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Effects of Analemma Water on Cellular Level

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ABSTRACT

Background: Water is a tasteless, odorless, and nearly colorless chemical substance that is the main constituent of most living organisms. From the strictly academic point of view, water is just H2O and exists in three different phases, namely liquid state, solid state and gaseous state. Water possesses a number of unique properties which can be explained by scientific knowledge only in part. According to the manufacturer, Analemma water is a stable, coherent water which improves the energy production in cells. The aim of this study was to use animal-free cell biological test methods to investigate the effects of Analemma water on cellular level in comparison to the initial local tap water.

Experimental: By using cultured organ-specific cells (intestinal epithelial cells, IPEC-J2) and functional neutrophils (differentiated HL-60 cells), the effect of Analemma water on cell metabolism, regeneration, cell viability/survival after oxidative stress and radical formation during inflammatory processes was investigated. Data given here represent mean values ± standard deviations of at least four independent experiments with biological replicates each. Statistical analyses were done with the two-tailed Wilcoxon-Mann-Whitney test.

Results: Analemma water resulted in an average stimulation of 22.8 ± 6.5 % at all test concentrations in direct comparison to the initial tap water ($p \leq 0.01$; n = 5). The medium increase in regeneration achieved by Analemma water compared to the initial tap water was 47.2 ± 20.5 ($p \leq 0.01$; n = 4). Intestinal epithelial cells cultured with Analemma water had a 25.8 ± 13.0 % better cell viability compared with cells cultured with initial tap water ($p \leq 0.01$; n = 4). Finally, Analemma water also stimulated the basal cell metabolism of functional neutrophils by 10.2 ± 9.6 % compared with initial tap water (not statistically significant). The formation of reactive superoxide anion radicals by the functional neutrophils occurred in a dose-dependent manner with an average increase of 23.0 ± 4.2 % for all concentrations between 10 and 40 vol% ($p \leq 0.01$; n = 4).

Conclusion: The in vitro results presented here suggest that Analemma water supports cellular processes such as metabolic activity, regeneration following injury, resistance to oxidative stress, and the formation of reactive oxygen species released by neutrophils. These results expand the knowledge of previous findings in clinical studies on human participants and studies on plants and soil.

Keywords: Quantum Electrodynamics; Quantum Biology; Coherence; Intestinal Health; Regeneration; Innate Immune Defense; Oxidative Stress; IPEC-J2 Cells; HL-60 Cells; Cell Culture

Introduction

Water is a tasteless, odorless, and nearly colorless chemical substance that is the main constituent of most living organisms. From the strictly academic point of view, water is just H_2O and exists in three different phases, namely liquid state, solid state and gaseous state. Water has a number of unique properties which can be explained by scientific knowledge only in part. According to the manufacturer, Analemma water has been conceived 2014 in a laboratory in The Netherlands, and mimicks the cycles of water in nature. Analemma water has undergone a year-long exposure to various physical and geometric forces which bring it into different patterns of oscillations of the coherent state. This water is filled into a wand made of quartz glass. When the Analemma wand comes into contact with regular water, it transforms its state and brings it into a stable coherent state which improves the energy production in cells. In a number of controlled double-blind clinical studies with human participants it has been shown that Analemma water has positive effects on human biological systems, including energy levels, intestinal health, immune function, and brain activity. In addition to the research with human participants, the effects on plants, soil microbiomes, and biophotonic processes were also investigated. In plants, stress resistance and physiological parameters were improved; in tomatoes and grains, biophotonic changes and energy efficiency were observed. All statements of this section are based on unpublished personal communications of the manufacturer.

Coherent water is not merely a transport medium in living biological systems, but can influence the functioning of biomolecules, the communication between cells and the balance of complex biological systems [1-5]. Water has also been linked with vital health and longevity [6]. Moreover, quantum electrodynamics is used as a framework for quantum biology, i.e. the idea that some biological structures and functions may arise from coherent electromagnetic interactions [7]. Its main biological claims are:

(1) Liquid water in cells does not act as a uniform fluid, but as a mix of coherent and non-coherent phases;

(2) Coherence domains in water, where molecules supposedly oscillate in a coordinated way;

(3) Efficient biochemical reactions, where only molecules with matching oscillation frequencies participate;

(4) Hormesis, meaning that very weak electromagnetic stimuli may produce a biological response, while stronger ones can disrupt coherence, and

(5) Morphogenesis and development, suggesting that weak fields may influence pattern formation.

The aim of this study was to use animal-free cell biological test methods to investigate the effects of Analemma water on cellular level in comparison to the initial local tap water.

Materials and Methods

Preparation of Analemma Water and Test Concentrations

1.000 mL of fresh local tap water (temperature 10-12°C) was structured by stirring clockwise with the Analemma water wand with stainless steel holder for 1 minute. The wand was provided by Water and Light B.V., NL – 3732 HW De Bilt, The Netherlands, for the current experiments. The initial local tap water and the structured Analemma water were added to cell culture media or reaction mixtures at a volume fraction ranging from 0 to 40 vol% in all repetitions. The experiments were performed over a period of several weeks always using freshly prepared Analemma water.

Cell Culture

The investigations were performed with two different cell types:

(1) Porcine intestinal epithelial cells (IPEC-J2 cells; ACC-701; Leibniz Institut, DSMZ, Braunschweig, Germany). The cells were routinely grown in a mixture (1:1) of Dulbecco's Modification of Eagle's Medium and Ham's F12 supplemented with 10 % growth mixture and standard amounts of antibiotics. The cells were routinely cultivated as mass cultures and were regularly subcultured twice a week with fresh culture medium.

(2) Human promyelocytes (HL-60 cells; ACC 3; Leibniz Institute, DSMZ, Braunschweig, Germany) which were differentiated to functional neutrophils able to undergo an oxidative burst upon phorbol ester stimulation similar to primary and freshly isolated neutrophils [8-10]. HL-60 cells were cultivated as suspended mass cultures in RPMI 1640 medium supplemented with 10 % growth mixture and standard amounts of antibiotics and were regularly subcultured twice a week with fresh culture medium. Cell cultivation was performed in an incubator at 37°C in a humid atmosphere of 5 % CO_2 and 95 % air.

Cell Metabolism

IPEC-J2 cells were seeded into 96-well culture plates (200 µL culture medium/well) at a cell density of 100,000 cells/well and incubated for 48 hours until complete adhesion and recovery of cellular metabolism was achieved. Thereafter, a reaction mixture consisting of phosphate buffered saline with calcium and magnesium, 15 mM glucose as an energy source, the test concentrations of the water samples (10 to 40 vol%) as well as a water-soluble tetrazolium dye (WST-1; 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt; Sigma-Aldrich, Deisenhofen, Germany) was added to the cells. Through the activity of the enzymes responsible for energy production in the mitochondria, the dye was cleaved and the resulting color change was measured quantitatively as an indicator of cellular metabolism. The WST-1 assay does not directly measure the number of living cells, but rather their metabolic dehydrogenases activity [11-13]. The optical density was measured as a differential measurement ΔOD = 450 and 690 nm at definite time points with an Elisareader (BioTek ELx808 & Gen 5 version 3.00) and analyzed using Microsoft Excel. Five independent experiments (n = 5) with biological replicates were performed.

Cell Regeneration

Intestinal epithelial cells were seeded at a density of 200,000 cells/mL into the four individual compartments of silicone 4 well-culture inserts (ibidi, Gräfelfing, Germany). The individual compartments are separated from each other by 500 µm thick silicone bars. Due to the special adhesive surface of the silicone frames, these adhere firmly to the bottom of a culture dish, thus forming a cell-free area which the cells can colonize by migration and proliferation after removal of the silicone frames. Immediately after removal of the silicone frames, 25 vol% of the water samples were added to the culture medium and cells were incubated for colonization of the cell-free area. After 12 hours of continuous incubation, cell cultures were washed with phosphate-buffered saline, fixed with methanol, stained with Giemsa's methylene blue solution (Sigma-Aldrich, Deisenhofen, Germany) and were air-dried. Micrographs documenting the residual cell-free area were done at different locations for each sample. A total of 4 measurements of the remaining cell-free area was done for each sample. IKOSA AI software with artificial intelligence (Kolaido, Altenrhein, Switzerland) was used to examine and calculate the residual cell-free area for the cell cultures. Four independent experiments (n = 4) with biological replicates were performed.

Hydrogen Peroxide-Induced Oxidative Stress

Cells were seeded into 96-well culture plates (200 µL culture medium/well) at a cell densitiy of 100,000 cells/well) and incubated for 24 hours until the cells had completely adhered and restored their metabolism. Then, cells were treated with hydrogen peroxide concentrations ranging from 0.5 to 2 mM in the culture medium together with 25 vol% of the water samples for the duration of the experiment [14-16]. After 24 hours of hydrogen peroxide exposure, cell viability was examined by incubation of the cells to a reaction mixture consisting of 180 µL of culture medium and 20 µL of the water-soluble tetrazolium dye XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide; Xeno metrix, Allschwil, Switzerland). The cleavage of the dye is used as a colorimetric method for the quantification of cell viability [17-19]. The optical density was measured as a difference measurement ΔOD = 450 and 690 nm at definite time points with an Elisareader (BioTek ELx808 with Gen 5 version 3.00) and analyzed using Microsoft Excel. Four independent experiments (n = 4) with biological replicates for each experiment were performed.

Endogenous Radical Formation

Promyelocytes were cultivated as suspended mass cultures in cell culture flasks with a vented cap (75 cm^2 growth area; TPP, Switzerland). The cultured promyelocytes were differentiated into functional neutrophils by adding 1.5 vol% dimethyl sulfoxide for 6 days. Finally, the cells were harvested by centrifugation (190 x g for 6 minutes) and were repeatedly washed in phosphate buffered saline with calcium and magnesium and centrifugation steps. Cells were resuspended in phosphate buffered saline with calcium and magnesium containing 10 mM glucose. 40 µL of the cell suspension aliquots were pipetted to a reaction mixture containing the test concentrations of the water samples (10 to 40 vol%), glucose solution, the tetrazolium dye WST-1 (Sigma-Aldrich, Deisenhofen, Germany) and phorbol-12-myristate-13-acetate (PMA; Sigma-Aldrich, Deisenhofen, Germany) for induction of an oxidative burst [20-23]. Cells without addition of the phorbol ester served as internal controls reflecting the basal cell metabolism without stimulation. The formed reactive superoxide anion radicals in the reaction mixture caused the cleavage and color change of the dye. The amount of superoxide anion radicals present in the reaction mixture was directly related to this color change. The change in optical density was recorded at various time points up to 40 minutes as a differential measurement ΔOD = 450 and 690 nm by an Elisareader (BioTek ELx 808 with software Gen 5 version 3.00) and calculated with Microsoft Excel. Four independent experiments (n = 4) with biological replicates for each experiment were performed.

Statistical Analysis

Data are given as mean values ± standard deviations. Single experiments with biological replicates were averaged within each independent experiment before statistical testing. Statistical analyses were done using the parameter-free two-tailed Wilcoxon-Mann-Whitney rank sum test and significance was determined at the 1 % ($p \leq 0.01$) level.

Results

Cell Metabolism

Analemma water resulted in an average stimulation of 22.8 ± 6.5 % at all test concentrations in direct comparison to the initial tap water. The difference of Analemma water to the initial tap water was always statistically significant for all test concentrations ($p \leq 0.01$). The maximum stimulation of Analemma water was observed at a volume concentration of 30 vol% present in the culture medium. The measurement data are presented in Figure 1 in detail.

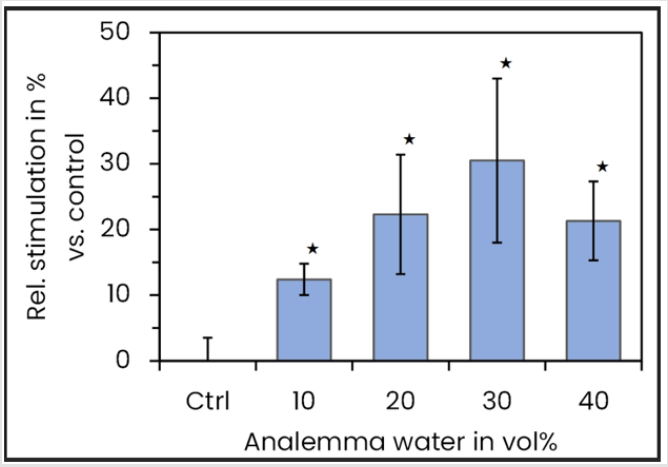

Figure 1: Graphical presentation of the stimulation of basal metabolism of cultured intestinal epithelial cells (IPEC-J2) after exposure to various concentrations of Analemma water in the culture medium. Ctrl = Control cells exposed to the same amount of initial tap water without treatment. Data represent mean values ± standard deviations of five independent experiments (n = 5) with biological replicates. *$p \leq 0.01$, two-tailed Wilcoxon-Mann-Whitney test.

Cell Regeneration

Based on the residual width at the end of the experiment, the medium increase in regeneration achieved by 25 vol% of Analemma water in the culture medium compared to 25 vol% of the initial tap water was 47.2 ± 20.5 %, demonstrating a better restoration of intestinal barrier integrity and functionality (Figures 2 & 3). Despite the large standard deviation, the difference of Analemma water to the initial tap water was statistically significant ($p \leq 0.01$).

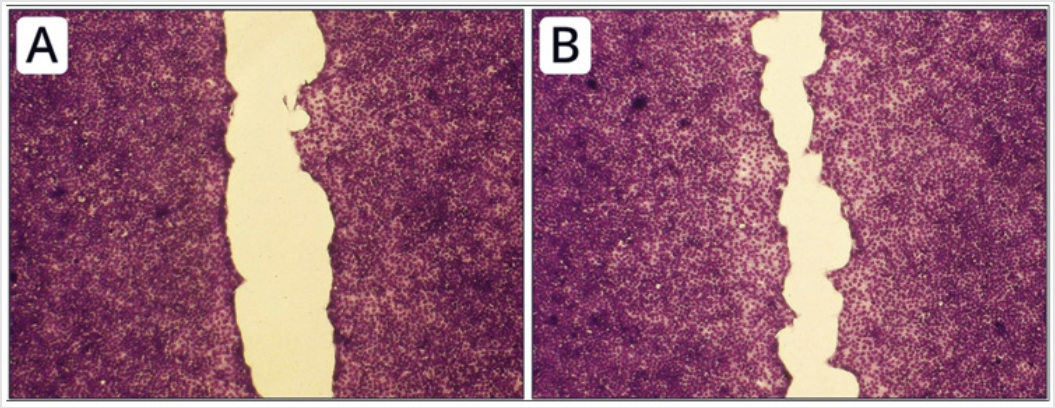

Figure 2:
Representative micrographs of the residual cell-free area after 12 hours of regeneration for intestinal epithelial cells (IPEC-J2) treated with 25 vol% of initial tap water (A) and cells which were treated with 25 vol% of Analemma water present in the culture medium for the duration of the experiment (B). Olympus IX50 inverted microscope with Olympus Planachromat 10x and an Olympus E-20 camera at 5 megapixel. Brightfield illumination of fixed and stained samples.

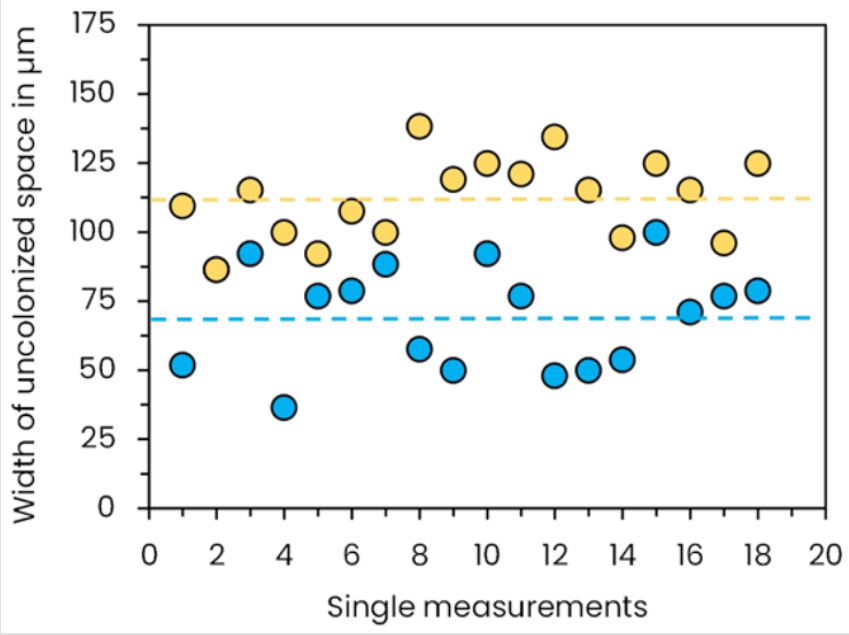

Figure 3: Graphical presentation of the single measurement data for both water samples for one experiment. The blue circles represent the width of the residual cell-free area for 25 vol% of Analemma water and the yellow circles for 25 vol% of initial local tap water. The colored dashed lines give the mean values for both water samples in this experiment. Please note: The smaller the area without cells, the more strongly regeneration is stimulated.

Hydrogen Peroxide-Induced Oxidative Stress

Intestinal epithelial cells cultured with 25 vol% Analemma water had a 25.8 ± 13.0 % better cell viability compared with cells cultured with initial tap water. The difference of Analemma water to the initial tap water was statistically significant ($p \leq 0.01$).

Endogenous Radical Formation

Analemma water stimulated the basal cell metabolism of functional neutrophils by an average of 10.2 ± 9.6 % compared with initial tap water. However, this difference was statistically not significant. The formation of reactive superoxide anion radicals by the functional neutrophils occurred in a dose-dependent manner with an average increase of 23,0 ± 4,2 % for all volume concentrations between 10 and 40 vol% present in the culture medium. This difference of Analemma water to the initial tap water was statistically significant ($p \leq 0.01$). The measurement data are presented in Figure 4 in detail.

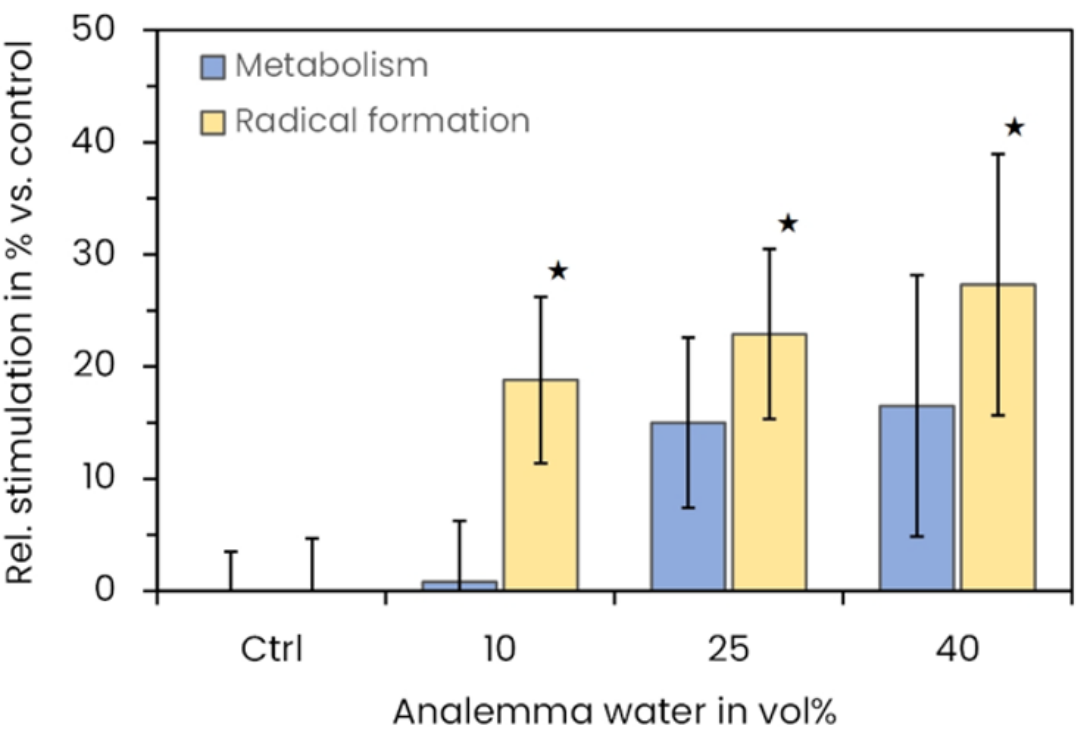

Figure 4: Graphical presentation of the relative basal metabolism of functional neutrophils (blue columns) and the superoxide anion formation after stimulation with a phorbol ester (yellow columns). Ctrl = Control cells exposed to the same amount of initial tap water without treatment. All Analemma water concentrations show a significant stimulation in radical formation compared to control cells (*$p \leq 0.01$; two-tailed Wilcoxon-Mann-Whitney test). Data represent mean values ± standard deviations of four independent experiments (n = 4) with biological replicates.

Discussion

In this study we investigated the cellular effects of freshly prepared Analemma water on selected properties of cultured cells with the focus on the gastrointestinal tract which comes into the first direct contact with the Analemma water after drinking. Therefore, the IPEC-J2 cell line was chosen for the experiments, because it is "unique and derived from the small intestine of a pig and is neither transformed nor tumorigenic in nature. IPEC-J2 cells mimic the human physiology more closely than any other cell line of non-human origin" [24]. The advantage of the IPEC-J2 cell line as an in vitro model originates from its morphological and functional similarities with intestinal epithelial cells in vivo. The epithelial cells of the intestinal barrier have a high turnover rate, because they are quite sensitive against alterations of their endogenous environmental conditions involving a deficiency of the epithelium and immune/inflammation mediating cells [25]. As shown in this study, the cell metabolism of the IPEC-J2 cells was stimulated by the addition of Analemma water with a peak at a volume concentration of 30 vol% present in the culture medium. Cell metabolism is the sum of all chemical reactions that enable cells to stay alive, grow, divide, and respond to their environment, thereby allowing them to maintain their structure, repair damage, and perform specialized functions. In particular, the intestinal epithelium is a multicellular interface whose inner layer consists of intestinal epithelial cells that absorb nutrients and form a physical and immune-mediated barrier against harmful components of the luminal environment.

Furthermore, the intestinal epithelium is the most regenerative tissue in the human body and serves as a protective mechanism against injury and infection, as well as for maintaining homeostasis [26,27]. Cell metabolism is directly linked to cell regeneration, a fundamental biological process that enables organisms to replace damaged or dead cells [28]. Promoting the regeneration of intestinal epithelial cells, as demonstrated here for Analemma water, can lead to a faster restoration of the integrity and functionality of the affected mucosal area. In most regenerative processes, closing a defect in a specific structure requires the formation of new cells through cell proliferation and migration [29]. As shown here, the increase in metabolic and regenerative activity acts as a cell protective mechanism against external or internal influences damaging the mucosal tissue. Especially oxidative stressors can increase the production of reactive oxygen species in the body, overwhelming the antioxidant defense system and leading to oxidative damage of cell components such as DNA, proteins, and lipids. A common in vitro model to simulate exogenous oxidative stress is the use of hydrogen peroxide as a donor for reactive oxygen species acting on cultured cells [30,31]. This in vitro model was also used to examine whether Analemma water might be able to reduce oxidative stress acting on intestinal epithelial cells. The measurement data demonstrate that the Analemma water has the potential to counteract oxidative stress and helping to promote and maintain the integrity and functionality of the intestinal barrier. Neutrophils are one of the body's most important first-line defenders in the innate immune system. They are a type of white blood cell that responds rapidly to infection or tissue damage, especially against bacteria and fungi. Unlike adaptive immune cells, neutrophils act quickly by the formation of reactive oxygen species to damage or kill invading organisms [32-34]. We observed that the coherent water increased the radical formation of functional neutrophils which might also be able to strengthen the innate immune defense in vivo.

Conclusion

The in vitro results presented here confirm and expand previous findings on Analemma water and demonstrate that it is able to support cellular processes such as metabolic activity, regeneration following injury, resistance to oxidative stress, and the formation of reactive oxygen species released by neutrophils in the blood. Since the intestine plays an essential part for the systemic health situation, the cellular effects observed in this study by Analemma water could also promote the health status of the body.

Conflict of Interest

The present study was funded by Water and Light B.V., NL – 3732 HW De Bilt, The Netherlands. The company made the Analemma water wand available for the experiments.

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