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The Role of Variability in Evaluating Ultra High Dilution Effects: Considerations Based on Plant Model Experiments

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Key Words
Homeopathy · Arsenic trioxide · Plant models · Ultra high dilution · Variability

Summary
A series of experiments, performed on plant models with ultra high dilutions (UHD) of arsenic trioxide at 45th decimal potency has been reviewed with a particular focus on variability. The working variables considered are: the number of germinated seeds out of a fixed set of 33, the stem length of wheat seedlings and the number of necrotic lesions in tobacco leaf disks inoculated with tobacco mosaic virus (TMV). A thorough comparison between treatment and control group has been proposed, considering the two main sources of variability in each series of experiments: variability within and between experiments. In treated groups, a systematic decrease in variability between-experiments, as well as a general decrease, with very few exceptions, in variability within experiments has been observed with respect to control. Variability is traditionally considered as control parameter of model systems. Our hypothesis, based on experimental evidences, proposes a new role of variability as a target of UHD action. This hypothesis may help interpret unanswered questions that keep rising in basic and clinical research in homeopathy.

Schlüsselwörter
Homöopathie · Arsentrioxid · Pflanzenmodell · Ultrahohe Verdünnung · Variabilität

Zusammenfassung
Eine Reihe von Experimenten, die an Pflanzenmodellen mit ultrahohen Verdünnungen (UHD) von Arsentrioxid 45D vorgenommen wurden, wurde in Hinblick auf die Variabilität gesichtet. Die betrachtete Arbeitsvariablen sind: die Anzahl auskeimender Samen aus einem festgesetzten Satz von 33, die Stängellänge von Weizenkeimlingen und die Anzahl nekrotischer Läsionen an Tabakblättern nach deren Inokulation mit dem Tabakmosaikvirus (TMV). Ein sorgfältiger Vergleich zwischen behandelten und Kontrollgruppen wird vorgeschlagen, der die Hauptursachen der Variabilität in jeder Experimentserie betrachtet: die Variabilität zwischen Experimenten und die Variabilität innerhalb eines Experiments. Im Vergleich zu den Kontrollgruppen wurde in den behandelten Gruppen eine systematische Abnahme der Variabilität zwischen den Experimenten sowie – von wenigen Ausnahmen abgesehen – eine allgemeine Abnahme der Variabilität innerhalb der Experimente beobachtet. Variabilität wird normalerweise als Kontrollparameter von Modellsystemen angesehen. Unsere Hypothese, die auf experimentellen Befunden basiert, legt eine neue Funktion der Variabilität als Ziel der UHD-Aktivität nahe. Diese Hypothese könnte helfen, offene Fragen der homöopathischen Grundlagen- und klinischen Forschung zu interpretieren.

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Introduction

Variability is the attitude of a natural or social phenomenon to assume different modalities, i.e. values or attributes, from an individual (a 'statistical unit') to another, or in the same individual dependent upon space or time context. For instance, if we are studying the weight of human adults, we have to face both kinds of variability, since weight varies from person to person, and one and the same person may become thinner or fatter from one measurement to another. Therefore, we can distinguish between an intra-individual variability and an inter-individual one.

In experimental research, variability is usually evaluated in order to check the stability of a test system, in a single experiment (within-experiment) and in its repetitions (between-experiments). A small variability is the most desired goal in every experimental design. According to this point of view, variability plays the role of a 'control parameter' of average or median values, referred to a specific working variable, but is not considered as an 'essential parameter' in the result evaluation. Generally, variability changes with a random pattern; otherwise, a systematic increase or decrease may reveal an underlying cause.

A more prominent role of variability has been suggested by Davids et al. [1] who applied the dynamical system theory to sports medicine, contradicting traditional views of variability as noise or error. This new perspective in movement variability is seen as functional in allowing individuals to adapt to the multitude of unique constraints (personal, task and environmental) on performance.

Research in homeopathy has surely underrated the role of variability. The attention has been focused on the hypothetical mechanism of action of homeopathic treatments [2–4] and on the lack of repeatability [5, 6]. Particularly the latter aspect has generated serious doubts on treatment efficacy and stimulated explanations of ultra high dilution (UHD) effects, mostly using complexity theory [7]. Homeopathy is a widely followed medical practice despite its controversial scientific basis. Besides clinical trials, costly and ethically problematic, much efforts have been made in investigating UHD effects on in vitro and in vivo biological models [8, 9]. That approach could provide deeper insight into UHD mode of action, thus giving hints for medical investigations.

During more than a decade, starting in the early 1990s, we have tested the UHD effects of arsenic trioxide (As_2O_3) on different plant models. With 'ultra high dilution' we mean serial dilutions up to above Avogadro number (thus having no molecules of original substance any more), spaced out with vertical succussions, according to Hahnemann's methodology [10]. On the basis of the experimental evidences in wheat and tobacco models [11, 12], our hypothesis is that a systematic reduction of variability might be one of the peculiar actions of UHD. Therefore we propose to consider variability as a central theoretical issue worthy of study in its own right.

Analysis of Variability in Wheat and Tobacco Models

Our basic model was focused on wheat seed germination [13, 14] and growth [12, 15]. Treatments were prepared following a standardized protocol [for details, see 12]; two kinds of experiment, with and without preliminary stress of the seeds with sub-lethal doses of As_2O_3, were carried out. We observed repeated significant effects both on germination and growth, particularly relevant with As_2O_3 at the 45th decimal potency (45x).

As far as the variability is concerned, we have essentially taken into account inter-individual variability, adopting indices belonging to the two classes usually considered in experimental research: standard deviation (SD) and coefficient of variation (CV), that is a relative index resulting from the ratio between SD and average value (m), thus allowing comparisons between variability of different phenomena.

Re-analysing all our previous results, we have reported in table 1 the mean (average) values, jointly with the respective standard errors (SE), and the percentage comparison of such mean values between control (unpotentised water) and As_2O_3 45x. In the wheat germination model (labelled A, if unstressed, and B, if stressed) we considered the number of germinated seeds in a standard trial of fixed size (n = 33), while in the wheat growth model (labelled C) the working variable was the stem length (in mm). The stimulating effects on mean values ranged, approximately, from 2 to 12% for germination and sensibly more (near 25%) for seedling growth. In stressed seed groups, the effects on germination are more relevant than in corresponding non-stressed groups, because the stress creates the right experimental conditions for the outgoing of the curative power of the treatment [16, 17].

We also developed a phytopathological model based on tobacco plants/tobacco mosaic virus (TMV) interaction [11], labelled D, in which tobacco leaves were inoculated with the virus (biotic stress) and then treated with UHD of As_2O_3. Once more, the 45x treatment yielded the most remarkable effects (compared to unpotentised water control), inducing a relevant decrease in the average number of necrotic lesions, indicating a better plant resistance to the virus.

Then we considered in detail the effects on variability. In table 2 we have reported the standard deviations of each control and treatment group, divided in its complementary sources: variability within and between experiments. If we indicate with y_{ik} the k-th observation of the i-th experiment, with n_i and m_i being the sample size and mean value of the i-th experiment, with n and m being the overall sample size and mean value, we then have:

$$SD\ (\text{overall}) = \sqrt{\frac{\sum_i \sum_k (y_{ik} - m)^2}{n}}$$

$$SD_W\ (\text{within experiments}) = \sqrt{\frac{\sum_i \sum_k (y_{ik} - m_i)^2}{n}}$$

Table 1. Results obtained with As_2O_3 45x (As) on different plant models: average value (M) and corresponding standard error (SE); difference in average (Avg. Diff. %) with respect to control (C)

Code	Model	Stress	C		As		Avg. Diff. (%)
			M	SE	M	SE	
A	*Wheat germination*						
A1	1992/93 [13]	no	31.21	0.20	31.96	0.18	+2.4
A2	1993/94 [14]	no	31.46	0.18	32.06	0.17	+1.9
A3	1995/96 [14]	no	31.00	0.23	32.13	0.26	+3.6
B	*Wheat germination*						
B1	1993/94 [14]	yes	27.97	0.41	29.75	0.37	+6.4
B2	1995/96 [14]	yes	26.42	0.71	29.63	0.49	+12.1
B3	2001 (data not published)	yes	27.29	0.36	28.50	0.27	+4.4
C	*Wheat growth*						
C1	1993/94 [15]	yes	3.17	0.16	3.94	0.17	+24.3
C2	2000 [12]	yes	6.18	0.38	7.51	0.47	+21.5
D	*Tobacco/TMV*						
D1	2001/02 [11]	yes	96.04	3.65	74.91	3.18	–22.0

Working variables are: number of germinated seeds (wheat germination), stem length in cm (wheat growth), number of necrotic lesions (Tobacco/TMV). Reference number in square brackets.

Table 2. Results obtained with As_2O_3 45x (As) on different plant models: standard deviation (SD), standard deviation within experiments (SD_W) and between experiments (SD_B), percentage comparisons (Diff. %) with respect to control (C)

Code	Number of experiments	SD			SD_W			SD_B		
		C	As	Diff. %	C	As	Diff. %	C	As	Diff. %
A1	16	1.35	0.89	–34.3	1.04	0.60	–42.3	0.86	0.65	–24.3
A2	12	1.33	1.03	–22.8	1.12	0.86	–23.4	0.71	0.56	–21.4
A3	8	1.58	1.27	–19.8	1.32	0.94	–28.6	0.87	0.85	–2.4
B1	12	2.37	2.14	–9.7	1.32	1.46	+10.8	1.97	1.56	–20.6
B2	8	3.50	2.41	–31.1	3.09	2.27	–26.5	1.64	0.81	–50.8
B3	8	2.47	1.86	–24.8	2.26	1.62	–28.2	1.01	0.91	–10.0
C1	8	1.98	2.03	+2.4	1.79	1.91	+7.0	0.85	0.67	–21.3
C2	3	3.63	2.57	–29.3	3.48	2.46	–29.4	1.03	0.74	–28.0
D1	3	59.99	51.18	–14.7	57.52	49.83	–13.4	17.06	11.69	–31.5

Code explanation can be found in table 1.

$$SD_B \text{ (between experiments)} = \sqrt{\frac{\sum_i (m_i - m)^2 n_i}{n}} \qquad \gamma = \frac{\sum_i \sum_k (y_{ik} - m)^3}{S^3}$$

We used standard deviations because they have the same unit of measurement of the original data, but due to the square root effect, the sum of $SD_W + SD_B$ is not equal to SD any more.

Looking at table 2, we can easily notice that the variability between experiments (SD_B), in treated groups, is smaller, in many cases dramatically smaller, in all plant models considered. Moreover, the variability within experiments (SD_W) is also generally smaller, with only two exceptions. The resulting overall standard deviation is then decreasing with a single exception with an almost immaterial increase.

In table 3 we finished our statistical analysis by reporting the CV (in %) and the index of skewness γ, defined as follows:

Looking at table 3, we can see a systematic decrease in CV, even in experiment B1 (where the standard deviation was slightly increasing). The only exception is in the tobacco/TMV model (D1), where the CV of the treated group is increased, possibly due to the sensible decrease of the average number of necrotic lesions which stands as the denominator of CV (see again table 1). Regarding skewness, the percentage differences seem to indicate an uncontrolled variability; yet, this is not surprising, if we consider that we are comparing indices that may even be null or negative. Nevertheless, the absence of a regular pattern in skewness comparisons between control and treated groups is evident. On the other hand, we may con-

Table 3. Results obtained with As_2O_3 45x (As) on different plant models: coefficient of variation % (CV), percentage comparisons (Diff. %) with respect to control (C), skewness (γ).

Code	CV			Skewness (γ)		
	C	As	Diff. %	C	As	Diff. %
A1	4.3	2.8	–35.9	–0.886	–0.275	–69.0
A2	4.2	3.2	–24.2	–0.228	–0.729	+219.7
A3	5.1	3.9	–22.6	–0.696	–1.705	+145.0
B1	8.5	7.2	–15.1	–0.018	–1.171	+6405.6
B2	13.2	8.1	–38.5	–0.384	–0.661	+72.1
B3	9.1	6.5	–28.0	–1.120	–0.224	–80.0
C1	62.3	51.4	–17.4	–0.321	–0.861	+168.2
C2	58.8	34.2	–41.8	–0.468	–1.495	+219.4
D1	62.5	68.2	+9.2	0.672	0.628	–6.5

Code explanation can be found in table 1.

sider that none of our working variables was expected to follow a symmetric model. For instance, Poisson distribution, which is proved, in our studies, to be a suitable model for germination, is a skewed model.

Discussion

The re-analysis presented here focused on the inter-individual variability, where the statistical unit is either a Petri dish containing 33 wheat seeds (wheat germination model), a single wheat seedling (wheat growth) or a tobacco leaf disk inoculated with TMV. A tendency toward a variability reduction in treated groups has always been evidenced. In a recent independent study, in which the experimental conditions of our wheat growth model were reproduced [18], a trend toward a decrease in variability between experiments was observed as well. Interestingly, this trend seemed to be uncoupled with the direction of the treatment effect, in fact in the reproduction trial the result is a complete reversal of the original (i.e. significant depression of shoot growth instead of increase).

In order to find an interpretation to the decrease of variability observed in all our model systems, we can refer to the traditional difference between 'aggregate' and 'system' deeply discussed by Humphrey [19], a well-known Canadian philosopher and expert of system theory. We define a simple aggregate as a complex of elements that are not connected by specific relations, whereas a system is a complex of elements connected by a network of relations. Humphrey stated that '… the system is thus a whole of parts, the wholeness being given by the relations existing between the constituent elements …' Given this definition, whether an action is directed to an aggregate or a system would lead to completely different responses. In experimental models, when a treatment has an effect on a bio-object and induces a modification, we can say that the treatment itself has essentially a 'local effect'. In this context, the bio-object is supposed to behave like an aggregate rather than a system. This happens, for example, in our wheat growth model, as we observe an increase of stem length in treated groups: each seedling has an individual interaction with treatment, regardless of the other seedlings. But all seedlings share the same biological features, from genetic level to phenotypic shape and size, which make it that specific kind of organism. Therefore, the set of individual seedlings can be considered not only as a mere aggregate, but as a very system, which is a wholeness connected by an organic kind of relations between elements. We would thus like to recall Humphrey, who wrote that 'any material system is regarded from the dynamical point of view, as constituted of a number of particles subject to inter-connections and constraints of various kinds'. The dilution/dynamization process, typical of the homeopathic procedure, would confer to the treatment's 'skill' to have an effect on the constraints, with a dynamic that still needs to be elucidated, and would allow that bio-objects (or their parts) would behave as a system, and not merely as an aggregate. On that basis, we hypothesize that the decrease of variability is an expression of the system's existence. We could therefore describe the effect of the UHD as 'systemic'.

Finally, we may argue that the concept of constraint can be related to that of 'equilibrium point' of a system, as discussed in a previous study [11]: the homeopathic treatment would lead the bio-objects towards this point, thus inducing a decrease of variability. Consequently, when the parameter considered is higher than the system equilibrium point, the treatment would induce a reduction of its value; on the other side, when the parameter is lower than the equilibrium point, the treatment would lead it to increase. This interpretation could give a possible solution to some of the questions rising up in basic research and clinical practice in homeopathy: (1) the usually less intense effect obtained with homeopathic treatments compared to allopathic ones [20] could be attributed to a 'local' effect, but considering the 'systemic' action on variability a more relevant result could be observed; (2) the lack of repeatability often reported with homeopathic treatments could be interpreted as a tendency towards the equilibrium point.

We suggest that our hypothesis be tested on the basis of a reanalysis of the existing results in homeopathic literature; moreover, further evidence should be given by means of specifically designed experiments.

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