ten chemical substances presenting the greatest threat to public health, as it occurs naturally in groundwater in many countries, including Argentina, Bangladesh, Chile, China, India, Mexico, and the United States. The main routes of exposure are drinking water, crop irrigation with contaminated water, and foods prepared with this water. In Bangladesh, for instance, significant attention has been placed on arsenic since it was discovered 30 years ago that the element was prevalent in drinking water wells. Around 20 million people are exposed to arsenic at concentrations greater than 50 µg/L. Up to five million children born between 2000 and 2030 are expected to die as a result of arsenic in the water supply.[51,52]

The case of arsenic-contaminated wine in Extremadura

In 1971, a somewhat famous winemaker was directly and personally running the company "Vinos El Raposo" in a wine-producing region near the city of Zafra, in the province of Badajoz. He processed both his own grapes and those of other growers. The wine was sold both at wholesale and to individuals. Around that time, he purchased an undetermined amount of sodium arsenate, which he stored in a transparent plastic bag in a cupboard at the winery, alongside other products including citric acid.[53] The latter product was used to acidify must and wine; this causes absorption of iron, preventing precipitation and improving the freshness and sweetness of the wine before bottling. Sodium arsenate, on the other hand, was used to treat the dreaded esca or parasitic grapevine apoplexy. Visually, both products are very similar.

In June 1978, "[…] believing that he was picking up the citric acid, he took the bag of sodium arsenate and poured three and a half kilos into the barrels at the winery."[53] This contaminated wine was subsequently distributed. Ingestion of the wine caused 11 deaths, with 335 individuals showing symptoms of varying severity. Particularly severe outbreaks occurred in the nearby towns of Valencia del Ventoso, Fuente de Cantos, Jerez de los Caballeros, Fregenal de la Sierra, other villages in south-western Extremadura, and, to a lesser extent, in Seville.[53,54] Most patients were male, aged between 20 and 70 years, and were regular drinkers with limited economic resources.[54] Many families were thrust into desperate circumstances due to the fatal outcomes of some cases and the severe sequelae of others.[54]

The Toxicology Institute of Seville analysed samples from different confiscated bottles of the wine, finding high concentrations of arsenic, ranging between 7 and 93 mg/L; this is far higher than the maximum limits allowed by the International Organisation of Vine and Wine (0.2 mg/L at the time).[56]

The case was heard by the young judge Julio Márquez de Prado, who years later became president of the Superior Court of Justice of Extremadura. The cadavers of individuals who had died with the symptoms of potential arsenic poisoning were examined, and exhumations were even ordered in some cases. The summary trial considered the crime of reckless endangerment. The opinion of the court was issued by Javier Gómez de Liaño, who years later was one of the National Court magistrates charged with trying the "rapeseed oil affair," one of the most important agri-food fraud cases in Spanish legal history.[57]

Such was the severity of the case that the Spanish Congress passed a motion on 17 September 1981 declaring the poisoning by arsenic-contaminated wine a "regional catastrophe." One of the members presenting the motion was Rodríguez Ibarra, a representative for Badajoz.[58]

The judgement of 22 April 1987 of the Supreme Court upheld the guilty verdict, finding the winemaker guilty of manslaughter and severe injury through negligence. The court also stressed the fact that storage of lethal products together with products used exclusively in winemaking was prohibited in the Statute of Vine, Wine, and Alcohols.[53,56] Several months later, on 25 May 1987, the Regional Ministry for Health and Consumer Affairs, by agreement with the Extremaduran regional government, decided to issue a grant of nearly 30 million pesetas "to the persons affected, or to their next of kin, for the poisoning caused by ingestion of wine mixed with arsenic."[59] The amount awarded in each case was based on the assessment of sequelae and the duration of inability to work. The sums awarded ranged from 15 000 to 776 000 pesetas. The families of the deceased victims received 500 000 pesetas.[59]

Figure 4. Prof González Dorrego speaking at a meeting of the Extremaduran Society of Neurology. Personal archive of Dr José María Álvarez Suárez Bárcenas.

A narrative summary of the clinical, neurophysiological, and neuropathological data on arsenic neuropathy reported in the doctoral thesis (1983)

The Residencia Sanitaria Nuestra Señora del Perpetuo Socorro hospital in Badajoz began operating in 1956, and was the most important hospital in the region until the inauguration of Hospital Infanta Cristina in 1987. The development of medical specialties was asymmetrical, with the majority being included under internal medicine; this was the case for neurology. At the time, the only neurologist in Badajoz was Dr González Dorrego, who had graduated with a degree in medicine and surgery from the University of Santiago in 1972, and specialised in neurology at the Autonomous University of Barcelona in 1974. He won his position at the hospital on 27 October 1975 (Spanish Official State Gazette of 18 November) after a public merit-based competition held by the general delegation of the National Institute of Social Insurance. He was one of the few full professors in Spain in the 1980s and 1990s, and was a founding member of the Extremaduran Society of Neurology (Figure 4).

Between July and August 1978, a total of 165 patients with suspected arsenic poisoning and neurological symptoms were studied at the Residencia Sanitaria de Badajoz. They were all from a specific geographical region in south-western Extremadura.

Of the total series, 71 were excluded from the analysis for a range of reasons, generally because they presented another disease that may better explain the neurological symptoms. Of the 94 patients included, 92 were men and 2 were women; ages ranged from 22 to 70 years. Of these, 61 were hospitalised. At the hospital, it was not initially possible to rule out an infectious cause of the outbreak, and organisational changes were needed, with the creation of a monographic ward for the isolation and management of the numerous patients admitted.

The epidemiological survey of all the patients was not able to clearly establish the total amount of toxin ingest-

ed or the exact time of ingestion, although it was estimated to have been more than 20 days prior. All the patients assessed came to hospital due to neurological symptoms, although medical history interviews revealed that they had previously presented general symptoms, with cutaneous/mucosal, gastrointestinal, and respiratory manifestations. Neuropathy was not the initial symptom in any case.

The severity of neuropathy was evaluated according to the intensity of motor involvement, and classified as mild (group I, not evident), moderate (group II, lower limb involvement), or severe (group III, generalised). Table 1 summarises the characteristics of each group, based on data from the doctoral thesis (Figure 5).[15]

Generally, from ten days after onset of general symptoms, patients presented subjective neurological symptoms such as paraesthesia, dysaesthesia, and muscle pain, followed soon after by progressive, symmetrical, distal weakness predominantly affecting the lower limbs, tone and trophic changes in muscle, and alterations in deep tendon reflexes and sensitivity. Cranial nerve involvement was not reported in any patient. Deep (vibratory and proprioceptive) sensory involvement was the most frequent sensory manifestation, and weakness was most pronounced in distal areas of the lower limbs. This was the most characteristic clinical course of subacute arsenic neuropathy.

Electroencephalography studies revealed no relevant abnormalities in groups I and II, whereas four of the 13 patients in group III presented diffuse cerebral dysfunction; they all had severe neuropathy, reported intense headache, and presented cerebrospinal fluid abnormalities.

Patients were treated according to their clinical severity. Patients in group I received D-penicillamine and vitamin B complex; those in group II were treated with intramuscular dimercaprol and vitamin B complex, and those in group III received all three treatments. Three patients required ventilatory support. Patients in groups II and III received rehabilitation therapy from the first week after admission, lasting three to four months in all cases.

The mortality rate in the series was 4.4%: two patients died due to respiratory failure, one due to arsenic encephalopathy, and one due to hypovolaemic shock. Autopsy studies were conducted in three cases, revealing arsenic levels in the liver of 1.6, 2.4, and 4.2 mg per 1500 g tissue, respectively.
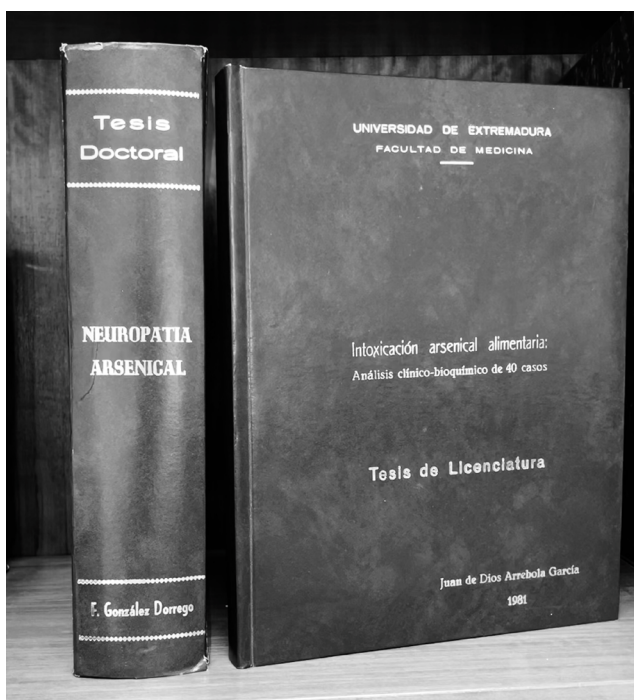

Figure 5. The spine of Prof González Dorrego's doctoral thesis (1983) and front cover of Dr Juan de Dios Arrebola García's degree dissertation (1981).

The 90 surviving patients were followed up for two years, with periodic reviews at two, six, 12, and 24 months. No patients were lost to follow-up. At two months, no patient in any of the three groups presented a clinical improvement. The more severe patients (22 in group I and 15 in group III) presented a clinical deterioration in the initial period, with paralysis ascending (never descending) but no cranial nerve involvement. After the first two months, deficits ceased to progress, entering a phase of clinical stability lasting 10-20 days. Subsequently, patients began a slow recovery, generally a reversal of the course of the disease (ie, descending). At 12 months, no cases of relapse or worsening of symptoms were observed. However, a significant number of patients (12 in group II and 7 in group III) presented a similar clinical status to that recorded at admission. At 24 months, 7% of patients were dependent in activities of daily living and could not walk unassisted.

Neurophysiological studies were performed with two systems, a two-channel Van Gogh electromyography machine equipped with a Tektronix oscilloscope, and

Table 1. Summary of the general characteristics of each clinical group in González Dorrego's doctoral thesis.[15] Compiled by the author.

	Total N = 94	Group 1 n = 19 (20.0%)	Group 2 n = 50 (53.2%)	Group 3 n = 25 (26.5%)
Age in years, mean (SD)	47.2 (1.2)	41.3 (2.5)	48.3 (1.6)	51.8 (2.2)
Gastrointestinal				
- Nausea	43 (45.7%)	6 (31.6%)	16 (32.0%)	21 (84.0%)
- Diarrhoea	61 (64.8%)	10 (52.6)	27 (54.0%)	24 (96.0%)
- Melaena	3 (3.2%)	1 (5.2%)	0 (0.0%)	2 (8.0%)
- Hepatomegaly	40 (42.5%)	1 (5.2%)	19 (38.0%)	20 (80.0%)
Cutaneous/mucosal				
- Conjunctivitis/facial oedema	85 (90.4%)	17 (89.4%)	44 (88.0%)	24 (96.0%)
- Urticaria	48 (51.0%)	11 (57.8%)	24 (48.0%)	13 (52.0%)
- Vesicles	22 (23.4%)	3 (15.7%)	7 (14.0%)	12 (48.0%)
- Melanoderma	39 (41.4%)	1 (5.2%)	10 (20.0%)	18 (72.0%)
- Hyperkeratosis	26 (27.6%)	0 (0.0%)	12 (24.0%)	14 (56.0%)
- Mees lines	26 (27.6%)	1 (5.2%)	13 (26.0%)	12 (48.0%)
Respiratory				
- Common cold	43 (45.7%)	8 (42.1%)	21 (42.0%)	14 (45.7%)
- Pneumonia	3 (3.2%)	0 (0.0%)	0 (0.0%)	3 (12.0%)
- Respiratory failure	3 (3.2%)	0 (0.0%%)	0 (0.0%)	3 (12.0%)
CNS				
- Headache	88 (93.6%)	18 (94.6%)	45 (90.0%)	25 (100%)
- Aseptic meningitis	1 (1.1%)	0 (0.0%)	0 (0.0%)	1 (4.0%)
- Encephalopathy	1 (1.1%)	0 (0.0%)	0 (0.0%)	1 (4.0%)
Vegetative				
- Sexual impotence	14 (15.4%)	0 (0.0%)	4 (8.0%)	10 (45.0%)
- Orthostatic hypotension	2 (2.2%)	0 (0.0%)	0 (0.0%)	2 (8.0%)
- Urinary sphincter dysfunction	6 (6.4%)	0 (0.0%)	0 (0.0%)	6 (24.0%)
- Cardiac arrhythmia	2 (2.2%)	0 (0.0%)	0 (0.0%)	2 (8.0%)
CFS study, mean (SD)				
- Cell count (cells/mm³)	-	1.2 (1.7)	3 (2.4)	19.6 (36.7)
- Glucose (mg, %)	-	58.4 (6.9)	54.4 (8.8)	65.7 (13.5)
- Proteins (mg, %)	-	20.2 (3.7)	28.4 (19.7)	36.4 (28.8)
Mortality	4 (4.4%)	1 (5.2%)	0 (0.0%)	3 (12.0%)
Final clinical outcome (2 years)				
- Mild sequelae	26 (27.6%)	0 (0.0%)	22 (44.0%)	4 (16.0%)
- Moderate sequelae	24 (25.6%)	0 (0.0%)	12 (24.0%)	12 (48.0%)
- Severe sequelae	6 (6.4%)	0 (0.0%)	0 (0.0%)	6 (24.0%)

a DISA 1500 system. The study was measured directly on the oscilloscope screen or on polaroid film. Dr Castilla Garrido, head of the neurophysiology department at Hospital Clínico de Sevilla, and Dr Rodríguez Albariño, head of the neurophysiology department at Residencia Sanitaria de Badajoz, advised and assisted Dr González Dorrego in performing the studies. To briefly summarise the neurophysiology findings, we may note that patients with mild or moderate neuropathy showed exclusively axonal lesions characterised by reduced evoked potential amplitudes and the presence of spontaneous activity and polyphasia. Patients with severe forms concurrently presented a demyelinating process with marked reductions in nerve conduction velocity and frequent conduction block. Some presented "dying back" axonopathy.

Anatomical pathology studies were performed with the collaboration of Dr Navarro, head of the neuropathology department at Hospital Vall d'Hebron (Barcelona), and Dr Gómez de Tejada, head of the anatomical pathology department at Residencia Sanitaria de Badajoz. Histological analysis of the sural nerve and gastrocnemius muscle was conducted in eight cases, with patients from all three groups and at different stages of disease progression. Regarding nerve involvement, nerve fibre dysfunction preceded the structural abnormality at an early phase, "probably because the toxin alters axonal transport in some way; study findings are normal." At more advanced stages, very severe axonal degeneration was observed, with signs of myelin destruction and a dramatic reduction in large-calibre myelinated fibres. The damage was so severe that, at two years, the nerve continued to display a near-total lack of large-calibre myelinated fibres, with very scarce signs of remyelination and significant endoneurial fibrosis. Muscle autopsy typically revealed muscle atrophy by fascicles, with small fibres presenting angular or polygonal transverse sections, with preserved striation in longitudinal sections, and no interstitial alterations; in other words, findings indicated pure neurogenic atrophy. The thesis contains numerous optical and electron microscopy images, which are highly illustrative of the cases studied, enabling comprehensive neuropathological review.

From a therapeutic perspective, the study demonstrates that the treatment proposed neither cured nor meaningfully changed the progression of the neuropathy; neither did it influence clinical progression. One of the conclusions of the study was that:

In the light of histological findings demonstrating continued nerve degeneration despite suppression of the toxin and the evidence that arsenic remains strongly adhered to tissues, we are inclined to think that chelation therapy must be started very early for it to be effective.

Relevance of the study by Prof González Dorrego

The series reported by Prof González Dorrego is important for several reasons. Firstly, it was one of the largest series to be reported since the early 20th century, and also the most homogeneous: the aetiology was the same in all cases, and all patients were from the same geographical area and presented similar socioeconomic circumstances. Previously, the largest published series were those by Heyman et al. (1956; 451 cases),[60] Jenkins (1966; 57 cases),[61] Chhuttani et al. (1967; 40 cases),[62] and Goldstein et al. (1975; 24 cases).[63] All these series report cases of arsenic poisoning of different types (pharmacological, accidental, or industrial) collected over periods of years, and included patients from very different geographical areas and social classes.[15]

Secondly, the nosological classification of chronic-subacute poisoning was a novelty, with the majority of previous publications referring to acute and chronic forms. Over a prolonged period, patients had periodically been ingesting different amounts of contaminated wine, with clinical manifestations appearing after an initial asymptomatic period. According to previous studies, the most typical finding was acute neuropathy, generally associated with a single large dose 5 to 10 days earlier, which subsequently progressed over a period of weeks even in the absence of further exposure. In some cases, neuropathy resulted in flaccid paralysis resembling Guillain-Barré syndrome.

Furthermore, the general symptoms of poisoning and their relationship with neuropathy had not previously been described so specifically, systematically, or exhaustively as in González Dorrego's thesis. The author describes the entire constellation of symptoms, reflecting involvement of multiple organs, which clearly demonstrates the role of arsenic as a systemic protoplasmic poison. The thesis supervisor was very complimentary in his authorisation for the author to submit the thesis: "performed with commendable technical ability and great scientific rigour."

Neurological involvement presented with symmetrical distal weakness, myalgia, cramps, and hypoactive or absent stretch reflex, associated with distinctive clinical characteristics, such as hyperpathia, pigmented dermatitis, Mees lines, and hyperkeratosis of the palm of the hand and/or the sole of the foot. Similar findings were described a few years later by Donofrio et al.[64]

Finally, perhaps the most novel finding was that younger patients presented milder disease, whereas those older than 50 years had more severe, more aggressive forms, with nerves presenting degeneration of the vasa nervorum, partial demyelination, and discreet axonal degeneration. A few years earlier, Arnold and Harriman[65] had studied healthy nerves by single-axon dissection in order to provide a control series for biopsy studies of nerve disease; those authors recognised the influence of older age over internode length and the incidence of Wallerian degeneration,[65] which may increase vulnerability to any toxin or disease that may damage the nerve.

Most previous electrophysiological studies of patients with arsenic neuropathy reported single measurements of nerve conduction velocity, showing a mild reduction in motor nerve conduction velocity and lack of sensory nerve action potentials.[58,61] The study by Prof González Dorrego described the temporal profile of electrophysiological changes, from the early stages of disease to partial recovery. He observed that sensory nerve action potentials remained absent throughout the follow-up period, demonstrating severe involvement of sensory nerve fibres. The alterations in motor conduction velocity changed over time, with an initial progressive decrease, reaching the lowest point several months after exposure, and subsequently a gradual increase from that time. The active denervation observed in electromyography studies and the mild-to-moderate slowing of motor nerve conduction velocity from the onset of neuropathy were correlated with pathological findings of axonal degeneration in biopsy studies of the sural nerve.

Very few previous works had described neurophysiological findings from patients who developed neuropathy several weeks after exposure to high doses of arsenic. Le Quesne and McLeod[66] described four such patients. Two of these presented absence of sensory responses, reduced conventional motor nerve conduction velocity, marked prolongation of distal motor latencies, and a reduction of at least 50% in motor action potential amplitude. Murphy et al.[67] published data from two patients with similar clinical presentation and electrophysiological results to those of the other patients described above. Both patients developed subacute polyneuropathy; and electroneurography performed weeks after arsenic exposure showed a lack of a sensory response, with moderately reduced motor nerve conduction velocity.[67] A similar case was described by Feit et al.,[68] who observed moderately decreased motor nerve conduction velocities at three weeks after exposure. Many of these patients were initially diagnosed with Guillain-Barré syndrome, before the final results of the toxicology evaluation were available.[64]

Most histology studies of peripheral nerves revealed very severe axonal degeneration and secondary myelin damage, predominantly involving large-calibre myelinated fibres. However, such contemporaries as Chhuttani (1978) and Chopra (1975) described differing degrees of segmental demyelination and remyelination in nerve fibre preparations, and argued that segmental demyelination and axonal degeneration may be the salient pathological characteristics of arsenic-induced neuropathy.[69]

We know today that, from a pathological perspective, arsenic can directly damage the axon, resulting in primary axonopathy with irreversible alterations to cytoarchitectonic organisation and axon function. Nonetheless, axonal damage can also be secondary, potentially triggering persistent neuropathy due to primary damage to the soma of peripheral neurons or primary demyelination (secondary axonopathy). This reflects the fact that larger and longer axons are the last to be affected, and are not the primary critical target. Demyelination may also result from damage to Schwann cells or from the "dying back" phenomenon.[70,71]

In addition to dose, the development and severity of neuropathy are influenced by such other factors as age, individual susceptibility, idiosyncrasy, and presence of certain concomitant diseases or conditions (eg, malnutrition). Although the role of interactions between diseases remains unclear today, this was one of the hypotheses posited by Prof González Dorrego.

Toxicity mechanisms of arsenic: current evidence

The exact metabolic pathways of arsenic have not yet been confirmed either in humans or in animals intended for human consumption.[72] Neither do we fully understand the mechanisms underlying arsenic-induced neurotoxicity, although several mechanisms have been proposed, mainly based on animal experimentation.[73]

Arsenic metabolites exert their toxic effect by inactivating a wide variety of enzymes, particularly those involved in cell energy metabolism and in DNA synthesis and repair.[74] Several mechanisms (oxidative stress, thiamine deficiency, and reduced acetylcholinesterase activity) seem to play an important role in arsenic-induced neurotoxicity.[75]

One of the most important mechanisms involved in arsenic neurotoxicity is the substance's capacity to cause oxidative stress and mitochondrial dysfunction.[76] Studies of the central nervous system in experimental animals revealed decreased activity of mitochondrial complexes I-IV and increased levels of reactive oxygen species (ROS). Accumulation of ROS causes damage to the lipid layer and dysfunction of membrane potentials.[77]

Thiamine deficiency is known to cause neurological complications; arsenic can cause thiamine deficiency and inhibit pyruvate decarboxylase, which is responsible for converting glucose into energy. Arsenic inhibits enzymatic complexes through the action of ROS. ROS production causes oxidation-mediated inactivation of pyruvate dehydrogenase. Arsenic-induced axonal neuropathy resembles "dry" beriberi neuropathy induced by thiamine deficiency and pyruvate decarboxylase inhibition.[74,75]

Acetylcholinesterase is one of the enzymes needed for the correct functioning of the nervous system. In rats, arsenic trioxide causes a significant, dose-dependent decrease in the activity of serum acetylcholinesterase. This reduction in cholinergic activity may be associated with peripheral neuropathy.[78]

The physiological process of nerve impulse propagation requires the axon structure to be intact in terms of anatomical organisation and molecular and biochemical homeostasis. Axonopathy results from alterations to axon cytoarchitecture and/or function. One very interesting effect that may explain the damage to the peripheral nervous system is the fact that arsenic causes changes in the composition of nerve proteins; for instance, reducing neurofilament expression. In experimental studies with animals, arsenic exposure reduced the expression of neurofilament protein and induced cytoskeleton destabilisation and structural alterations, which may eventually lead to degeneration of peripheral nerve axons.[79] Arsenic-induced axonopathy may be caused by alterations in alpha- and beta-tubulin assembly and disassembly dynamics in axons, which maintain microtubule organisation and functioning, as well as by the dysfunction of actin microfilaments and neurofilaments (neuronal intermediate filaments).[79]

Conclusions

Arsenic continues to be a significant health issue. Our understanding of arsenic exposure and its toxicological effects has grown significantly over the last 20 years. However, its metabolism and neurotoxic mechanisms are not yet fully understood. Forty-five years ago, the first established neurologist in Badajoz had the opportunity to conduct a flawlessly thorough study of patients with neuropathy due to arsenic poisoning. The results and conclusions of his work should be mandatory reading today.

The best way of concluding the present study, and praising the work of Prof González Dorrego, is to quote the final conclusion of his thesis:

> The clinical, electrophysiological, and neuropathological study of this large series of patients with arsenic poisoning yielded novel data that may be of diagnostic, prognostic, and therapeutic value in arsenic poisoning, but may also be relevant to other toxic polyneuropathies.

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