

Original Article

Transfer of the effect of potentized mercuric chloride on α -amylase from one test tube to another through capillary water

Atheni Konar, MSc^{1,3}, Tandra Sarkar MSc^{1,3}, Nirmal Chandra Sukul*, PhD^{2,3}, Anirban Sukul PhD³, Indrani Chakraborty, PhD.^{3,4}

¹Molecular Homeopathy Research Unit, Dr. Bholanath Chakravarty Memorial Trust, Kolkata, West Bengal, India

²Department of Zoology, Visva-Bharati University, Santiniketan, WestBengal, India

³Sukul Institute of Homeopathic Research, Santiniketan, West Bengal, India

⁴Department of Zoology, Belda College, West Midnapur, West Bengal, India

*Corresponding author: NC Sukul, ncsukul@gmail.com

Abstract

Objective: In a series of experiments we showed that treatment of a plant or animal with a diluted and agitated substance might affect other plants or animals connected to the former by the capillary water in cotton threads. The aim of the present study was to establish whether drug effect could be transferred in a cell-free medium.

Design: Two test tubes, each containing 1 ml of 1% starch solution and 1 ml of α -amylase, were connected by means wet cotton threads encased in a polythene tube. One of the tubes also contained *Mercurius corrosivus* (*Merc-c*) 30 cH and the other ethanol solution (control). After 15 min, the enzyme activity was stopped with DNSA, and the breakdown product of starch, maltose, was estimated. A third, separate tube contained all the tested materials except for *Merc-c* and the control solution. In a second experiment two tubes, one containing 1,200 ppm and the other 200 ppm of maltose, were similarly connected over 15 min. Both experiments were repeated 20 times.

Results: In the first experiment, the amount of maltose was similar in both connected tubes, but it was significantly lower in the unconnected tube. In the second experiment, maltose concentration in both tubes remained unchanged.

Conclusion: The information of *Merc-c* 30 cH was effectively transferred through capillary water between two tubes in cell-free medium. This effect was not due to physical transfer of either solvent or solutes. Water seems to be the most probable carrier of information in diluted and agitated solutions.

Keywords: Water, α -amylase, *Mercurius corrosivus* 30 cH, starch, maltose, high dilutions



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Introduction

In a series of experiments we demonstrated that the effect of diluted and agitated substances was transferred from one plant to another,^{1,2} and also from one animal to another through the capillary water in cotton threads.^{3,4} The studies indicate that water behaves as the carrier of the information contained in homeopathic drugs. To exclude the possible interference of a living body with the transfer of the drug effect, we devised a cell-free model.

In an earlier study, Sukul *et al*⁵ found that *Mercurius corrosivus* (*Merc-c*) 30 cH, accelerated *in vitro* hydrolysis of starch by α -amylase in a cell-free medium. That model was adapted to conduct the transfer experiment in a cell-free medium. Boyd⁶ first reported that *Merc-c* 30 cH enhanced the *in vitro* hydrolysis of starch by diastase. Potentized mercuric chloride was reported to increase the α -amylase activity⁷. Positive effect of *Merc-c* 30 cH was also reported in systematic reviews.^{8,9}

Materials and methods

Merc-c 30 cH (Dr. Reckeweg, Germany) was purchased as a 10-ml sealed vial from the local market, Kolkata, India. The drug was prepared in 90% ethanol as shown in the label. The control consisted of 90% ethanol was prepared from absolute ethanol (Merck,

Germany). Both control and drug were diluted with sterile distilled water in proportion 1:500 two hours before each experiment. In an earlier study, we established that diluting a homeopathic drug in proportion 1:1,000 conserves the specific efficacy of the drug, while it eliminates the effect of the diluent medium, ethanol.¹⁰

Preparation of reagents

DNSA reagent was prepared with 1 g dinitrosalicylic acid (DNSA), 0.2 g crystalline phenol and 0.5 g sodium sulfite. DNSA was then dissolved in 100 ml sterile distilled water with 1% NaOH, and stored in the dark at 4 °C. Rochelle salt was prepared with 40 g potassium sodium tartrate dissolved in 100 ml distilled water, and then placed in sealed glass vials and stored at 4 °C. Soluble starch (SRL, Mumbai, India) was dissolved in 0.1 M sodium acetate buffer (pH 4.7) to prepare 1% starch solution. Porcine α -amylase was obtained from SRL.

The activity of α -amylase in terms of maltose release resulting from the hydrolysis of starch was measured by a standard biochemical procedure.^{5, 12} Maltose is a disaccharide of α -1, 4 linked glucose.³ One milliliter of α -amylase was mixed with 100 μ l of *Merc-c* 30 cH 1:500 or control I (90% ethanol, 1:500) solution and kept in test tubes. The test tube containing enzyme and drug solution was



connected by means of a wet cotton thread to another test tube, which contained the enzyme and, instead of the drug, an equal amount of distilled water (Figure 1). A piece of 15-mm thick, 80-cm long sterile cotton

thread was soaked in sterile distilled water and encased in a flexible 37-cm long polythene tube so that the free ends of the thread remained uncovered (Figure 1).



Figure 1. Test tube containing Merc-c 30cH (far left) connected to a test tube without the drug (second from left). Test tubes containing ethanol control I (third from left) and distilled water control II (fourth from left). Test tube on the far right was used as reference.

Each test tube was 15-cm high and 4-mm wide inside. The experiment included two further test tubes, one containing enzyme and ethanol solution (Control I) and the other enzyme and sterile distilled water (Control II)

in the same amount as the drug or control I). Ten min later, 1ml of starch solution was added to each test tube (the connected pair and controls I and II) and kept for 15 min. The 2 connected tubes were kept at the same



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level for the solution not to move from one to the other through the wet thread. After 15 min, the connecting thread was carefully withdrawn from the paired tubes. Next, 2 ml of DNSA reagent was simultaneously added to each tube to stop the enzyme activity. The 4 tubes were placed in boiling water for exactly 2 min and then cooled to room temperature (27 °C). Next, 5 ml of distilled water were added to each tube. The optical density (OD) of the solution in each test tube was measured at 540 nm in a UV-VIS spectrophotometer (Shimadzu, Japan). The breakdown product, maltose, was quantified on a standard curve plotted with 6 different concentrations of maltose (200, 400, 600, 800, 1,000 and 1,200 ppm). The experiment was repeated 20 times. To exclude the possibility of direct transfer of solutions between the 2 connected test tubes through the capillary water in the connecting thread we performed another experiment. Two similar test tubes containing maltose solutions of different concentration (200 and 1,200 ppm) only were connected in a similar manner by means of wet cotton thread. After 15 min, the connecting thread was quickly withdrawn and the concentration of maltose in each test tube was measured in the spectrophotometer. Also the second

experiment was repeated 20 times. All the experiments were randomized by changing the relative position of test tubes except the connected ones, and the examiner was blinded as to the test tube contents. The results were analyzed by means of one-way ANOVA. All the experiments were conducted at room temperature (27 °C) and 60% relative humidity.

Precautions essential for consistent results

1. The DNSA reagent should be added to the drug containing and connected tubes simultaneously to stop the starch breakdown at the same time.
2. The test tubes should receive uniform heat in the water bath.
3. The cotton thread should remain properly immersed in the test tube liquid and the covering tube should remain upright.
4. Drug and ethanol solution (1:500) should be prepared freshly before each experiment.

Results

The amount of maltose released following 15-min reaction of α -amylase with starch in the 4 test tubes is presented with the corresponding standard error and results of statistical analysis in Figure 2.



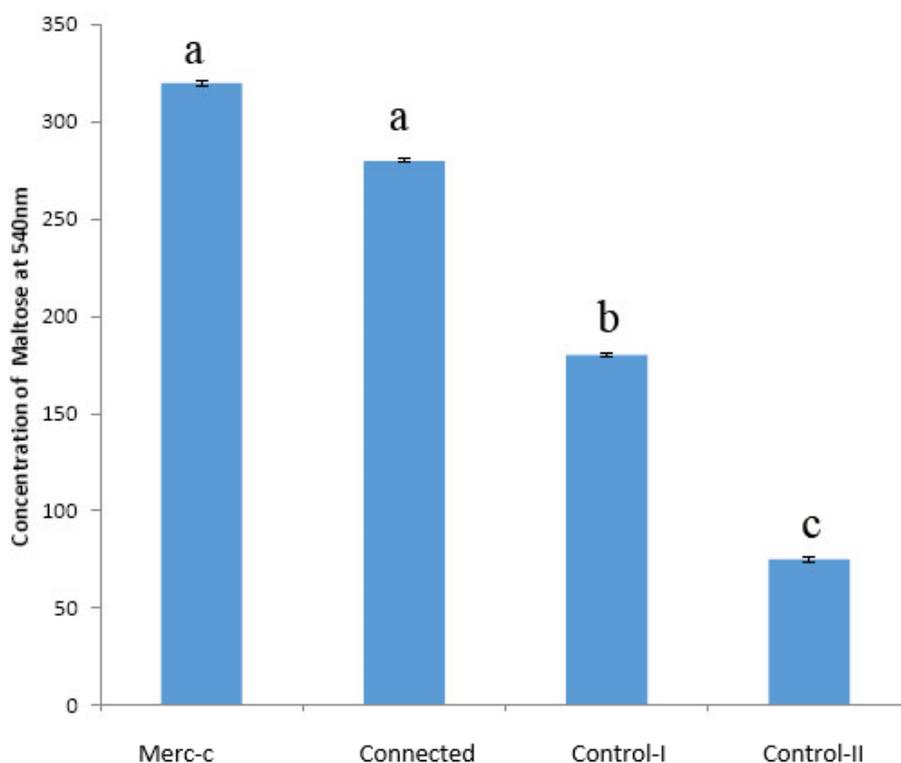


Figure 2. 1. Direct treatment with *Merc-c* cH, 2. Test tube without *Merc-c* 30cH, but connected to tube 1 I by a cotton thread. 3. Test tube containing diluent ethanol only (Control I). 4. Test tube containing distilled water instead of drug or ethanol (Control II).

a, b, c : different lowercase letters indicate significant difference ($p < 0.01$) by one-way ANOVA followed by Student's t-test. $n = 20$.

The data were analyzed by means of one-way ANOVA followed by Student's t-test. The amount of maltose in the tubes containing *Merc-c* 30 cH did not differ significantly from that in the connected tubes ($p < 0.05$). The tubes containing water (Control II) showed the lowest amount, while the ones containing ethanol (Control I) showed a significantly higher value compared to the ones with water ($p < 0.01$). Both the tubes containing *Merc-c* 30 cH and the connected ones exhibited the highest amount of released maltose, which

was significantly different compared to the ones containing ethanol ($p < 0.01$). The results of the second experiment showed that the concentration of maltose solution in the connected pair of tubes remained unchanged after 15 min: the initial and final concentrations were 200 and 1,200 ppm, respectively.

Discussion

The results provide further confirmation of our earlier results showing that diluted and



agitated *Merc-c 30cH* promotes α -amylase-induced hydrolysis of starch.⁵ However, other authors did not find any effect of *Merc-c* on enzymatic hydrolysis of starch.¹⁴ Those authors prepared *Merc-c* with distilled water, while the efficacy of a homeopathic drug diluted in pure water deteriorates quickly.^{5,6} In addition, aqueous preparations might produce anomalous results. In turn, use of hydrated ethanol is problematic, as it produces some effect of its own. However, the effect of ethanol is substantially minimized by diluting aqueous ethanol in water in proportion 1:500 or 1:1,000.¹⁰

The results of the present study provide evidence on that water behaves as an effective carrier of the imprint of the starting materials. The results of the second experiment clearly demonstrate that there was no physical transport of solvent (water) or solute (maltose) between the test tubes connected by capillary water. We recently reported that high dilutions of different drugs differ as to their hydrogen bonding and free water molecules.¹⁵ The present *in vitro* study once again indicates that water seems to be the most probable carrier of information of the starting materials in homeopathic high dilutions.

Conclusion



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The information of *Merc-c 30 cH* was effectively transferred through capillary water between 2 tubes in a cell-free medium. That effect was not due to physical transport of either solvent or solute between the tubes. Water seems to be the most probable carrier of the information in homeopathy high dilutions.

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The authors declare that there is no conflict of interest.

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