Experimental

ON ENHANCING NICOTINAMIDE ADENINE DINUCLEOTIDE (NAD) ACTIVITY IN LIVING SYSTEMS

Michael J. Kohane & William A. Tiller, Ph.D.

ABSTRACT

Nicotinamide Adenine Dinucleotide (NAD) is a key molecule in intermediary metabolism and for the flow of energy in biological systems. It has been shown that a significant association exists in *Drosophila melanogaster*, the fruit fly, between larval development time, NAD and Adenosine Triphosphate (ATP)/Adenosine Diphosphate (ADP) ratio. Furthermore, variation in NAD concentration has been associated with both AIDS and Alzheimer's disease in humans. Recent studies have favorably implicated NAD as a medication for patients suffering from both dementia of the Alzheimer type and HIV-1 infection. In this paper, we have examined the possibility that both EMFs and intention-augmented EMFs significantly affect (1) the activity of NAD in solution over time and (2) the condition of the water used for preparing NAD solutions to be subsequently utilized for in vitro energy assays in *D. melanogaster*. Here, we show that NAD activity and the (ATP)/(ADP) ratio significantly depended on the treatments given to the water. Our results indicated (1) that a reduction in exposure to EMFs increased NAD activity and (2) that electronic devices that had been exposed to a human intention concerned with energy metabolism, positively influenced both the activity of NAD and the ATP/ADP ratio in vitro.

KEYWORDS: Human intention, Nicotinamide Adenine Dinucleotide (NAD), Adenosine Trisphosphate (ATP), Adenosine Diphosphate (ADP), electromagnetic fields, intention imprinted electronic device (IIED), Alzheimer's, AIDS

INTRODUCTION

A recent study has favorably implicated the coenzyme nicotinamide adenine dinucleotide (NADH) as a medication for patients suffering from dementia of the Alzheimer type.[1] In all patients evaluated in that study, an improvement in their cognitive dysfunction was observed. In another study it was shown that HIV-1 infection of human cells in vitro leads to significant decreases in the intracellular concentration of NAD.[2] This decrease has been shown to vary with viral load and HIV strain. They conclude from their study that HIV induces a state of intracellular pellagra, which is reversed by the administration of nicotinamide which, in turn, maintains increased intracellular NAD concentration in HIV infected cells.[2]

Very recently, we have shown that it is possible to influence the thermodynamic activity of NAD and the election transport chain to increase the (ATP)/(ADP) ratio in developing fruit fly larvae and thus, significantly increase the fitness of these larvae.[3,4] In addition, using the same basic technique, the thermodynamic activity of the liver enzyme alkaline phosphatase (ALP) has been significantly increased.[3-5] Because of the potential applicability of this new technique to AIDS and Alzheimers diseases, a study to be reported on here has been undertaken with the in vitro fly model system.

Based on the present paradigm, the conventional viewpoint is that humans cannot meaningfully interact, via their intention, with specific target experiments. Even more strongly, one would state that human intention cannot possibly be captured in a simple electronic device and *then* have the device meaningfully interact with these target experiments. Over the past two years, three specific and very different target experiments have been conducted using intention imprinted electronic devices (IIED's) and found robust interaction between these simple devices and the target experiments in complete opposition to the prevailing paradigm.[3-8]

On the experimental side, for each target experiment, one starts with two identical physical devices, isolates one from the other to be the control and "charges" one with the specific intention for the particular experiment. This device is then named an Intention Imprinted Electronic Device (IIED) and is described in detail in reference 3. This charging process involved the services

of four meditators to impart the intention (imprint) to the device with the specific intention. The devices with the same imprint were then wrapped in aluminum foil and stored in electrically grounded Faraday cages until the next step in the process. The Faraday cage blocks electromagnetic radiation of wavelength greater than the spacing of the wire mesh used in the cage construction. The aluminum foil blocks electromagnetic radiation of even shorter wavelength, including photons. Next, when needed, the aluminum foil wrapped devices were separately shipped on different days, via Federal Express, to their laboratory destination about 2,000 miles away. On arriving there, they were immediately placed in separate, electrically grounded Faraday cages until use in the actual target experiment conducted by others (see Figure 1).

For the specific target experiments, the general intentions were (1) to decrease (increase) the pH of water by one full pH unit, (2) to increase the (ATP)/(ADP) ratio in fruit fly larvae so as to significantly decrease the development time and (3) to significantly increase the thermodynamic activity of the liver enzyme ALP.[3-8] The strong success of these three experimental studies promises to have important consequences for future medicine. The present paper continues the vector of progress for this new technique.

In this paper we show that these electronic devices significantly alter the condition of the water used for preparing NAD solutions to be utilized for in vitro fruit fly studies. Here, both NAD activity and the (ATP)/(ADP) ratio are shown to significantly depend on the four treatments given to the water.

EXPERIMENTAL METHODS

TREATMENTS

The study involved three simultaneous experimental variants with respect to the control exposure (C) with all four placed adjacent to each other in an incubator at 4°C. The three variants were (1) an identical exposure in a 30cm diameter Faraday Cage with no device present (F), (2) the same as (1) but with an unimprinted device (IIED) at cage center present (*d,o*) and (3) the same as (1) but with an imprinted device at cage center present (*d,i*). The Faraday cage represents a shielded environment which facilitates

exposure to both fewer and specific EMFs (see above). The experimental layout is shown in Figure 1.

In our experiments, comparisons between *(d,o)* and *(d,j)* indicate the effects of exposure to the two different electronic devices—the intention effect. Comparisons between (C) and (F) indicate the effect of a reduction in exposure to random environmental EMFs—the Faraday cage effect. Comparisons between (F) and *(d,o)* and *(d,j)* indicate the effects of the addition of specific EMFs to the Faraday cage environment—the oscillator effect.

Electronic Devices

Our experiments utilized a triple oscillator electronic device producing frequencies of 5.0 MHz, 8.0 MHz and 9.3 MHz. The devices were powered by line voltage to 9V DC. A detailed description of the devices, including circuit diagrams, is given in references 3 and 5. We studied two categories of EMFs produced by these devices. The first category involved devices *(d,o)* which had not been exposed to human informational influences. The second category involved IIED devices *(d,j)* which had been exposed to human informational influences (see below). Thus, *(d,o)* and *(d,j)* constituted physically identical pairs of devices which differed only in the fact that one of the pair, *(d,o)* and *(d,j)* respectively, had been exposed to the human informational influence. The devices were individually wrapped in aluminum foil and stored in separate Faraday cages and were fabricated to be identical to those produced commercially.[9]

Intention

The actual imprinting procedure was as follows: (i) place the device along with its current transformer on a table around which the imprinters sit, (ii) four people (two men plus two women) who were all accomplished meditators, coherent, inner-self managed and readily capable of entering an ordered mode of heart function and sustaining it for an extended period of time, sat around the table ready to enter a deep meditative state, (iii) a signal was then given to enter such an internal state, to cleanse the environment and create a sacred space for the intention.[3,10] Then, a signal was given by one of the four to put attention on the table-top objects and begin a mental cleansing process to erase

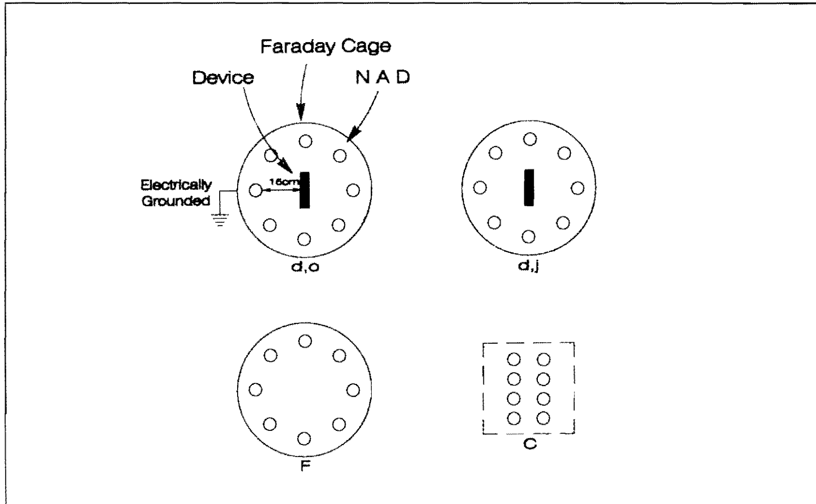

Figure 1a. *Experimental layout.*

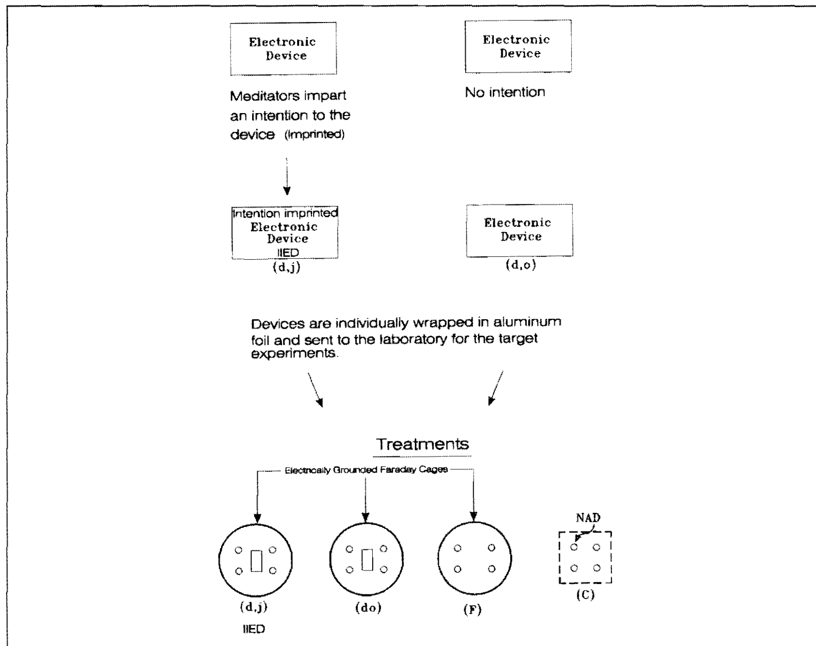

Figure 1b. *Flow diagram showing the creation of the IIED and the target experiments.*

any prior imprints from the device, (iv) after 3 or 4 minutes, another signal was given to begin focussing on the specific prearranged intention statement for about 10-15 minutes, (v) next, a final signal was given to shift focus to a closing intention designed to seal off the imprint projected into the device and minimize the leakage of the essential energy/information from the devices. This completed the process so the four people withdrew from the meditative state and returned to their normal state of consciousness.

The specific intention, here, was "to activate the indwelling consciousness of the device (*d,j*) so as to increase the concentration of NAD plus the activity of the available enzymes, dehydrogenases and ATP synthase in the mitochondria so that production of ATP is significantly increased relative to that produced in the unimprinted device (*d,o*)."

EXPERIMENTS

(NAD) Change Over Time

We initially exposed purified water (ASTM Type I) to the treatments (*d,o*), (*d,j*), (F) and (C) for 2 weeks.[7] We named these treated waters W,o, W,j, W,F and W,C. We then dissolved NAD (Sigma), at 0.01M, in 5 ml of the respective waters. We named these solutions NAD,o, NAD,j, NAD,F and NAD,C. We placed these NAD solutions in the four treatments and measured the NAD concentration over 200 days using standard procedures and High Perfomance Liquid Chromatography (HPLC).[11,12]

On days 78 and 105 respectively, we also prepared four replicate NAD solutions using untreated purified water, without prior exposure to the treatments. These treatments were named (Lab-F and Lab-C respectively). Lab-F replicates were subsequently stored in the Faraday cage without devices (F). Lab-C replicates were subsequently stored in the control environment of the incubator (C). Finally, using standard procedures and HPLC, we measured the concentration of ATP, a breakdown product of NAD in each solution.[11,12] We commenced the ATP assay at day 100 for NAD,o, NAD,j, NAD,F and NAD,C and at days 0 and 20 for C-C and C-F respectively. The (NAD) and (ATP) are given as absorbance units, which are the integrated areas of the respective peaks (see Figure 2).[11,12]

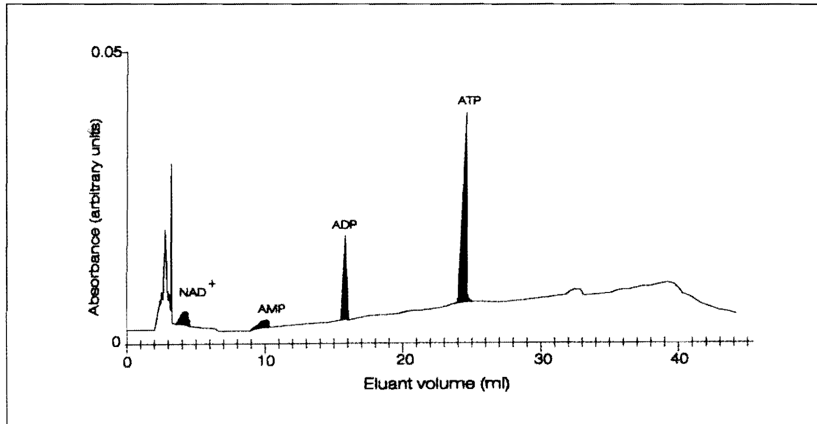

Figure 2. *HPLC separation of NAD, AMP, ADP and ATP from* ***D. melanogaster*** *larval homogenates. All molecules were detected at 260 nm. and analytical conditions and instruments are described in references 11 and 12. (NAD) and (ATP) were reported as absorbance units, calculated as the integrated area of the respective peaks. The (ATP)/(ADP) ratios were calculated using these values.*

In Vitro Experiments: (ATP)/(ADP) Ratio in Homogenates of *Drosphila melanogaster*:

The fruit fly in vitro model system measures the (ATP)/(ADP) ratio in third instar larval homogenates which are supplemented with NAD. The procedure has been described in detail in and is summarized below.[11] Larvae for experiments were derived from cultures of an isofemale, maintained at 18°C and 55% Relative Humidity (RH) using standard food.[11] Adult and larval density were kept constant throughout the experiments and the generation time was 14-16 days.

Source Water-NAD Solution Combinations. At convenient times during Experiment (a) above, we assessed the influence of the source waters and the NAD solutions upon the (ATP)/(ADP) ratio in larval homogenates. We used the source waters W,o, W,j, W,F and W,C described above to prepare third instar larval homogenates at 2°-4°C.[11] Aliquots from these homogenates were then added to equal volumes of the respective NAD solutions NAD,o, NAD,j, NAD,F and NAD,C at 2-4°C and the (ATP)/(ADP) ratio plus (NAD) were

subsequently determined. For example, homogenates were prepared using 250ul of ice cold W,o. 50ul of this homogenate was then immediately added to centrifuge tubes containing 50ul of ice-cold NAD,o. After a 7 minute reaction period, during which the added NAD drives the electron transport chain in vitro and increases the (ATP)/(ADP) ratio, metabolic activity was stopped by freezing the centrifuge tubes in liquid nitrogen. ATP and ADP were then extracted and quantified using standard procedures and HPLC.[11,12] The (ATP)/(ADP) ratios were calculated on the basis of the absorbance units, which were the integrated areas of the respective peaks (see Figure 2).[11,12]

All experimental steps were carried out on ice at 2-4°C and on a laboratory bench in a room adjacent to the room in which the incubator and treatments were located. We assayed all possible combinations by randomly performing the above procedure in a time period from day 30 through day 38 of (a) above. We adjusted larval mass in each homogenate to be approximately 20 mg and there were no significant differences between homogenates ($p > 0.100$). There were 8 replicate assays for each source water-NAD solution combination. Thus, the (ATP)/(ADP) ratio was determined, through time, for each initial water, in combination with the corresponding NAD solution—W,o with NAD,o; W,j with NAD,j; W,F with NAD,F and W,C with NAD,C.

Source water only. Finally we assessed the influence of the source waters W,o, W,j and W,F, without additional NAD, upon the (ATP)/(ADP) ratio in larval homogenates as follows: we transferred sufficient third instar larvae (20 mg larval mass) to centrifuge tubes. We then added 250 ul ice cold source water (W,o, W,j and W,F) to the tubes and stored the tubes on ice for 1 minute. We then homogenized the larvae at 0-2° C for 30 seconds and again, immediately stopped metabolic activity by freezing the tubes in liquid nitrogen. The (ATP)/(ADP) ratios were determined as above. There were no significant differences in larval mass between homogenates ($p > 0.100$) and there were 8 replicate assays for each source water.

Device History

In late February, 1998, the IIED (*d,j*) was charged with the intention and placed together with (*d,o*) in the Faraday cages in the incubator. The IIED (*d,j*) was re-charged with the intention in July, 1998. Experiment (a) was

initiated in February, 1999 and completed in September, 1999. Experiment (b) (i) was carried out in March, 1999 and Experiment (b) (ii) in April, 1999. Finally, from July, 1998 to February, 1999, the devices were used in other experiments and hence, they were in the "on" condition throughout this time.

Statistical Procedures

We have presented our experimental results in the following fashion: For the change in concentration of NAD and ATP over time, we show means for each treatment in Figures 3 and 4, and assess these data with both visual inspection and Analysis of Variance (ANOVA), where the data were log-transformed prior to analysis.

For the in vitro experiments, we show all of the primary data as notched box plots. 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. Outside values are represented by an asterisk and far outside values, by an open circle. We assess the data by both visual inspection and ANOVA, where the data were transformed (square root or arc sine) prior to analysis. Based on the ANOVA, we examine pair-wise comparisons with TUKEY post hoc tests. This test provides protection for testing many pairs of means simultaneously allowing us to determine which treatment means differ significantly from another. Statistical analysis followed standard procedures and we used SYSTAT. This is a multi-purpose statistical program that is designed for use with Macintosh Version 5.2.1 for the Macintosh.[13-15]

RESULTS

(NAD) Change Over Time

The change in (NAD) activity over a period of 200 days is given in Figure 3. Considering the first 100 days, it is clear that the (NAD) activity changes with time in an oscillatory pattern and much more so than the two controls. Overall,

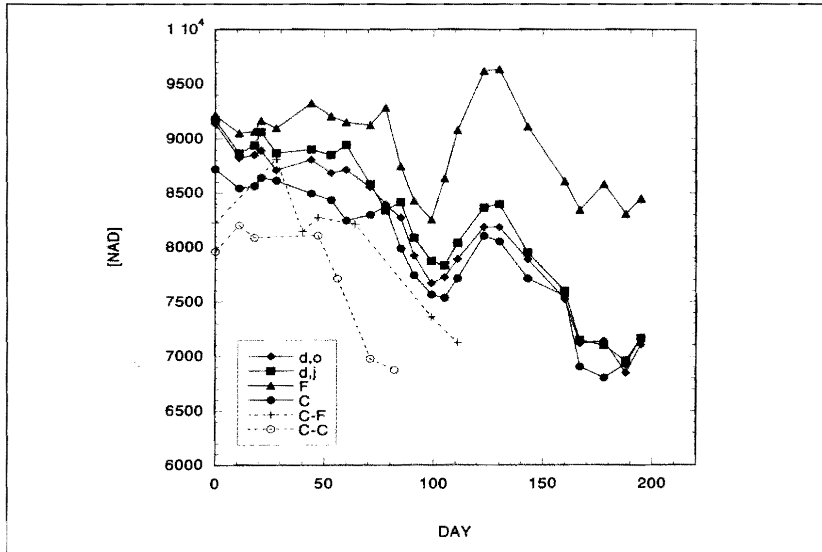

Figure 3. *Change in (NAD) with time for the various treatments. (NAD) is given as the area of the NAD peaks detected via HPLC.*

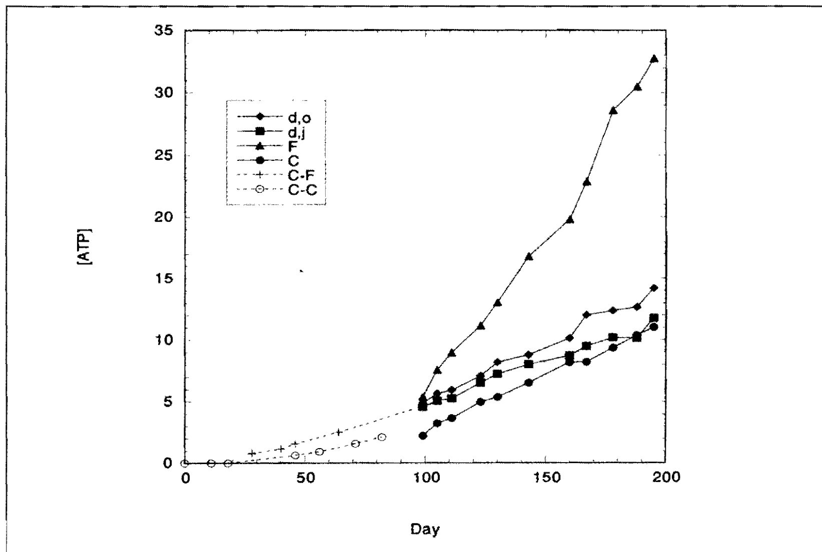

Figure 4. *(NAD) "breakdown product" change with time. Means are given for levels (area, via HPLC) of ATP in the 0.01M solutions of NAD of Figure 3.*

the general trend was a decrease in (NAD) activity with significant treatment rankings of (F) > (*d,j*) > (*d,o*) > (C). All treatments were assayed in random order over a 36 hour period and the "day of assay" in Figure 3 refers to the time of the first assay in the sequence. We assessed treatment effects for each day of assay using ANOVAS and TUKEY post hoc tests (not given).

Considering day zero, significantly ($p < 0.001$) higher values were observed for (F), (*d,j*) and (*d,o*) in comparison to (C) whereas the first three did not significantly differ. ANOVAs were significant ($p < 0.001$) for all subsequent days of the assay. The highest and lowest (NAD) activity were uniformly observed for (F) and (C), respectively, at ($p < 0.001$). Values for (*d,j*) were significantly ($p < 0.001$) greater than for (*d,o*) for assay days 21, 28, 53, 60, 85, 91 and 99 but not for days 11, 18, 44, 71 and 78.

During days 100-150, (F) and (C) continued to exhibit the highest and lowest NAD activity values, respectively. All ANOVAs were significant with a single maximum value around day 130. Around day 160, the NAD values for (*d,j*), (*d,o*) and (C) converged and did not differ significantly thereafter. (F) remained significantly higher than the other treatments during this final period with some suggestion of oscillatory behavior still being present. We suggest that the imprint "charge" in (*d,j*) had totally leaked away by day 130. Finally, considering (C-F) and (C-C), the former maintained its higher NAD activity level for a longer period than the latter.

Finally, Figure 4 shows the time evolution picture for ATP, one of the "breakdown products" in the NAD solution. Once again, we observe the strong EMF-shielding factor associated with the use of Faraday cages in this work.

IN VITRO EXPERIMENTS: (ATP)/(ADP) RATIO IN HOMOGENATES OF *DROSPHILA MELANOGASTER*:

All of our data are shown as box plots in Figure 5. The ANOVA and TUKEY post hoc comparisons are given in Table I. Visual inspection of the figures and the statistical analysis indicated that both (a) the source water-NAD solution combination and (b) the source water alone significantly ($p < 0.001$) influenced the (ATP)/(ADP) ratio in the larval homogenates. Treatment

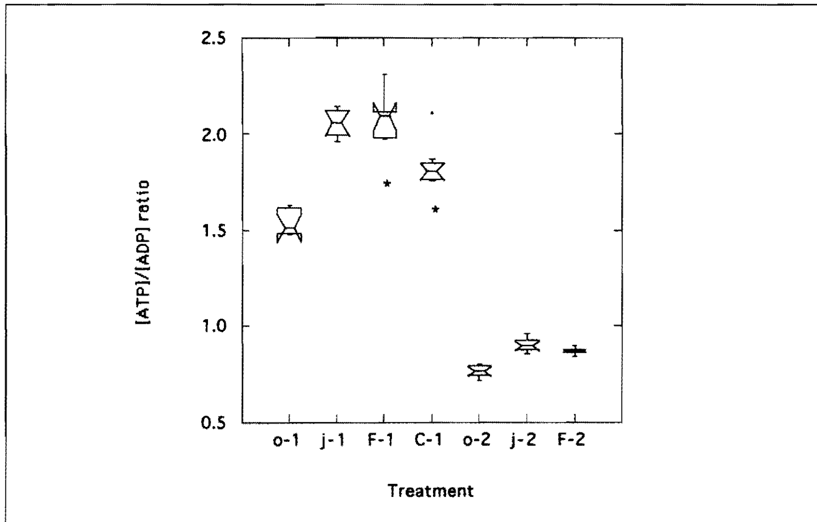

Figure 5. *Boxplot data display of (ATP)/(ADP) ratios in the* ***D. melanogaster*** *in vitro homogenates for source-water-NAD combinations (treatments are labeled o-1, j-1, F-1 and C-1) and source-water only (treatments are labeled o-2, j-2 and F-2).*

rankings for (a) were (F) ≥ (*d,j*) > (C) > (*d,o*) whereas, for (b), they were (*d,j*) > (F) > (*d,o*).

DISCUSSION

EMF Effects

We first consider our results in the context of the effects of EMFs on our experimental system and focus on the treatment comparisons (F) versus (C), (*d,j*) and (*d,o*). We then discuss some ideas regarding the possible influences of EMFs on biological systems and the possibility that EMFs function as a biological stress. Finally, we enlarge our scope to incorporate the specific intention effects, examining the treatment comparisons (*d,j*) versus (*d,o*). Overall, we found that (F) maintained higher NAD levels over time than (C), (*d,j*) and (*d,o*). Faraday cage shielding from ambient environmental EMFs significantly maintained NAD levels while the low power/specific frequency

Table I

(ATP)/(ADP) ratio in the in vitro fruit fly homogenates: (a) source-water-NAD combinations and (b) source-water only.

(a) Source-water-NAD combinations

ANOVA

Source	df	Mean Square	F
Source water-NAD solution	3	0.072	49.761**
error	28	0.001	

Tukey post hoc tests significant at $p < 0.05$

Comparison	p value
d,o	
< *d,j*	$p < 0.001$
< F	$p < 0.001$
< C	$p < 0.001$
d,j	
> C	$p < 0.001$
z	
> C	$p < 0.001$

(b) Source-water only

ANOVA

Source	df	Mean Square	F
Source water-NAD solution	2	0.142	38.641**
error	21	0.004	

Tukey post hoc tests significant at $p < 0.05$

Comparison	p value
d,o	
< *d,j*	$p < 0.001$
< F	$p < 0.001$
d,j	
> F	$p < 0.05$

** indicates $p < 0.001$

EMFs from an unimprinted device significantly reduced NAD levels. The specific intention imprint of the IIED more than compensated for these normal EMF effects and raised the NAD level. Thus, it has been clearly shown that the observed IIED effects on water, fitness and energy metabolism in vivo[3-7] can be extended to an in vitro biological material, NAD.

EMF MECHANISMS

In the past decade or so, many studies have appeared indicating that weak electromagnetic fields (EMFs) can influence water and biological systems.[10,16-24] Furthermore, low frequency EMFs influence specific RNA transcripts in human cells and transcription in *Drosophila melanogaster* cells and Ho et al. have shown that weak static magnetic fields cause abnormalities in first instar larvae of *D. melanogaster*.[22,25,26] Hence, our data supplements this growing list.

The detailed mechanisms responsible for EM radiation effects in biological systems have not yet been delineated. At the atomic level a qualitative picture of two likely and specific pathways can be readily articulated. EM photon interaction with any biological body can occur either directly as a field/ion coupling with the tissues and fluids or indirectly via piezoelectric coupling, wherein, the EM oscillations generate strain oscillations (sound waves) in the tissues. The absorption cross-sections involved in these two modes of interaction determine the degree of EM-field stimulus to the biological system. For the direct coupling mode, fairly large absorption cross-sections are expected for EM frequencies in the GHz to THz range. For the indirect coupling mode, since the velocity of sound in biological systems is approximately 10^{-4} to 10^{-6} times the velocity of light, reasonably large absorption cross-sections are expected in the sub MHz through the MHz frequency range.

It is perhaps interesting to note that protein chains, made from monomer peptides, are generally helical in structure and of considerable length, while collagen has an amino-acid sequence forming an α-helix. Piezoelectricity has been observed in many polypeptides, where it arises from the α-helical backbone of these chains and, in some cases, exhibits values as large as quartz. These absorbed signals are expected to generate a set of chaotic stress waves

that excite and eventually fatigue the cells, synaptic responses, neurological function and the immune system of the biological moiety under consideration and hence, function as a biological stress, as discussed below.

At the cellular level, Eichwald and Walleczek propose a kinetic model whereby external EMFs interact with cellular systems at the level of enzymes involved in intracellular Calcium-signaling processes.[24] Modulation of Ca entry may affect other cellular processes that are Ca-dependent e.g. DNA synthesis. The magnetic field is expected to alter the kinetics of a specific step within the relevant enzyme-reaction cycle on the basis of the radical pair mechanism.[27] Biphasic response behavior (either stimulatory or inhibitory) is accounted for by including cooperative steps within the enzyme cycle.

Additionally, Jouaville et al. have demonstrated the physiological role of the mitochondrial energy metabolism in intracellular calcium signaling in *Xenopus laevis* oocytes.[28] In particular, modulation of cytochrome oxidase activity was shown to alter Ca signaling. Nossol et al. reported magnetic field influences on the in vitro redox activity of this enzyme.[29] Thus, the possibility arises that a magnetic field effect on mitochondrial cytochrome oxidase also affects cellular Ca signaling. Importantly, these results also indicate a likely EMF effect upon energy metabolism and Menendez has suggested that an electromagnetic coupling process may explain the proton translocation mechanism in cellular energy transfer.[30]

In this context of cellular energetics, and on the basis of the altered development time we observe, we suggest that the EMF may directly interact with cellular energy metabolism, in particular NAD and the electron transport chain; and gene expression.[11] The idea of a functional relationship between cellular energetics and gene expression is supported by increasing evidence which shows that the metabolic status and integrity of mitochondria and chloroplasts can influence gene expression and that NAD may have a transcription level regulatory role.[31-33]

Furthermore, a significant association has been observed between stress, larval development time, the cofactor nicotinamide adenine dinucleotide (NAD) and the (ATP)/(ADP) ratio in *D. melanogaster.*[11] Thus, the decrease in larval development time observed for the reduction in EMF exposure here may be due to altered energy metabolism and gene expression. We suggest that EMFs

and intention-augmented EMFs, modify fitness by directly interacting with cellular energy metabolism, the (ATP)/(ADP) ratio and gene expression via NAD and the electron transport chain (see Figure 6).[3,4]

In conclusion, a number of models are available to explain the action of EMFs on biological systems, suggesting that EMFs may produce their effects in a variety of ways. This view may be useful in understanding the possibility that EMFs which have been subjected to human influences (augmented EMFs) may specifically modify biological processes.

EMFs and Stress

The Faraday cage represents a somewhat shielded environment with respect to EM radiation and facilitates exposure to fewer frequencies in the absence of devices and to specific EM frequencies in the presence of devices. Thus, a reduction in exposure to EMFs of specific and various frequencies increased an important fitness component, larval development time in *D. melanogaster.* It is possible that this fitness effect was a consequence of an alteration in energy metabolism, as indicated by the change in the (ATP)/(ADP) ratio. A shorter development time leads to higher fitness and we therefore suggest that exposure to EMFs may represent a biological stress.[34,35]

Associations between rapid development time, a long-life and success in mating can be predicted on the basis of stress levels encountered in natural populations, the observation that stress targets biological energy metabolism and the high energy efficiency underlain by stress-resistance genotypes. Fitness at one stage of the lifecycle should therefore correlate with fitness at other stages.[36] Hence, the changes observed here for development time in response to EMF exposure are likely to manifest for other fitness components and may have important evolutionary consequences.

Differences observed for the (ATP)/(ADP) ratios were not large, but were statistically significant, indicating that EMFs modified energy metabolism. Further, a higher ratio was observed for the treatments which produced a shorter development time. This strong negative correlation between development time

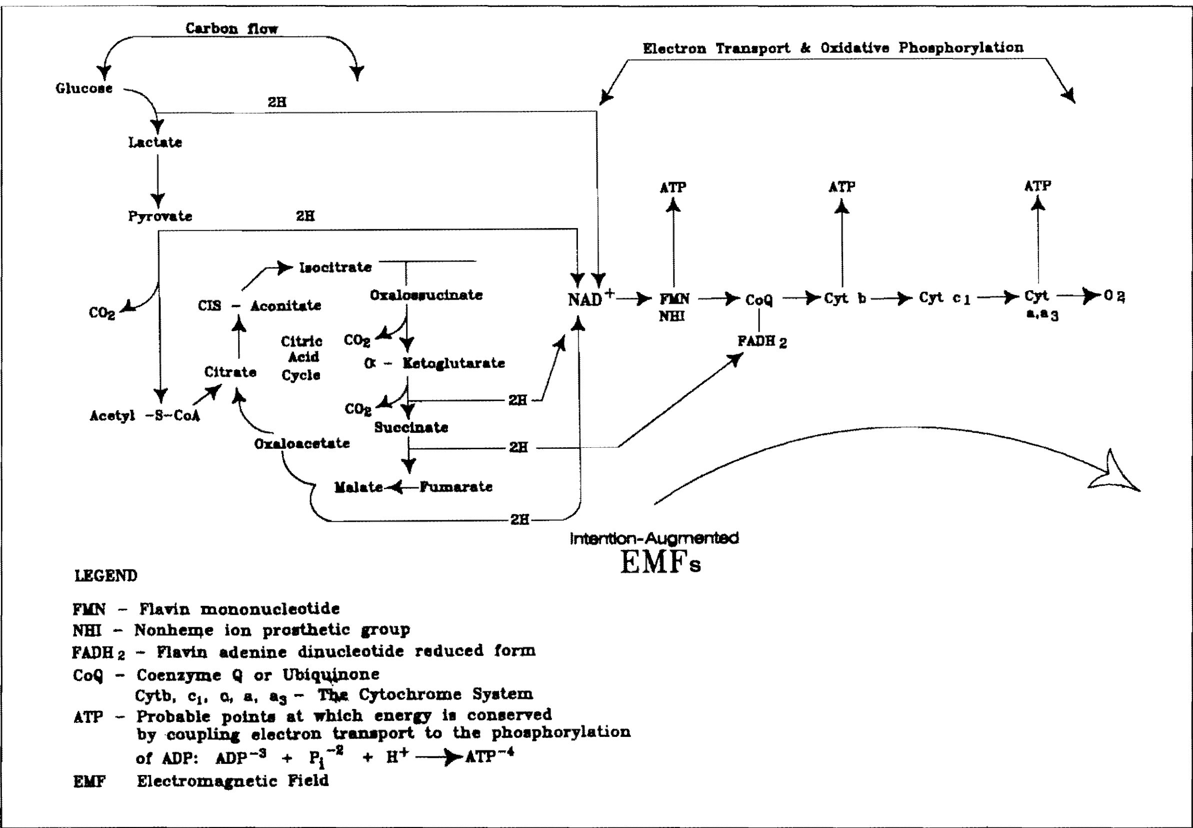

Figure 6. *Diagram of Intermediary Metabolism, illustrating the pivotal role of NAD and the suggested interaction with intention-augmented EMFs. Modified from Kohane.*[45]

and the (ATP)/(ADP) ratio provides further evidence for a relationship between energetics, fitness and possibly, stress.[36,37] Various enzymes involved in energy metabolism have been associated with development rates in a number of species.[36,37] Thus, it may be worthwhile to consider the functional role of EMFs in relation to this sort of enzyme variation.

The hypothesis that EMFs may function as a stress, possibly via alteration of energy metabolism, is important in an evolutionary context since interactions between stress and energy metabolism may modulate fitness variation and evolutionary change.[38,39] Certainly, our results indicate that EMFs influence both fitness and energy metabolism. The disturbance of normal development in a stressful environment may be associated with an increased energy requirement.[39] Under stress, energy availability for growth and reproduction is expected to be reduced in relation to the maintenance function. High metabolic rate, with more energy available for growth and reproduction may lead to greater adaptive specialization in comparison to low metabolic rates.

Finally, survival under stress is likely to be associated with metabolic rate since stress requires the continuous expenditure of energy. Further, variation in metabolic rate can be reduced to the adenine nucleotide level and electron transport chain activity.[39] The stress response must depend upon the primary targets of selection in natural populations and how this is affected by extreme stress. The above suggests that the primary targets of selection under EMF stress may be initially considered to be energy carriers and changes in their concentration.[38,39]

Human Intention, IIEDs and Augmented EMFs:

Overall, our results indicate that the IIED's (*d,j*) gave significantly better results on (NAD) and the (ATP)/(ADP) ratio than did the unimprinted devices (*d,o*) in the otherwise shielded environment. Thus, the in vitro effect on the homogenates by the four water treatments, with or without the supplemental NAD, has been shown to generate a very significant influence on the (ATP)/(ADP) ratios for the homogenates. Clearly, the various water treatments by themselves produced a statistically significant effect while NAD, appeared

to largely act as an amplifier for the water treatment effect. From this, we may deduce that treated source water itself carried the enhancement factor which was amplified by the additional NAD. It is probably the EMF factor that produces the slight shift in treatment rankings.

As noted above, a number of models are available to explain the action of EMFs on biological systems, suggesting that EMFs may produce their effects in a variety of detailed ways. This fact may enable human influences to register effects at the level of EMFs and allow them to be transferred to biological systems. That is, EMF effects on biological systems may be explained by a variety of models and biological processes. Thus, the human, who manifests these biological processes, possibly utilizes such processes to influence and augment the EMFs produced by the devices.

There should be a direct relationship between the internal biological mechanism in the human who influences the EMF environment and the biological mechanism in the fruit fly which generates the EMFs' effects. We have shown that EMFs influence energy metabolism and thus, it is possible that this is the mechanism whereby the human influences the EMFs. In summary, humans may tap the diversity of mechanisms which are available to explain the effects of EMFs on biological systems and utilize these to augment EMFs, which subsequently influence biological processes via the same mechanisms. A useful analogy may the influence of temperature on fitness. Temperature affects components of fitness and biological processes; and humans utilize temperature to modify these.

Thus, in this paper we have explored a new dimension of an age-old question, "Are there no meaningful interactions between human experimenters and the biological entities that they are studying?" Here we let the experimental data speak for itself. Our experiments supplement (1) behavioral research indicating that experimenters may influence their results and (2) studies indicating that the influence of the placebo effect may be profound.[40,41] Furthermore, our study adds to the growing number of studies which address the direct and non-device mediated influence of humans, primarily healers, upon experimental systems similar to ours.[42] We suggest that the IIED's utilized herein have elevated human intention to the level of an independent thermodynamic variable of the experimental system.

To further develop a theoretical understanding of how EM energies, and the more subtle energies associated with the intention-incorporating process, impact our experimental systems it is useful to consider the general thermodynamic perspective (see Appendix A). We suggest that the primary effect of intention on biological systems is via a modification of the standard state electrochemical potential which is related to the ground state energy of either the neutral or charge carrying species.

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CORRESPONDENCE: Michael Kohane • Ditron, Ltd. • PO Box 70 • Excelsior, MN 55331

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APPENDIX A

Standard thermodynamic theory[43] shows us that the electrochemical potential, η^j_A, of a particular molecule or moiety, *j*, in medium A is given by:

$$\eta^j_A = \mu^j_{oA} + kT\ \ln(a^j_A) + z^j eV + 1/2(e_A \overline{E}^2 + m_A \overline{H}^2). \quad (1)$$

The last term represents the applied EMF contribution to the standard electrochemical potential. Here, μ_o = standard state chemical potential, T = temperature, k = Boltzmann's Constant, a = chemical activity, ($a = \gamma c$ where c = concentration and γ = activity coefficient), e = electron charge, z = valence, V = electrostatic potential, ε = contribution to the electrical permittivity, m = contribution to the magnetic permeability, $\overline{E}$= electric field and $\overline{H}$ = magnetic field. One finds that z^j = +1 and -1, respectively, for H+ and OH-. However, $z^j = 0$ for electrically neutral atoms and molecules. Using a per mole basis, rather than a per molecule basis, k becomes R (the gas constant), e becomes F (the Faraday Constant) and the other parameters in EQ. 1 change accordingly.

From a kinetic perspective, the transport flux, F^j, of the j species in medium A will be given in terms of its mobility, M^j, by:

$$F^j = M^j_A\ \nabla\eta^j_A. \quad (2)$$

where $M^j_A = D^j_A C^j_A/kT$ and D is the diffusion coefficient. In all likelihood, the ultimate chemical limitation in many biological processes depends upon the sum or difference in F^j and F^k for two key species j and k. In others, it is the activation energy for primary reactions that is the limiting step.[24]

It has been postulated elsewhere that directed human intention is capable of altering the condition of subtle substance coherence in the physical vacuum which, in turn, should change the ground state energy of fundamental particles, atoms, molecules and biological moieties.[10] Dirac's original concept for the formation of an electron from his "negative energy sea" of the vacuum[10] led to a ground state electron energy determined by the interaction between the physical stuff that constitutes the electron and the non-physical stuff that constitutes the negative energy sea of the vacuum.

Dirac used Relativistic Quantum Mechanics (neglecting particle self-energy) to evaluate the interaction between a physical electron and the vacuum state. His equations predicted not only the existence of positive energy states, but also allowed the existence of negative energy solutions. For a free Dirac electron, the available positive and negative energy levels are symmetric about the defined energy position. Dirac

postulated that the vacuum consisted of all these negative energy states and that these states were all filled (such negative energy states are unphysical and, therefore, unobservable by present day instrumentation).

Nevertheless, by stimulating the negative energy states via a photon with sufficient energy, a particle (electron) may be promoted into one of the positive energy states and become physically *real.* The holes, left behind in the negative energy state spectrum are the antiparticles (positron). Thus, he proposed that we live in a sea of virtual (unobservable) particles - the Dirac Sea. Since all physical observations represent finite fluctuations in energy and charge with respect to the vacuum state, this leads to an acceptable model. For example, if one negative energy particle (electron) is absent from the Dirac Sea, we have a "hole" relative to the normal vacuum:

$$\text{Energy of "Hole"} = -(E_{neg}) \rightarrow \text{Positive Energy}$$

$$\text{Charge of "Hole"} = -(Qe) \rightarrow \text{Positive Charge.}$$

Thus, the absence of a negative energy electron is equivalent to the presence of a positive energy positron. To date, antiparticles have been found experimentally for all the particles known to physics.[44] Today, the vacuum is seen as a chaotic sea of boundless energy (energy density equivalent to approximately 10^{94} gms per cc) at the Quantum Relativity level with incredibly large destructive interference of virtual particle wavefunctions. An interaction occurs between this virtual particle sea and physical matter. Thus, if from a deep meditative state, the human's intention can shift this degree of coherence in the virtual particle sea even a tiny amount, the ground state energy of the electron would be appreciably altered. This is the basis for suggesting that μ^j_{oA} in EQ.1 may be affected by directed human intention. In fact, it should also be recognized that evolutionary change in collective human consciousness may cause some drift in the degree of coherence of the vacuum and thus some drift in μ^j_{oA}. Below, we show how this changes the effective chemical activity, $a^{j'}_A$, of the j-species in medium A.

We propose as discussed above that changes in the degree of coherence of the vacuum may occur as a consequence of individual human intention or collective human consciousness. These effects can easily be incorporated into EQ.1. Indeed the results of our experiments require an approach such as this to extend the conventional thermodynamic treatment. Thus, let us define μ^j_{oA} and V as the appropriate values of these terms in the presence of directed human intention while μ^j_{ooA} and V_o are the values in the total absence of human intention. Therefore, we also define

3a) $$\mu^j_{oA} = \mu^j_{ooA} + \Delta\mu^j_{oA}$$

where $\Delta\mu^j_{oA}$ becomes non-zero by the application of directed human intention and

(3b) $$\Delta V = V - V_o$$

again where V is defined only when human intention is applied.

EQ.1 can be rewritten in terms of an effective chemical activity, $a^{j'}_A$, in the following fashion

(4a) $$\eta^j_A = \mu^j_{ooA} + kT \ln (a^{j'}_A) + z^j eV_o.$$

where

(4b) $$\frac{a^{j'}_A}{a^j_A} = \exp \left\{ \frac{\Delta\mu^j_{oA} + z^j e\Delta V = 1/2 \ (\varepsilon^j_A \bar{E}^2 + m_A \bar{H}^2)}{kT} \right\}$$

In this fashion, one can see how both EMF effects and human intention effects can influence η^j_A. Here, $a^{j'}_A$ is the effective chemical activity of species j in medium A, V_o is the baseline macropotential in A, while μ^j_{ooA} is the baseline standard state chemical potential in the absence of directed human intention.[43] It should be recognized that evolutionary changes in collective human consciousness may cause a drift in μ^j_{ooA}.

One can see via the definition of $a^{j'}_A$ above (equation 4b), a clear thermodynamic basis for both positive EMF effects and positive intention-augmented EMF effects on biological systems (since any change in the magnitude of μ^j_a or $\bar{E}^2, \bar{H}^2$ alters the magnitude of $a^{j'}/a^j$). With the passage of time and more detailed experiments, specific pathways producing these thermodynamic effects may be elucidated. The final apportioning of the total effect between the thermodynamic and the kinetic categories awaits further research.

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