REAPPRAISAL OF A CLASSICAL BOTANICAL EXPERIMENT IN ULTRA HIGH DILUTION RESEARCH. ENERGETIC COUPLING IN A WHEAT MODEL

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SUMMARY

A. The effects of three different dilutions of silver nitrate log 24, log 25 and log 26, specially prepared according to the instructions for the preparation of "homoeopathic" remedies, following a historical protocol (1926) on germination and growth of coleoptiles of wheat seedlings were investigated. A typical effect pattern was found, with the dilutions log 24 and log 26 significantly enhancing development. The consistency with a previous historical study is discussed.

B. In experiments with dilution log 24, it was found that both unprepared solvent (water) as well as analogously "homoeopathically" prepared water is suitable for reference. The consistency with a previous multicenter study is discussed.

C. The effects of the dilutions, as described in study A, were investigated when the dilutions were kept in sealed glass vials that were brought in permanent contact with the water the grains were submerged with. The typical effect pattern already described in study A was found.

ZUSAMMENFASSUNG

A. Es wurden die Wirkungen dreier verschiedener Verdünnungen von Silbernitrat log 24, log 25 und log 26 auf Keimung und Koleoptilwachstum von Weizensamen untersucht. Die Verdünnungen wurden entsprechend der "homöopathischen" Vorschrift eines historischen Protokolls (1926) zubereitet. Ein typisches Wirkungsmuster, bei dem die Verdünnungen log 24 und log 26 die Entwicklung deutlich förderten, wurde beobachtet. Die Übereinstimmung mit einer früheren historischen Studie wird diskutiert.

B. In Experimenten mit Verdünnung log 25 wurde gefunden, daß sowohl nicht eigens präpariertes Lösungsmittel (Wasser) als auch analog "homöopathisch" zubereitetes Wasser als Kontrolle verwendet werden können. Die Übereinstimmung mit einer früheren multizentrischen Studie wird diskutiert.

C. Es wurden die Wirkungen der in Studie A beschriebenen Verdünnungen untersucht, wenn diese Verdünnungen in versiegelten Glasphiolen eingeschlossen waren, die in permanentem Kontakt mit dem die Samen bedeckenden zugegossenen Wasser waren. Das bereits in Studie A beschriebene Wirkungsmuster wurde erneut beobachtet.

INTRODUCTION

An increasing number of studies report different effects of diluted substances, depending on whether or not those were submitted to a "homoeopathic" agitation process during dilution (see the examples discussed in this book). There is clinical evidence that even in very high dilutions, when these were submitted to a special preparation process, specific information can be stored [1,2].

In the background of such more or less therapy-orientated studies, important work has been done in research on Ultra High Dilutions (UHD) on plant models [3-22]. In many of

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these studies [e.g. 14,15], but also in other fields of UHD research [23], it was a.o. claimed that, from a logarithmic range of dilutions (e.g. 10^{-24}, 10^{-25}, 10^{-26}), only certain dilutions are able to exert biological activity.

The aim of the study discussed here is the critical evaluation of the reliability of a test system which has been quoted as a basic model for the research on "homoeopathic" drugs, and has been discussed for decades [8,10,11,12,14,15,21].

The efficacy of a "homoeopathic" drug commonly used, namely silver nitrate, was investigated in the model system of the germination and the development of leaves of wheat grains. The study is based on the facts a. that silver nitrate in a common molecular dose inhibits life processes [24] and b. that inverse - i.e. stimulating - effects of specially prepared high dilutions of silver nitrate have been reported [8,10,11,12,14,15,21], especially from "homoeopathically" prepared dilutions log 24 and log 26 (but not log 25) [14].

In order to evaluate the respective protocol by Kolisko [14] critically, a study was performed to compare the effects of different steps of the dilution of silver nitrate, namely log 24, log 25 and log 26. Unprepared solvent (distilled water) was used as an additional control (**A**).

Furthermore, the dilution log 24 was tested against analogously "homoeopathically" prepared distilled water (**B**). Data from a respective study, organized by the biological editor of this book and performed in three independent research institutes (see discussion), have been published earlier [22].

Furthermore, an additional approach was added: it concerned the evaluation of the exposure to the test dilutions in a way that they were not mixed with the water the grains were submerged with. For this purpose, the test dilutions silver nitrate log 24, log 25 and log 26 were applicated in closed glass vials (**C**).

METHODS

Study A

Plants: Unbroken, unsorted grains of winter wheat (sort: Mephisto), grown without an application of herbicides or pesticides, were used.

Sites involved: All experiments were performed by one researcher, but at two different sites, at the Institut für strukturelle medizinische Forschung, Graz and the Ludwig Boltzmann Institut für Homöopathie, Graz, respectively.

Observed development: According to the protocol of 1924 [14], germination and the initial development of stalks (coleoptiles) were observed. It is reported that biological systems in such circumscript developmental transitions show maximal sensitivity [25,26, see the contribution of Endler et al.]. The parameters observed according to [14] seem to be adequate for a first quantification of the development of the plants.

Preparation of test solutions: The grains were observed under the influence of an aqueous solution $1:10^{24}$ part of weight of silver nitrate (Merck), specially prepared according to "homoeopathic" instructions. The stock solution had a concentration of 1:100 part silver nitrate of weight, as described in [14], and it was diluted in distilled water in steps of 1:10. The solutions, including the stock solution, were agitated according to standardized instructions [27]: at every step a sterile bottle is partly filled with the dilution,

and is pushed down at short regular intervals (e.g. against a rubber impediment) to create mechanical shocks. The test dilution prepared in this way is called dilution silver nitrate D24. In addition to the dilution log 24, dilutions log 25 and log 26 part silver nitrate of weight were analogously prepared. Three analogously prepared sets of ranges of the dilutions were used. Untreated distilled water was used as an additional control.

Independent solution coding: All sets were applied blindly. The sets were coded at the Institut für strukturelle medizinische Forschung, Graz, and at the Institut for Plant Physiology of the University of Graz, respectively.

Exposition to probes according to [14]: The grains with the germination furrow downwards were put into glass dishes (diameter 11cm), each containing 20 ml of the respective probe. The number of germinated grains and the stalk length were measured after 5 days. Natural light was used. The experiments were performed inside in the summer season at a temperature of 20°C (comparatively long coleoptiles). The position of the dishes was rotated in the course of the experiment.

Avoidance of contamination by the probes: Before use, the dishes were treated with dry heat for 45 minutes and splashes of the dilutions into the surroundings, when they occurred, were removed with soapy water according to the instructions of Boyd [28].

Data base: Three sets of dishes for the treatment with dilution silver nitrate D24, D25 and D26, respectively, plus dishes for additional water control were always used for the experiments. 30 grains were put into one dish; the number of grains was equal in each experiment (for total numbers, see below).

Evaluation of the data: The number of germinated seedlings was compared to the number of non-germinated seedlings for the groups according to the treatment in a four-field table by the chi-square test. For a description of the data on stalk lengths, the statistical mean was used, and the lengths were compared by one way variance analysis. S.E. of the arithmetic mean was calculated. According to [14], the groups of seedlings treated with the different silver nitrate probes were mutually compared (see figures). Furthermore, they were compared to the water control (see tables).

Study B

Researchers involved: Two sets of experiments were performed by two independent researchers (Endler, Pongratz) from the Ludwig Boltzmann Institut für Homöopathie, Graz, at two different laboratory sites.

Preparation of test solutions was done as described for study A (dilution silver nitrate D24). Analogously prepared distilled water (dilution water D24) was used for control. Two analogously prepared sets of the dilutions were used.

Data base: 16 sets of dishes for the treatment with dilution silver nitrate D24 and water D24, respectively, were always used for the experiments. 20 or 30 grains, respectively, were put into one dish; the respective number of grains per dish was equal for both groups (for total numbers, see below).

The experiments were performed inside in the winter season at a temperature of 15°C (comparatively short coleoptiles). For further conditions, see study A.

Study C

Exposition to the probes: The dilutions silver nitrate D24, D25 and D26, as well as those for control, respectively, were sealed in glass vials. The coded vials were first all hung

into a common water bath to make sure that they were not individually contaminated outside and then hung into the water submerging the corresponding sets of grains.

Ampoules used: The dilutions were sealed in hardglass vials with an optical transmission spectrum starting from about 350 nm, the optical transmission spectrum was limited by the properties of the submerging water to wavelengths less than about 2500 nm.

The experiments were performed inside in the summer season at a temperature of 20°C (comparatively long coleoptiles). For further conditions, see study A.

RESULTS

Study A

Experiments with a total of 890 grains treated with silver nitrate D24, 890 grains treated with silver nitrate D25 and 890 grains treated with silver nitrate D26 were performed. In addition, one group with 686 grains was treated with unprepared distilled water. The comparison of the germination rates as well as of the mean stalk length showed a certain pattern of the effects of the three different dilutions. All the parameters observed indicate that development is enhanced by the probes silver nitrate D24 and silver nitrate D26 as compared to the probe silver nitrate D25 (see Fig. 1 and Tab. 1).

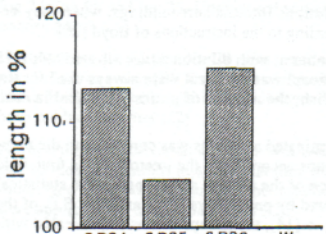


Fig. 1: The influence of the test dilutions silver nitrate D24, D25 and D26, compared to unprepared water, on wheat germination. Ordinate: mean stalk length. 100% refers to the stalk length under the influence of unprepared water. Abscisses: S D24 - D26, silver nitrate D24 - D26; W, unprepared water.

Tab. 1: Stalk lengths, study A

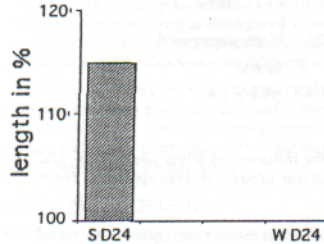
$AgNO_3$ D24	$AgNO_3$ D25	$AgNO_3$ D26	Water unprepared
60.1 ± 1.4	55.5 ± 1.4	61.1 ± 1.4	53.1 ± 1.4
**	-	*	

Tab. 1: Mean stalk length of wheat under the influence of silver nitrate D24, D25, D26 and unprepared water, respectively. Median (mm) ± SEM. **, $P < 0.01$; *, $P < 0.05$; -, not significant: the effect of the test substance compared to the effect of unprepared water.

The differences could be found both for the pooled data as well as for most of the 16 successive experiments. They were also found when the statistical median was used for the calculations instead of the statistical mean.

Study B

Experiments with a total of 400 grains treated with silver D24 and 400 grains treated with water D24 were performed by the two researchers. When the data were pooled, the germination rate of grains treated with the test dilution silver nitrate D24 was above that for



reference at about 5 % ($P > 0.05$), and the mean stalk length in the group treated with the test dilution was above that for reference at about 15% ($P < 0.001$, see Fig. 2).

Fig. 2: The influence of the test dilution silver nitrate D24, compared to water D24, on wheat germination. Ordinate: mean stalk length. 100% refers to water D24. Abscissa: S D24, silver nitrate D24; W D24, water D24.

Tab. 2: Stalk lengths, study B

$AgNO_3$ D24	Water D24
20.0 ± 2.5	17.4 ± 2.5
**	

Tab. 2: Mean stalk length of wheat under the influence of silver nitrate D24 and water D24, respectively. For further information, see Tab. 1.

Experiments with a total of 200 plus 200 grains were performed by Endler. Both the germination rate as well as the mean stalk length for the grains treated with the test dilution were above those for reference (however, only the difference in the stalk lengths was statistically significant - $P < 0.01$ -).

Experiments with a total of 200 plus 200 grains were performed by Pongratz. Both the germination rate as well as the mean stalk length for the grains treated with the test dilution were above those for reference (however, only the difference in the stalk lengths was statistically significant - $P < 0.05$ -). The differences were also found when the statistical median was used for the calculations instead of the statistical mean.

Study C

One experiment with 600 grains treated with silver nitrate D24, sealed in glass vials, 600 grains treated with silver nitrate D25, sealed in vials and 600 grains treated with silver nitrate D26, sealed in vials, was performed. In addition, one group with 600 grains was treated with unprepared distilled water.

The comparison of the germination rates as well as of the mean stalk length showed the same pattern of the effects of the three different dilutions as was found in study B. All the parameters observed indicate that the development is enhanced by the probes silver nitrate D24 and silver nitrate D26 as compared to the probe silver nitrate D25, when the dilutions were sealed in glass vials (see Fig. 3 and Tab. 3)

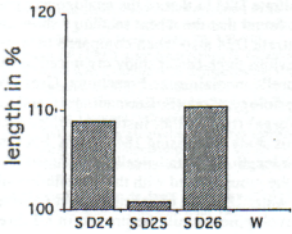


Fig. 3: The influence of the test dilutions silver nitrate D24, D25 and D26, sealed in glass vials, compared to unprepared water, sealed in a glass vial, on wheat germination. For further information, see Fig. 1.

Tab. 3: Stalk lengths, study C

$AgNO_3$ D24 in vial	$AgNO_3$D25 in vial	$AgNO_3$D26 in vial	Water unprepared in vial
65.1 ± 1.0 *	60.3 ± 1.3 -	66.1 ± 1.3 *	59.8 ± 1.4

Tab. 3: Mean stalk length of wheat under the influence of silver nitrate D24, D25, D26 and unprepared water, respectively, when all test substances were applied in sealed glass vials. For further information, see Tab. 1.

The differences were also found when the statistical median was used for the calculations instead of the statistical mean.

DISCUSSION

The findings of study **A** show a variable growth of wheat seedlings under the influence of the three different "homoeopathically" prepared dilutions of silver nitrate log 24, log 25 and log 26, "D24", "D25" and "D26". This is consistent with the description by Kolisko (1926) [14]. All Kolisko - data, too, show more growth under the influence of silver D24 and D26 than under the influence of D25 (Fig. 4).

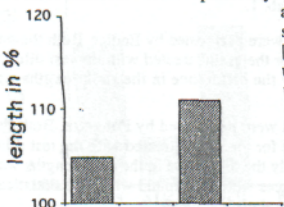


Fig. 4: The influence of the test dilutions silver nitrate D24, D25 and D26 in the experiments of Kolisko in 1924. Abscissa: 100% refers to silver nitrate D25. For further information, see Fig. 1. (Adopted from [14], modified.)

Furthermore, when in our study the silver nitrate dilutions were compared to the unprepared solvent, a statistically significant enhancement of growth was found under the influence of D24 and D26, but no difference at D25. This is consistent with our expectations derived from the Kolisko-study.The importance of the study discussed here is the critical proof of the reliability of a test system which has been quoted as a basic model for the research on "homoeopathic" drugs, and has been controversially discussed for decades.

In the study above, the effect of silver nitrate D24 was compared to that of D25 (according to Kolisko) and to that of untreated water (by laboratory convenience). Furthermore, we compared the effect of silver nitrate D24 to that of the analogously prepared solvent, i.e. water D24. In study **B**, it was found that the wheat seedlings show enhanced growth under the influence of silver nitrate D24 also when compared to wate D24. This is consistent with the findings of a previous three-center study organized by th writers and performed at the Institut für strukturelle medizinische Forschung, Graz (W Pongratz), the University Institute for Plant Physiology, Graz (E. Bermadinger), and th University Institute for Botanics, Vienna (F. Varga) (two further institutes contribute data based on different observation modes). In this study comprising 1840 grains, both th differences in germination rate as well as in stalk length were statistically significant (P 0.05 and P < 0.001, respectively), the values for the group treated with the test dilution sil ver nitrate D24 being above those for reference water D24. An enhancing effect of silve nitrate D24 on the observed stage in wheat development could be proven in all thre laboratories [22].

The findings of study **C**, where again silver nitrate D24, D25 and D26 were mutually compared and tested versus unprepared water as in study A, show the same typical pattern of variable growth of wheat seedlings under the influence of the three dilutions as described in study A, also when the dilutions were sealed in glass vials.

At present, we have only very limited insight into the physical properties of such test dilutions and into their physiological interconnections with the living system. However, with regard to study C, a non-molecular interconnection between the *UHD* and the living system such as an electromagnetic field can be postulated. (For further information, see the contribution by Endler et al.)

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ANNOTATION

A more detailed description of the experiments discussed here is in preparation for print. The authors thank Z. Bentwich for his collaboration.

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