Experimental

ANOMALOUS ENVIRONMENTAL INFLUENCES ON IN VITRO ENZYME STUDIES *Part* **1:** *Some Faraday Cage & Multiple Vessel Effects*

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ABSTRACT

In these papers, we experimentally explore the connection between a detector, which is a small vessel containing an in vitro enzyme-alkaline phosphatase (ALP), and its local environment via monitoring the ALP thermodynamic activity as the local environment is changed. Based on purely space-time (D-space) physics, there should be no change in ALP activity as the environment of the incubator/refrigerator is systematically altered. However, based on reciprocal spacetime (R-space/D-space) interactive physics, changes can be expected under appropriate conditions. Here we report on (a) the effects associated with having no Faraday cage vs. an empty Faraday cage made from 1, 2, 3, 4, 5 or 6 layers of copper screen located on the shelf beside the detector and (b) the same as (a) but with an ALP-solution vessel at various D-space locations in the Faraday Cage. Experimentally, we find statistically significant changes in ALP activity as the environment of the control vessel is systematically changed. The territory currently being explored to explain these results involves selecting an appropriate higher dimensional basespace for viewing nature.

KEYWORDS: Enzyme activity, ALP, Faraday Cage, R-Space/D-Space Interactive Physics

INTRODUCTION

In a recent paper, intention-induced increases and decreases in pH via the use of an intention imprinted electronic device (IIED) were reported upon. This study, with its very significant results, supplemented a similar successful in vivo study using IIEDs to increase the [ATP]/[ADP] ratio in fruit fly larvae with consequent reduction in larval development time.^{1,2} In the fruit fly study, the experimental control sat adjacent to Faraday cages wherein specific treatment experiments were simultaneously occurring. Since the results of these experiments are so anomalous, there appears to be no ready explanation based upon the conventional scientific paradigm and one must begin to look elsewhere for a meaningful explanation. The territory currently being explored involves selecting an appropriate higher dimensional base-space for viewing nature.³⁻⁶

In this higher dimensional modeling a special 8-space comprised of dual 4 spaces plays a central role.³ One of these 4-spaces is our familiar distance-time or *(x,y,z,t)-space* which is labeled Direct-space (D-space). The other is its Reciprocal-space (R-space) or (x^1, y^1, z^1, t^1) -space. Since the general model is a lO-dimensional diffraction model, the conjugate quality map in R-space to a particular quality in D-space is given by the Fourier Transform (FT) of the map of that quality in D -space.³ The intensity or modulus of this map involves the incorporation and activation of a 9-space substance called Deltrons which act as a coupling medium for interaction between the electric monopole substance of D-space and the magnetic monopole substance of R -space.⁷ The recent experimental studies are thought to have their explanation via augmented electromagnetism which involves strong coupling to R-space substance and fields. $1,2$

In these papers, we experimentally explore the connection between a small vessel containing an in vitro enzyme-alkaline phosphatase (ALP) and its local environment via monitoring the ALP thermodynamic activity as the local environment is changed. Based on purely D-space physics, there should be no change in ALP activity as the environment of the incubator/refrigerator is systematically altered. However, based on R-space/D-space interactive physics, changes can be expected under appropriate conditions. Experimentally, we find statistically significant changes in ALP activity as the environment of the control vessel is systematically changed.

The thermodynamic activity of the ALP-solution in the control vessel is utilized as a detector of environmental influences. Here, in Part 1, we report on (a) the effects associated with having no Faraday cage vs. an empty Faraday cage made from 1, 2, 3, 4, 5 or 6 layers of copper screen located on the shelf beside the control vessel and (b) the same as (a) but with one to several ALP-solution vessels at various D-space locations in the Faraday Cage. In Part 2, we examine the influence of IIEDs on our detector. This interesting experimental data allows one to learn something about R-space and about the way nature utilizes the R-space/D-space connection to generate physical phenomena.

EXPERIMENTAL METHODS

ALKALINE PHOSPHATASE (ALP)

LP is present in nearly all tissues of the body, especially at or in the
cell membranes and at particularly high levels in intestinal epithelium,
kidney tubules, bone, liver and placenta.⁸ Although its precise cell membranes and at particularly high levels in intestinal epithelium, Kidney tubules, bone, liver and placenta.⁸ Although its precise metabolic function is not yet understood, this enzyme is closely associated with the calcification process in bone. The two or three variants of ALP present in the sera of normal adults originate in the liver or the biliary tract while up to half of the total activity comes from the skeleton. Serum ALP measurements are of particular interest in the investigation of hepatobiliary disease and bone disease associated with increased osteoblastic activity.

The response of the liver to *any* form of biliary obstruction is to synthesize more ALP (increase its thermodynamic activity). These increases in ALP activity can be greater than three-fold in the case of an obstructing stone or cancer. However, liver diseases such as infectious hepatitis show only normal levels or moderate elevations. Among the bone diseases, the highest levels of serum ALP are found as a result of the action of osteoblastic cells as they try to rebuild bone that is being reabsorbed by uncontrolled activity of osteoclasts (values of 10-25 times normal are not unusual). Physiological bone growth elevates ALP in serum to about 1.2 to 2.5 times normal adult values and transient elevations are often found during the healing of bone fractures. Thus, overall, the human body has a variety of natural pathways for increasing the thermodynamic activity of ALP.

ALP ACTIVITY

The most widely used substrate for assaying the thermodynamic activity
of ALP is 4-nitrophenyl phosphate (4-NPP). This ester is colorless,
but 4-nitrophenol (4-NP), the product of enzyme action, is yellow at
the pH of the of ALP is 4-nitrophenyl phosphate (4-NPP). This ester is colorless, but 4-nitrophenol (4-NP), the product of enzyme action, is yellow at the pH of the reaction. Thus, the enzyme reactivity can be followed continuously by observing the rate of increase of yellow color spectrophotometrically.

In our experiments, ALP activity was determined via reflective spectroscopy using the Johnson and Johnson Clinical Diagnostics technique. This is a system that is widely used in physician's offices, clinics, hospitals and reference labs. A detailed description of this system is given in Appendix A of Part 2 scheduled to appear in *Subtle Energies and Energy Medicine,* Volume 11 No 2.

The Johnson and Johnson Clinical Diagnostics technique utilizes the Vitros DT60 chemistry system-the DT60II Analyzer and the DTSC II Module (Appendix A).^{8,9} Vitros DT Control enzyme solutions, which are available as vials of frozen lyophilate and serum, were utilized as the experimental ALP solutions. These solutions are designed for use in monitoring the precision of this system. ALP was derived from porcine kidney and standard procedures were followed (Appendix A).^{8,9} Reporting units were U/L (where U is the international unit or quantity of enzyme that will catalyze the reaction of one umole of substrate per minute and L is litre).

FARADAY CAGES

A standard Faraday cage consists of a copper mesh screen enclosing a certain spatial volume. It is electrically grounded so the electromagnetic (EM) waves of wavelength larger than the mesh size, that impinge on the screen, mostly leak off to ground and only minimally penetrate to the interior space. Thus, the interior space has a greatly reduced EM integrated power density in the wavelength range larger than the copper mesh spacing except at very low frequency where the EM skin depth in copper is large. In the text and Figures we represent Faraday cage as F, $n = 0, 1, 2, 3, 4, 5$ and 6 where $n = 0$ refers to no Faraday cage and $n = 1$ -6 refers to the number of layers of copper mesh used in the cage construction. The Faraday cages used here were 61 cm x 11 cm x 20 cm in size and a one layer cage $(F, n = 1)$ can be expected

to reduce the EM field strength for these frequencies by a factor of approximately 10. In all cases the Faraday cages were electrically grounded.

ExPERIMENTAL LAYOUT AND DATA PRESENTATION

core experimental system was situated in an incubator at 4°C. The
system comprised 100ml quartz tubes placed in beakers containing
100ml pure water (see position 0 Figure 1). For the different experi-
ments (see below), th system comprised 100ml quartz tubes placed in beakers containing 100ml pure water (see position 0 Figure 1). For the different experiments (see below), the ALP activity was measured in an ALP "detector," designated (C) at position 0 fixed in the incubator. The ALP "detector" comprised stock ALP solution diluted as follows: 100ul ALP plus 150ul of pure water (Appendix B).

For the experiments conducted without the Faraday cage (F, $n = 0$), the IIEDs and ALP- solution vessels were located at least 13.5 cm away from the detector on the incubator shel£ When a Faraday cage was used, it was also located with its nearest surface 13.5 cm away from the detector on the same incubator shelf. The IIEDs and ALP-solution vessels were always placed at the same positions on the shelf in the absence of the Faraday cage and, when the Faraday cage was used, inside the cage at these same spatial positions. The distances between

Figure 1. *Experiment configuration with ALP detector (C) at position 0 and potential ALP-vessel sites at positions* (1), (2) *or (3).*

positions 1, 2 and 3 were 20 cm for the ALP-vessel solutions (see Experiment b). Positions were designated $(p0)$, $(p1)$, $(p2)$ and $(p3)$.

We adopted a randomized design and an exposure period of 30 minutes. We collected data at the same 5 times during one day and repeated this procedure over sequential days. The times during the day were as follows: (1) 9:3 minutes. We collected data at the same 5 times during one day and repeated this procedure over sequential days. The times during the day were as follows: (1) 9:30 a.m.; (2) 10 a.m.; (3) 12:30 p.m.; (4) 1 p.m., and (5) 1:30 p.m. A new ALP stock solution was used each day. Thus, at each time, we exposed 8 replicate tubes of (C) to the particular experimental treatment and measured the ALP activity in each replicate at the end of the exposure period. This was repeated on sequential days until sufficient replicates (a minimum of 16 replicates and a minimum of 2 days) were obtained for the particular experimental treatment.

We have presented our experimental results in the following fashion. We show our data as means and standard errors of the mean in Figures and in Appendix D as boxplots (see below). We log-transform the data and assess them with the ANOVA statistical procedure. We examine all pair-wise treatment comparisons with Tukey post hoc tests based on the ANOVA and state the conclusions from these analyses in the text. The ANOVAs and Tukey tests are given in full in Appendix C. Statistical analysis followed reference 10 and 11 and we used SYSTAT Version 5.2.1 for the Macintosh.^{10,11}

For the ANOVAs we adopt Matthew's proposal concerning level of significance for the assessment of anomalous phenomena. Hence "significant" in the text refers to ANOVAs with probability values less than 0.003 ($p < 0.003$).¹² In appendix C we give all Tukey post hoc tests for treatments and treatment combinations that were both significant and not significant in the ANOVAs.¹⁰ We show Tukey tests that were significant at $p < 0.05$ to enable the reader to consider comparisons at a conventional level of significance.¹¹ Thus, we provide the reader with all our data, organized in (i) Figures as both means with standard errors and boxplots (see below) and (ii) ANOVAs with Tukey post hoc tests to enable the reader to make his or her own interpretations and conclusions.

Finally, in Appendix D we provide notched box plot representations of all our data. A notched box plot provides a simple graphical summary of a batch of data and implements confidence intervals on the shown median values. The boxes are notched at the median and return to full width at the lower and

upper confidence interval values. If the intervals around two medians do not overlap, then one can be confident at about the 95% level that the two population medians are different.¹⁰ Outside values are represented by an asterisk and far outside values, by an open circle.

SETTING THE SCENE

Initially, we conducted three experiments to assess the behavior of our detector in the incubator. These experiments were designed to provide the "base-line" for our subsequent major experiments.

ALP activity variation with time of day

ur experimental design necessitated measurement of ALP activity in (C) at 5 times during one day. This experiment with no Faraday cage, no devices and no additional ALP vessels was to test the effect of (i) time of day and (ii) the use of a new ALP solution on each day. ALP activity in ALP solutions in tubes at position 0 of Figure 1 in the empty incubator was measured throughout one day, at the times given above, which were the same as those used in the subsequent experiments. For each time, 8 replicates of the ALP solution were exposed to the environment for 30 minutes and then their activity determined. These measurements were repeated on a second day using a new ALP stock solution.

ALP Activity in the detector (C) at positions 0, **1, 2** *and 3*

This experiment with no Faraday cage, no devices and no additional ALP vessels was to test the effect of moving the ALP detector to various locations in the incubator. Thus, we monitored only (C) at positions $(p0)$, $(p1)$, $(p2)$ and $(p3)$ in the incubator in the absence of both the additional ALP-solution vessels and the Faraday cage.

Exposure Period and Faraday Cage

This experiment with no devices and no additional ALP vessels was to test the effect of different exposure periods, $t = 15$, 30 and 45 minutes, for (C) located at position 0 in Figure 1. We also introduced the one layer Faraday cage (F, $n = 1$) in this experiment. Thus, in total we assessed ALP activity in the detector (C) for Δ *t* and for (F, *n* = 0 and 1), where (F, *n* = 0) refers to no Faraday cage.

EXPERIMENTS

Experiment (a): Faraday Cage Layer (F)

We first investigated changes in the ALP "detector", designated (C), located at position 0 in Figure 1 and an empty Faraday cage F, $n = 0, 1, 2, 3, 4, 5$ or 6 layers of copper mesh.

Experiment (b): ALP vessels, position and Faraday Cage

I n this experiment we placed an ALP-solution vessel, identical to (C), at position 1 and measured ALP activity in (C) for (F, $n = 0$ and 1). These measurements were repeated with a single ALP-solution vessel at positions position 1 and measured ALP activity in (C) for $(F, n = 0 \text{ and } 1)$. These (2) and then (3), next, 2 and 3 identical ALP-solution vessels were placed at each location.

RESULTS

SETTING THE SCENE

ALP activity variation with time of day

Figure 2 and the ANOVA indicated that both day and time of day did not influence (C). We did detect a significant time of day x day interaction in the ANOVA, indicating that there was some variation in our experimental system, possibly due to the use of new ALP solutions. However, the variation of ALP levels from day to day was low and well within the expected range. For all subsequent experiments we initially assessed the influence of day on our results.

ALP Activity in the detector (C) at positions 0, 1, 2 *and 3*

The ANOVA indicated that day did not significantly influence our results. Further, Figure 3 indicated that the ALP activity in (C) appeared to vary

Figure 2. Setting the Scene: ALP activity variation in the detector (C) with time of day *(Figure 2a) and day (Figure 2b).*

Figure 3. Setting the Scene: ALP activity in the detector (C) at positions 0, 1, 2 and 3.

with respect to position. However, this effect was not significant in the ANOVA.

Exposure Period and Faraday Cage

The ANOVA again indicated that day did not significantly influence our results. Further, exposure period did not alter the ALP activity for no **L** Faraday cage, $(F, n = 0)$ (see Figure 4). There was an indication that it did somewhat for $(F, n = 1)$, with higher values observed for the longer exposure period. However, the ANOVA and Tukey test indicated that these differences were not significant. Finally, the ANOVA indicated that the presence of the Faraday cage significantly increased the ALP activity of (C). The mean values were 142.98 IU for $(F, n = 0)$ and 152.71 IU for $(F, n = 1)$.

EXPERIMENTS

Experiment (aJ: Faraday Cage Layer

Figure 5 and the ANOVA indicated that the presence of the Faraday cage significantly modified the ALP activity of the detector (C). The largest number of significant pair-wise comparisons were observed for the 5 layer Faraday cage. From Figure 5, one sees that a single layer cage significantly increased the activity of the ALP detector while a 5-layer cage reduced it somewhat. When comparing the 5-layer cage with cages of different *n,* the most significant comparison was between $(F, n = 1)$ and $(F, n = 5)$ at $(p < 0.001)$. Finally, the ANOVA indicated that day did not significantly influence the results.

*Experiment (b*J: *ALP vessels, position and Faraday Cage*

Figure 6 and the ANOVA indicated that Faraday cage, position and vessel modified ALP activity in the detector (C). Further, the ANOVA indicated that day did not significantly influence our results. Position and vessel effects were relatively similar for $(F, n = 0)$ whereas differences were much more apparent for $(F, n = 1)$. This observation was supported by the fact that twice as many Tukey pair-wise comparison were significant for $(F, n = 1)$ in comparison to $(F, n = 0)$. Of interest here were the consistently low values for the 3 vessel circumstances.

Figure 4. *Setting the Scene: Exposure period and Faraday cage.*

Figure 5. *Experiment (aJ: Faraday cage layer.*

Figure 6. Experiment (b): (F, n = 0) refers to vessels in the incubator without a Faraday cage and $(F, n = 1)$ *refers to vessels in the Faraday cage.*

The ANOVA indicated that the position effect was uniform for all vessel numbers and the overall mean values were as follows: position $1-148.638$ IU, position $2-144.581$ IU and position $3-140.384$ IU. The Tukey post hoc test indicated that ALP activity in (C) was highest for position 1, which was significantly greater than both positions 2 and 3. The latter two positions were not significantly different.

Figure 7 shows our data from an additional perspective where the data were pooled for position. Considering Figure 7a and the case of (F, *n* = 0), results indicated that ALP activity in (C) was not significantly modified by vessel number and this was confirmed by ANOVA. Considering the case of $(F, n = 1)$, Figure 7a and the ANOYA indicated a significant effect on ALP activity in (C) for the number of vessels involved. The ALP activity detected in (C) for the 3 vessel experiment was significantly less than values observed for both the 1 and 2 vessel experiments.

Figure 7a and 7b. Experiment (b): Pooling the data for position. We show ALP activity measured in the detector (C) as a fonction of (a) the presence/absence of Faraday cage (F, *n* = *0,1) and (b) vessel number* (1, 2 *and* 3) *where the data were pooled for position.*

Considering the viewpoint of vessel number (Figure 7b), results indicated that for the 1 and 2 vessel experiments, ALP activity observed in (C) appeared to be increased for $(F, n = 1)$ in comparison to $(F, n = 0)$. This result was reversed for the experiment involving 3 vessels and these observations were confirmed as significant by ANOVA.

In summary, ALP activity values detected in (C) were significantly higher for position 1 than positions 2 and 3. Further, the three vessel experiment produced significantly lower ALP activity values in (C) than both the 1 and 2 vessel experiments. The use of the Faraday cage significantly increased ALP activity in (C) for both the 1 and 2 vessel experiments but significantly decreased the value for the 3 vessel experiment.

DISCUSSION

s a summary comment on this work, our "setting the scene" experi-
ments indicated no significant effects on our detector, in contrast to
the highly significant effects observed in our two experiments. Thus, ments indicated no significant effects on our detector, in contrast to we have noted some remarkable and statistically significant effects which appear to have no ready explanation based upon the accepted, present day, scientific paradigm.

Our results clearly indicated that the ALP solution can serve as a significant detector of novel energies, thought to be R-space, vacuum energy effects in its local environment. It clearly detected the presence of a Faraday cage in the incubator at an appreciable distance from itself (relative to normal atomic force field distances), presumably because the electromagnetic nature of the copper in the Faraday cage constructively/ destructively interferes with that of the incubator itself at the R-space level.³ Changing the number of layers of copper mesh in the Faraday cage significantly altered the ALP detector activity, presumably also by local interference interactions between the copper layers at the Rspace level. An exposure period effect is also indicated with ALP activity increasing with exposure period.

Placing one to several ALP-vessels at different positions in the Faraday cage provided statistically significant additional substantiation for the (F, *n* = 0 or 1) layer Faraday cage comparison effect with significant R-space interference effects coming from grouping 3 ALP vessels at the various positions.

Finally, the very robust ALP detector results shown here only scratch the surface of all the relevant factors involved in the R-space/D-space interactive physics. Much more work needs to be done in this area before serious conclusions can be drawn.

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ApPENDIX A VITROS **DT** SYSTEM

Vitros DT Controls, designed for use in monitoring the precision and accuracy of the Vitros DT Chemistry System, were used as the experimental material. The Vitros DT Controls are available as vials of frozen lyophilate and serum. Enzymes are present in the lyophilate and ALP was derived from porcine kidney. The lyophilate and serum vials were stored at 2-8°C for no longer than 6 months. Reconstitution was achieved as follows: the lyophilate and dilute vials were thawed at room temperature for 1 hour. A specific volume of diluent (see below) was then added to a lyophilate vial. This vial was then inverted and gently mixed for 30 minutes at room temperature. Expected values were then determined and verified prior to use of the solution in the experiments (see main text). A fresh solution was prepared and used for each experiment. However, if necessary, reconstituted Vitros DT Control solutions can be used for up to 7 days if stored at 2-8°C. Reporting units were U/L (where U is the international unit or quantity of enzyme that will catalyze the reaction of one umole of substrate per minute and L is litre).

The Vitros ALP DT slide is a dry, multilayered film in a plastic support. It contains all the reagents necessary to determine activity in 10ul serum or plasma. The reaction is based on alkaline phosphatase catalyzing the hydrolysis of p-nitrophenyl phosphate to p-nitrophenoL A 10ul drop of solution is deposited on the slide and evenly distributed by the spreading layer, which contains the p-nitrophenyl phosphate substrate and other components required for the reaction. The ALP in the solution catalyzes the hydrolysis of p-nitrophenyl phosphate to p-nitrophenol at alkaline pH as follows:

$$
\begin{array}{ccc}\n&\text{ALP} &\\ \text{p-nitrophenol phosphate} & \longrightarrow & \text{p-nitrophenol + H}_3\text{PO}_4\\ \text{Mg}^{2+} & &\end{array}
$$

The p-nitrophenol is then diffused into the underlying layer where it is monitored by reflectance spectrophotometry. The change in the reflection density is monitored at 37°C and the rate of change is used to calculate enzyme activity. Slide reagents were as follows: p-nitrophenyl phosphate, 2-amino-2-methyl-l-propanol and magnesium sulphate, polymer beads, binders, buffer, surfactants, polymer cross-linking agent and preservative. The wavelength used was 400 nanometers and the assay time and temperature was a maximum 5 minutes at 37°C. Standard curves are available from Johnson and Johnson.

ApPENDIX B CONSEQUENCES OF DILUTING THE **ALP** SOLUTION

Enzymatic activity determinations via the Vitros DT 60 system naturally involves concentrated solutions of the enzyme so that definitive and accurate results can be obtained in a short time. In such a case, one might presume that the chemical activity of the target enzyme approaches unity. On the other hand, one wishes here to demonstrate the effect of intention-augmented electromagnetic fields from the lIED used in the experiment which needs as much "thermodynamic room" as possible to allow the intention-induced thermodynamic pathways to significantly increase the chemical activity coefficient of the target enzyme. We proceeded to do this by diluting the ALP in purified water. This approach has a down-side which is an impairment of the Vitros DT 60 detection mechanism and the anticipated consequence is illustrated in Figure B1.

Figure RI. Experiments with ALP and the Vitros DT60 system: The anticipated consequences of diluting the target enzyme.

ApPENDIX C ANOVAs AND TUKEY POST HOC TESTS

1. Setting the Scene: ALP activity in the detector (C) with time of day and day.

2. Setting the Scene: ALP activity in the detector (C) at positions 0, 1, 2 and 3.

3. Setting the Scene: Exposure Period and Faraday Cage.

 $ATOII$

Tukey post hoc tests significant at $p < 0.05$

(i) Faraday cage layer:

(ii) Faraday cage layer and exposure period:

** indicates $p < 0.001$

4. Experiment (a): Faraday Cage Layer (F).

Tukey post hoc tests significant at $p < 0.05$

5. Experiment (b): ALP vessels, position and Faraday Cage.

Tukey post hoc tests significant at $p < 0.05$

(i) Position:

(ii) Position and vessel number.

Faraday cage, $n = 0$

Faraday cage, *n* = 1

ApPENDIX D NOTCHED BOXPLOT REPRESENTATION OF ALL OUR DATA.

1D. Setting the Scene: ALP activity in the detector (C) with time of day (a) and day (b).

2D. Setting the Scene: ALP activity in the detector (C) at positions 0, 1, 2 and 3.

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3 D. Setting the Scene: Exposure Period and Faraday cage.

4D. Experiment (a): Faraday Cage Layer.

5D. Experiment (b): ALP vessels, position and Faraday cage. (a) F, *n* = 0 (vessels in the incubator without a Faraday cage), and (b) F, $n = 1$ (vessels in the Faraday cage).

6D. Experiment (b): ALP vessels, position and Faraday cage. Pooling the data for position.

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