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A Study of the Effect of Decimal and Centesimal Dilutions of Arsenic on the Retention and Mobilization of Arsenic in the Rat

J. C. Cazin, M. Cazin, J. L. Gaborit, A. Chaoui, J. Boiron[1], P. Belon[1], Y. Cherruault[2] & C. Papapanayotou[2]

Laboratoire de Pharmacologie, Faculté de Pharmacie, 3 rue Laguesse 59045, Lille Cédex; [1]Laboratoires Boiron, 20 rue de la Liberation, 69110 Ste-Foy-Les-Lyon and [2]Université Paris 6, Médimat, 15 rue de l'Ecole de Medicine, 75270 Paris Cédex 06, France

Having developed a pharmacokinetic method for studying the fate of orally administered arsenious anhydride by a radioactive tracer method, the influence of Hahnemannian dilutions of arsenicum album on the elimination and retention of this toxin in the rat was then investigated. The effects of centesimal (cH) and decimal (dH) dilutions were studied.

All the dilutions studied were found to be active. The strongest effects were observed after the administration of dilutions corresponding to a concentration of 10^{-14} (14dH and 7cH). Overall, the decimal dilutions augmented the elimination of arsenic more than the centesimals.

The observed results were submitted to mathematical analysis. A mathematical model, which confirms that Hahnemannian dilutions have biological effects which are a direct function of the degree of dilution, was developed.

Introduction

The action of infinitesimal dilutions of toxins on the elimination of the same toxin, previously fixed in the organism, is now well established.

First observed, in the guinea-pig, with arsenic and bismuth, by Lapp & Wurmser[1,2]; this phenomenon was demonstrated again by Boiron & Cier[3] in the pigeon with arsenic and antimony. Subsequently, Mouriquand *et al.*[4] used variations in vestibular chronaxy as an objective index of the elimination of exogenous arsenic and antimony in subtoxic doses. In 1962, Cier & Boiron[3] studied the specificity of this action by examining whether there were cross-reactions between arsenic and antimony, which are chemically related, with a number of common biological properties.

The experiment presented here is a study of the retention of arsenic in the rat, and its mobilization under the influence of infinitesimal doses of the same toxin. It is a logical development from previous studies conducted in our laboratory, which have led to the development of a reliable experimental model permitting the study of the actions of homoeopathic dilutions.

The protocol for this experiment has been developed from previous studies using radionuclides of arsenic,[5–8] which have established the rat to be the animal which best lends itself to the study of the retention and mobilization of arsenic under the influence of infinitesimal doses of arsenic.

It is well established that, in the rat, blood is one of the preferred sites of arsenic fixation. By measuring arsenic elimination and blood arsenic concentrations, at selected time points, we have objectively documented the action of the 7th centesimal dilution of arsenicum album, in comparison to 'potentised' water.[9,10] On the basis of our initial results we were encouraged to extend our work to study other dilutions of arsenic. These dilutions were prepared, by the Hahnemannian method of serial dilution with succussion, from arsenious anhydride, also known as white arsenic, or arsenicum album.

In the method of dilution developed by Samuel Hahnemann, the founder of Homoeopathy, known as Hahnemannian dilution or potentisation,[11] one part of the original substance is dispersed in 99 parts of diluent. This is then subjected to a brief period of

vigorous shaking or succussion, to produce the first Hahnemannian centesimal dilution, or first cH. Repeating the same operation, starting from this first cH, produces the second cH; this process is repeated to give any desired centesimal potency.

In order to produce Hahnemannian decimal dilutions (dH), each successive dilution is of 1 part in 10. Thus, 10dH indicates that the original substance has been diluted in a very pure solvent (distilled water, or a water-ethanol mixture), 1:10, 10 times, with succussion at each stage.

In this experiment, we studied the effects of both decimal (10, 14, 18, 22, 26 and 30 dH) and centesimal (5, 7, 9, 11, 13 and 15 cH) dilutions of arsenic on the mobilization and retention of arsenic in the rat.

Materials and methods

Batches of 30 young male Wistar rats, each weighing approximately 70 g, were given, via oesophageal tube, a single dose of 10 mg/kg arsenious anhydride and a tracer dose of 100 μCi/kg ^{73}As in the form of arsenious acid (Amersham). These were suspended in 5% gum arabic syrup, and administered in a volume of 0.5 ml per 20 g body weight.

Twelve hours after oral dosing (T_0 + 12 h) the animals were given a single intraperitoneal injection of 1 ml of the Hahnemannian dilution of arsenicum album or the same volume of control (succussed distilled water). Following this the rats were isolated in metabolism cages and allowed water and normal feed *ad libitum*.

At T_0 + 20 h, 8 hours after the treatment dose, the rats were sacrificed and blood taken by intracardiac puncture for estimation of radioactivity. The urine and faeces, which were automatically separated by the cages, were collected, weighed and then sampled for estimation of radioactivity. Radioactivity was measured in an Intertechnique automatic gamma counter, model GC4000, with a 3-inch crystal. All results were corrected for counting efficiency, background noise and radioactive decay.

Two experiments were carried out using this experimental method, one devoted to Hahnemannian decimal dilutions, the other to Hahnemannian centesimal dilutions.

Results

Statistical analysis of the effect of arsenicum album versus potentised water was by Student's *t*-test.

Experiment 1: decimal dilutions

A study of Hahnemannian decimal dilutions of arsenicum album (10dH, 14dH, 22dH, 26dH, 30dH), compared with potentised water.

Blood levels (Table 1 and Figure 1). The mean blood arsenic concentrations of rats treated with different potencies are lower than the equivalent levels in potentised water-treated animals. The mean reduction is between about 2 and 7%, depending on the potency used. The greatest reduction was observed with the 14dH potency (6.64%)—this was significant at the 0.1% level. The smallest difference was obtained with the 30dH potency, this did not reach significance at the 5% level.

Excretion (Table 2 and Figure 2). Faecal and urinary arsenic excretion corroborates the blood results. In fact, the highest level of excretion was seen with the 14dH, and the lowest with the 30dH. The differences in excretion, in favour of the treated rats, were significant at the 0.1% level.

Experiment 2: centesimal dilutions

A study of the 5cH, 7cH, 9cH, 11cH, 13cH and 15cH potencies of arsenicum album, compared with potentised water.

Table 1 Arsenic concentration in μg/100 mg in blood, urine and faeces at T_0 + 20 (ie 20 h) after initial loading, and 8 h after homoeopathic dosing), for decimal potencies

	Decimal dilution (dH)						
	10	*14*	*18*	*22*	*26*	*30*	*Control*
Blood	9.844 ± 0.537	9.752 ± 0.530	9.792 ± 0.654	9.945 ± 0.611	10.013 ± 0.533	10.273 ± 0.710	10.446 ± 0.566
Urine	0.239 ± 0.038	0.209 ± 0.037	0.220 ± 0.045	0.182 ± 0.041	0.207 ± 0.046	0.201 ± 0.041	0.197 ± 0.027
Faeces	1.808 ± 0.245	2.067 ± 0.306	2.038 ± 0.352	1.931 ± 0.169	1.778 ± 0.241	1.678 ± 0.210	1.573 ± 0.190

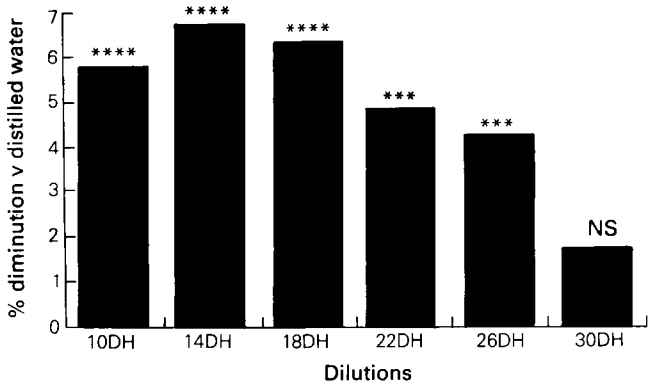

Figure 1 Variations in blood arsenic level, expressed as percentages, compared to potentised water. ****, $P \leqslant 0.001$; ***, $P \leqslant 0.01$; ns = not significant

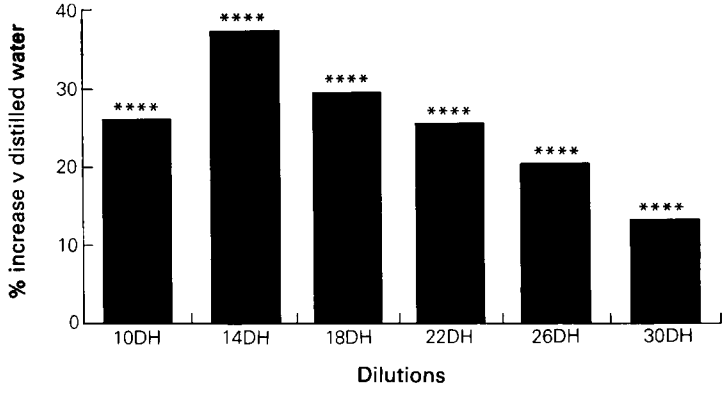

Figure 2 Variations in total excretion (urine and faeces) of arsenic, per rat, expressed as a percentage compared to potentised water
****$P \leqslant 0.001$

Table 2 Arsenic excretion in μg per rat during the period T_0 + 12 h to T_0 + 20 h, for decimal potencies

	Decimal dilution (dH)						
	10	*14*	*18*	*22*	*26*	*30*	*Control*
Urine	1.462 ± 0.140	1.337 ± 0.171	1.345 ± 0.239	1.190 ± 0.157	1.312 ± 0.219	1.196 ± 0.192	1.172 ± 0.133
Faeces	24.674 ± 2.996	27.081 ± 3.319	25.444 ± 3.974	24.799 ± 1.877	23.567 ± 3.060	22.236 ± 2.831	19.521 ± 2.178
Urine and faeces	26.136 ± 3.136	28.418 ± 3.491	26.789 ± 4.213	25.989 ± 2.034	24.879 ± 3.279	23.432 ± 3.022	20.693 ± 2.311

Blood levels (Table 3 and Figure 3). A difference in the retention of arsenic in the blood between the rats treated with homoeopathic potencies, and those receiving potentised water, was observed. In fact, the percentage reduction of blood arsenic concentration in the animals receiving active treatment, compared to those receiving potentised water, varied between 4 and 12%. The most effective dilution was the 7cH, which caused a reduction of 11.96% (statistically significant at the 0.1% level). For the 5cH dilution the reduction of blood lead concentration was 9.87%, also significant at the 0.1% level. For the other dilutions, despite the smaller reductions in concentration, the significance level varied between 1% and 0.1% for the 15cH potency.

Excretion. The elimination of arsenic in the period T_0 + 12−20 h (following a single intraperitoneal injection at T_0 + 12 h was increased in rats receiving the homoeopathic potencies when compared to those receiving potentised water. (See Table 4 and Figure 4).

Total arsenic excretion (faecal + urinary) per rat showed the greatest increase with the 7cH potency (38.89%), this was in agreement with the blood results.

Statistical analysis demonstrated that all potencies, except the 15cH, caused an increase in total excretion, in comparison to potentised water, significant at the 0.1% level.

Discussion

Experiment 1 (decimal dilutions)

All the decimal dilutions tested were found to have a greater effect on arsenic elimination than potentised water. The greatest differences were found with the lower dilutions (10dH, 14dH and 18dH), the most

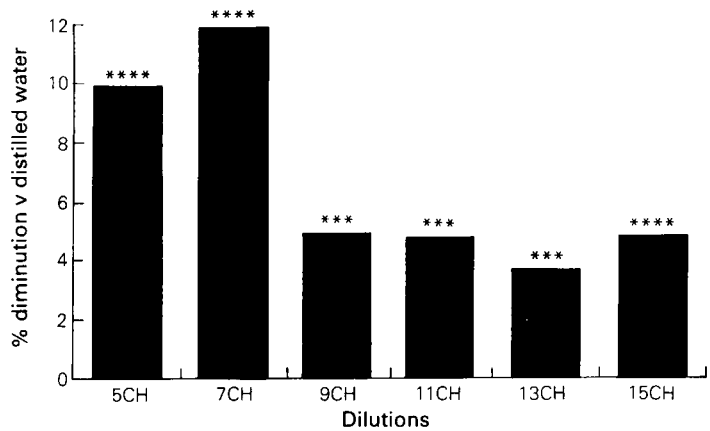

Figure 3 Variations in blood arsenic level, expressed as percentages, compared to potentised water, centesimal potencies **** $P \leq 0.001$, *** $P \leq 0.01$

Table 3 Arsenic concentration in μg/100 mg in blood, urine and faeces at T_0 + 20 h (i.e. 20 h after initial loading, and 8 h after homoeopathic dosing), for centesimal potencies

	Centesimal dilution (cH)						
	5	*7*	*9*	*11*	*13*	*15*	*Control*
Blood	9.359 ± 0.503	9.142 ± 0.430	9.873 ± 0.463	9.886 ± 0.593	9.998 ± 0.491	9.886 ± 0.436	10.384 ± 0.555
Urine	0.231 ± 0.019	0.243 ± 0.026	0.210 ± 0.017	0.211 ± 0.031	0.194 ± 0.028	0.200 ± 0.018	0.194 ± 0.025
Faeces	0.174 ± 0.157	1.856 ± 1.188	1.660 ± 0.109	1.649 ± 0.120	1.598 ± 0.111	1.583 ± 0.164	0.443 ± 0.145

Table 4 Arsenic excretion in μg per rat during the period T_0 + 12 h to T_0 + 20 h, for centesimal potencies

	Centesimal dilution (cH)						
	5	*7*	*9*	*11*	*13*	*15*	*Control*
Urine	1.439 ± 0.083	1.517 ± 0.133	1.289 ± 0.088	1.223 ± 0.186	1.198 ± 0.172	1.162 ± 0.170	1.165 ± 0.130
Faeces	22.952 ± 2.944	26.052 ± 2.431	22.662 ± 2.085	21.108 ± 1.422	20.963 ± 2.238	19.497 ± 2.283	18.684 ± 1.373
Urine and faeces	24.391 ± 3.027	27.569 ± 2.563	23.951 ± 2.173	22.331 ± 1.608	22.161 ± 2.410	20.659 ± 2.453	19.849 ± 1.503

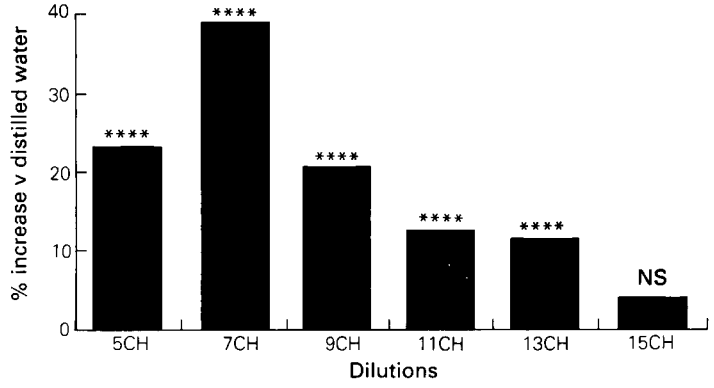

Figure 4 Variations in total excretion (urine and faeces) of arsenic, per rat, expressed as a percentage compared to potentised water, centesimal potencies
**** $P \leq 0.001$; NS, not significant

active dilution being the 14dH, which increased total excretion by 37.33%, compared to potentised water. The least active dilutions were the 26 and 30dH.

Experiment 2 (centesimal dilutions)
Here again the excretion of arsenic, following dilutions of centesimal potencies of arsenic was greater than that detected following administration of potentised water. Blood levels were correspondingly lower. The most active dilutions were the 5 and 7cH, the least active, 13 and 15cH.

The observed differences between the blood concentrations of treated and untreated animals are small but significant, except for the 30dH. In order to validate these results, taking into account the blood volume of each animal, we performed a mathematical study, assuming an uncertainty of ± 10% in the observed blood radioactivity. Each concentration data point was assumed to be subject to random variation of ± 10%, generating for each dilution 60 groups of 30 data points. We established that, even with such a margin of uncertainty, the differences between the animals treated with Hahnemannian dilutions and those treated with potentised water remained significant.

We also attempted to model the observed effects on urinary and faecal excretion as a function of potency. For this purpose the uncertainty was arbitrarily fixed at 2%. This low value is made possible by the fact that, throughout the experiment urine and faeces were collected separately as the animals were housed in metabolism cages.

Ten mathematical formulae of the form $Yi = F(Ii)$; i = 1.6, were tested. Two formulae gave the best approximation:

$$Yj = \sum_{i=0}^{1} \alpha_i [\log (Dj)]^i \quad j = 1,\ldots,6$$

$D = 10^{-2xcH}\ 10^{20}$ except potentised water

where, $\alpha_0 = 23.3729$
$\alpha_1 = 0.1190$
error = 0.0788

or

$$Yj = \sum_{i=0}^{1} \alpha_i [\log (Dj)]^i$$

$D = x$cH

where $\alpha_0 = 28.7243$
$\alpha_1 = -0.5215$
error = 0.0812

Unfortunately, neither of the formulae above permitted potentised water or the maximum effect. On the other hand, the following formula, which we finally adopted, permitted the potentised water results to be taken into account, and statistical analysis to be performed.

In the formula:

$$Y = \alpha_0 + \alpha_1 \log (1 + I) + \alpha_2 \log^2 (1 + I)$$

where, I represents the concentration of the Hahnemannian dilution, and α_0, α_1, α_2, constant coefficients for a series of data points, varying from series to series.

For cH:

$\alpha_0 = 19.848$
$\alpha_1 = 0.4771$
$\alpha_2 = -9.4567 \times 10^{-3}$
mean error % = 4.1949

For dH:

$\alpha_0 = 20.6925$
$\alpha_1 = 0.6897$
$\alpha_2 = -0.0155$
mean error % = 5.3969

Overall, the measured phenomena fit a mathematical formulation, with only small discrepancies between observed and theoretically predicted values.

There thus certainly exists a quantifiable and modellable biological effect due to Hahnemannian dilutions. The effects are a direct function of the degree of dilution.

Conclusion

The following conclusions may be drawn from this study of the effect of decimal and centesimal potencies on the retention and excretion of arsenic in the rat:

1 Arsenic excretion is predominantly faecal in the period 12–20 hours, irrespective of treatment (including potentised water control).

2 All the dilutions studied were active, increasing arsenic excretion.

3 The most active of the dilutions studied were those corresponding to a concentration of 10^{-14} (i.e. 14dH and 7cH). On the whole, the decimal potencies augmented excretion more than the centesimal.

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