Effect of Deuterium Depleted Water on Life

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This past year I was an REU working with Dr. Steve Koch and Anthony Salvagno. We wanted to better understand how water with varying amounts of Deuterium affects life forms. Because deuterium occurs naturally in small quantities, but is toxic in large quantities, we had a few questions: It is known that Deuterium in large quantities is toxic to living organisms, but at what point does it become toxic? During the research of this question, we began to wonder, “What is the mechanism of Deuterium/Hydrogen exchange?” And finally, while looking into these questions, a third question arose: Since Deuterium Oxide (D2O) occurs naturally, does it serve some, as yet, undiscovered purpose for life? And finally, what interested me most in Dr. Koch’s lab was that he and Anthony conducted their research using Open Notebook Science. This paper will discuss what I’ve learned over the past several months about these questions and what I’ve learned about Open Notebook Science.

Introduction to D2O

Because I am studying computer engineering, not bio-physics, there was a slight learning curve that I had to overcome at the beginning of my REU. I had taken a biology course the previous year, but had very little knowledge about deuterium or deuterium depleted water. In fact, I had never heard of heavy water before I started working with Dr. Koch.

First, I had to clearly define Deuterium and D2O. I already knew that Hydrogen is the most abundant element in the universe and that it is composed of one proton and one electron with an atomic mass of one. Deuterium is an isotope of hydrogen, with one proton, one neutron, and one electron and an atomic mass of two, twice the mass of the common hydrogen atom. It is so unique it has its own element symbol “D.” Deuterium Oxide, or heavy water, is composed of two deuterium atoms and one oxygen atom, with the chemical symbol D2O. Naturally occurring water has about a 17mM (millimolar) concentration of deuterium. Since water is one of the most abundant resources on our planet, and D2O occurs naturally, we wondered what is D2O’s purpose?

Repeating Crumley

As it turns out, we weren’t the first people to ponder these questions. In 1950 Helen A. Crumley et al performed an experiment testing plant seed growth in varying amounts of D2O.1 They used H2O and 33%, 66%, and 99% D2O mixed with H2O. They discovered that growth rates were drastically slower in increasing amounts of D2O. In order to answer our first question, at what point

Figure 1 Crumley results of influence of ordinary water, 33%, 66%, and 99% deuterium oxide on tobacco seed germination. Counts made at daily intervals for 39 days.

does D2O become toxic, we decided to see if we could repeat the Crumley experiments and get the same results (Figure 1).

We ran seven trials with slight modifications to the Crumley protocols. The Crumley experiments used a variety of seeds: Tobacco, Clover, Radish, and Kentucky bluegrass. Our first experiments used two varieties of tobacco seeds: Virginia gold and Havana, since we already had them available in the lab. Later we expanded our trials to include Arabidopsis – mustard – seeds. While the Crumley trials placed their seeds in the open, on wet cloths, we placed our seeds in sealed analyslides. Finally, instead of using 100 seeds per trial, we used 30-40 seeds per trial.

In Crumley’s paper, they based their findings and data on what they called “percentage of germination.” However, it was unclear how they measured this percentage. We counted the seeds that germinated and calculated the percentage based on the starting number of seeds, i.e., seeds germinated ÷ total number of seeds used. We also clarified what constituted a germinated seed. Figure 2 shows several seeds that have clearly grown a root. We counted a seed as germinated if even there was only a slightly visible root as shown in the pink box. The orange box shows a non-germinating seed; darker with no visible root.

Each of the seven experiments took two weeks. Most of the experiments used five water samples (one Deuterium Depleted Water (DDW) and four levels of D2O) and a control of deionized (DI) water without seeds (watching for possible mold growth) as outlined in Table 1.

<table>
<thead>
<tr>
<th>Experiment Name</th>
<th>Water Samples Used</th>
<th>Seeds</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>RC1</td>
<td>DI control w/out seeds; DDW; 33%, 66%, and 99% D2O</td>
<td>Virginia Gold Havana</td>
<td></td>
</tr>
<tr>
<td>RC2</td>
<td>DI control w/out seeds; DI; DDW; 33% and 66% D2O in DI; 33% and 66% D2O in DDW; 99% D2O (pure D2O)</td>
<td>Virginia Gold Havana</td>
<td></td>
</tr>
<tr>
<td>RC3</td>
<td>same as RC2</td>
<td>same as RC2</td>
<td></td>
</tr>
<tr>
<td>RC4</td>
<td>same as RC2</td>
<td>Virginia Gold Havana</td>
<td></td>
</tr>
<tr>
<td>RC5</td>
<td>same as RC2</td>
<td>same as RC2</td>
<td></td>
</tr>
<tr>
<td>RCD</td>
<td>six analyslides of 99% D2O</td>
<td>Virginia Gold Havana</td>
<td>validating that in 99% D2O seeds never grew; this trail lasted 35 days</td>
</tr>
<tr>
<td>RCW</td>
<td>CHTM, RoDI purified, Sigma Molecular Biology pure, and Tissue Culture pure</td>
<td>Virginia Gold Havana</td>
<td>trial of different DI waters to see if they grew differently... inconclusive</td>
</tr>
</tbody>
</table>

Table 1 Repeating Crumley Experiments
Our overall results matched the Crumley experiment with one glaring difference… nothing grew in pure D2O (Figure 3). This was an interesting finding. We suspected that there must have been some level of deuterium exchange during the Crumley experiment, since their seeds were grown in the open air and our seeds were sealed in analyslides. These results led us to question the rate of the naturally occurring Deuterium/Hydrogen exchange. We wanted to see if there was a difference between the amounts of D2O in H2O (or vice versa). We were also curious to see if DDW absorbs D2O naturally over time. Finally, just for fun we wanted to test the different DI water to see if there were any differences between them.

![Figure 3 Koch Lab RC results](image)

### Fourier Transform Infrared spectroscopy (FTIR)

Visually (to the naked human eye), all water looks the same. There is no way for the human eye to tell the difference between H2O and D2O. However, spectroscopically, these two different water types are very different. Dr. Sanjay Krishna and Stephen Myers at CHTM graciously granted me access to, and use of, their laboratory’s FTIR. Stephen trained me on its use and was also especially helpful for the spectroscopic interpretation.

What we wanted to understand was the mechanism of hydrogen / deuterium exchange. Why did Crumley have growth in 99% and we didn’t have any growth? D2O and H2O behave differently physically with oxygen bond. We don’t fully understand this yet, but the spectroscopy reveals the difference in these interactions.

We first took a look at the ‘shelf-life’ of our DDW, or at least look into the mechanism of Deuterium/Hydrogen exchange. We suspected that, over time, the DDW would eventually absorb deuterium from the atmosphere and be pretty much the same as regular water. In order to test this theory, we ran FTIR spectroscopy on DDW opened on different dates. Although we are
still unsure what all this data means, it seems clear that there is some deuterium exchange happening over time (Figure 4).

![Figure 4 FTIR on different water types](image)

**Deuterium Depleted Water (DDW)**

The last question we were studying was, “Since Deuterium Oxide (D2O) occurs naturally, does it serve some, as yet, undiscovered purpose for life?” In order to attempt to answer this question we ran experiments with DDW and tobacco seeds, e. coli., and yeast.

Recall, from my introduction to Deuterium, naturally occurring water has about a 17mM (millimolar) concentration of D2O. If it occurs naturally, wouldn’t that mean that nature has some use for it? We decided to look at this question by testing tobacco seed growth in pure DDW. At the end of my REU we had just started to move into testing e.coli growth in DDW and pure D2O. We chose e.coli because it is easy to grow and it is very hard to kill.

We ran five trials of tobacco seeds in DDW, adding arabidopsis seeds to the last few trials. The fourth trial was setup using three seed types: Virginia Gold, Havana 2000, and Columbia Arabidopsis. Five to six seeds of each type where put into twelve cuvettes. Ten of the cuvettes were filled with 3ml of DDW, one cuvette was filled with 3ml of tap water, and the final cuvette was filled with DI water. After about 24 hours the seeds would sink to the bottom of the cuvettes.
After about three days the seeds would begin to sprout and after approximately another five days roots where noticeably visible. The most interesting thing about the sprouting roots is that the seed’s roots in DDW would have noticeable, fine, root hairs (Figure 5). However, the seeds grown in tap water or DI water did not show the same amount of root hair growth (Figure 6).

![Figure 5 Dark Virginia seeds in tap water](image1) ![Figure 6 Virginia Gold seeds grown in DDW](image2)

Although we were excited about this development, this was not enough to conclude that life (at least tobacco seeds) had developed some need for D2O. We ran a fifth trial to see if we could get similar root hair growth using four different types of water. We used two kinds of pure water from Sigma: molecular biology grade water and double purified water for tissue cell culture, 1% D2O mixed with DDW, and pure DDW. For the seeds we used just the two types of tobacco. After 25 days of growth we had root hairs on our seeds. This was disappointing, but at least we realized that our results were inconclusive.

**Open Notebook Science**

As a computer engineering undergraduate, the Open Notebook Science portion of my experience was by far the most interesting to me. The skills and tools I’ve learned about will apply to any future endeavors that I will have. Open Notebook Science is the practice of recording everything related to the research project publically, in an easily available, electronic (online) media. Research is documented in real time – as it is recorded. According to Wikipedia, “Open Notebook Science is the practice of making the entire primary record of a research project publicly available online as it is recorded.” [http://en.wikipedia.org/wiki/Open_notebook_science]

This includes every step of the research path, from inception and planning, to data collection, experiment protocols, and the final conclusions. The tools used for Open Notebook
Science are all available free online. They are collaborative, which makes them great for open science.

The tools I used most often are in bold. I used Wordpress to created my online notebook and record my activities and data. I used Figshare to share data sets (FTIR readings). Social media tools like Facebook, Google +, and Twitter were great to share my daily research activities with everyone I know. Every time I posted a notebook entry, I would also post it on Facebook. Several of my non-scientist friends would comment or like my posts. I also had a few posts in my notebook from recognized experts in the bio-physics arena. My work was read, acknowledged, and collaborated on with people that I would not have access to with a traditional paper and pen notebook.