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INVESTIGATION OF THE BIOLOGICAL
EFFECTS OF PULSED ELECTROMAGNETIC FIELDS.

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I. Introduction

Research conducted during the initial year of study under Contract N 000 14-75-C-0334 has involved an investigation of the effects of electromagnetic pulsed fields (EMP) on the Dutch rabbit as well as in vitro study of electric field effects on bilayer lipid membranes (BLM). The object of the research is to determine the in vivo effects of EMP exposure and to develop model systems to investigate the basic mechanisms of interaction. Since extensive data on the biological effects of another type of electromagnetic stressor, namely microwave radiation, has been obtained in this laboratory and elsewhere, the effects of EMP exposure will be compared to microwave exposure effects using similar end-points.

Rabbits have been exposed in the EMP simulator at the Electromagnetic Radiation- Bio Effects Laboratory at the Naval Surface Weapons Laboratory, Dahlgren, Virginia. The animals used in this phase of the study are housed at the Medical College of Virginia and are transported to and from Dahlgren in an air-conditioned animal van. The staff of the Electromagnetic Radiation - Bio Effects Laboratory is hereby acknowledged for the use of their facilities and for their aid in carrying out this investigation. The in vitro bilayer lipid membrane research is being conducted in the laboratories of the Biophysics Department at Virginia Commonwealth University.

II In vivo Effects in the Dutch Rabbit

A study of the effects of EMP radiation on rabbit blood chemistry and drug-induced sleeping time in the Dutch rabbit has been initiated during the first year of this investigation. It has been ascertained, in this laboratory, that these dependent variables are affected by acute microwave exposures in the intensity range of from 5 to 25 mW/cm² and at frequencies of 2.45 GHz and 1.7 GHz (1,2). Since such effects are induced by relatively low levels of microwave exposure, they represent potentially sensitive indicators of EMP effects as well, although there are

obvious differences in these exposure modalities. The Dutch rabbit has been used for the in vivo study of EMP effects since the total serum pool is of sufficient volume to permit serial sampling prior to and immediately following exposure as well as at extended intervals following exposure. It is thus possible to obtain inter- as well as intra-animal treatment data for statistical analysis.

During the past nine months of contract support the major effort has been directed toward the study of the effects of EMP exposure on drug-induced sleeping time. The presently available animal exposure facility (EMP simulator) at the Electromagnetic Radiation - Bio Effects Laboratory has proven to be suitable for this phase of the study. Due to the size of this simulator, however, studies with upright unanesthetized animals would be more difficult to perform and, therefore, the blood chemistry and serum protein studies have been deferred until a larger EMP simulator, which is currently being constructed for use in the next few months, is available. Baseline values of the following blood chemistry parameters have been determined on the group of rabbits to be used in the EMP study: calcium, inorganic phosphate, glucose, blood urea nitrogen, uric acid, cholesterol, total serum protein, albumin, bilirubin and serum enzymes (LDH, SGOT, and alkaline phosphatase). The mean baseline values will be compared with data obtained from these animals immediately prior to and post exposure to EMP radiation. During the past six months techniques have also been developed for the assay of serum creatine phosphokinase isoenzymes, serum triglycerides, and serum free fatty acids and lipids for application to the in vivo study of the EMP stress response in the Dutch rabbit.

The EMP simulator used in the study of drug-induced sleeping times consists of two sheets of 0.8 mm copper, 61 cm wide by 114 cm long. The sheets which serve as the plates of the capacitor EMP simulator are placed such that one parallel section 56 cm by 61 cm is separated by an air gap of 19 cm and the other parallel section of 41 cm by 61 cm is separated by 6.4 mm of polyethylene. The capacitance of the section separated by the air gap is 16×10^{-12} farads and the section separated by

the polyethylene sheet is 7.7×10^{-10} farads. The characteristic frequency of the EMP simulator of 23.5 MHz is thus largely governed by the section separated by polyethylene. The effect of capacitance changes in the air gap section used for exposure of biological systems, which result from insertion of animals or other specimens into the field between the plates, has minimal effect upon the characteristic frequency of the simulator. Each pulse, which is triggered by a "free running" spark gap under nitrogen of 45psig, consists of an exponentially decaying cosine wave the amplitude of which decreases to one-half of its initial maximum value in 4 cycles. The total simulator pulse duration is thus approximately $1\mu\text{sec}$.

The pulse repetition frequency (PRF) in the rabbit exposures was either 24Hz at a plate voltage of 36KV (ie 1.9 KV/cm) or 10Hz at a voltage of 26KV (1.37 KV/cm). Since the spark gap was "free running" (i.e. untriggered), the PRF was not constant. At the higher voltage the range of the PRF was from 22 to 25Hz, while at 26KV the frequency varied within the range of from 8 Hz to 12 Hz. The mean interpulse duration at a PRF of 24 Hz was thus 42 msec; at a PRF of 10 Hz it was 100msec.

The investigation of the effect of EMP exposure at PRF's of 24 Hz and 10 Hz on drug-induced sleeping time was performed in an identical manner to studies previously conducted using 1.7 GHz or 2.45 GHz microwave radiation as the stressor. Two weeks prior to EMP exposure 8 to 10 litter-mate Dutch rabbits were anesthetized with a dosage of 22mg/kg sodium pentobarbital, a short acting, general, nonselective CNS depressant at a concentration of 60 mg/ml. Injection of the pentobarbital into the marginal ear vein of the rabbit produces rapid (ie within 30 seconds) anesthesia characterized by a loss of the righting reflex; the criteria used as the initial time of sleep. The time required for the animals to regain the righting reflex is then determined and is referred to as the "sleeping time". A baseline mean sleeping time is determined for the experimental and control animals. The effect of the EMP field is then determined by following the same anesthetization procedure and then immediately placing the animal in the center of the air gap in the EMP

simulator and redetermining the sleeping time during exposure. By a random order selection procedure control animals are also sham-exposed in the EMP simulator in an indential fashion, with the exception that the simulator is not pulsed. Two weeks following EMP (or sham-EMP) exposure a baseline sleeping time is redetermined for each experimental animal. The two levels of controls employed in this study thus provide an indication of both treatment effects and the "trip" effect involved in transporting the experimental animal to and from the Electromagnetic Radiation - Bio Effects Laboratory. To minimize any "trip" effects, the animals are transported to the Dahlgren laboratory at least three days prior to EMP exposure and remain for an additional 2 to 3 days following treatment.

The results of previous sleeping time studies have indicated that there is a statistically significant correlation between mean sleeping time and body weight in the Dutch rabbit (2). Since it is known that there is a significant positive correlation between body weight and age in the rabbit age range used in this study (ie. 6-16 months), the dependancy of sleeping time on body weight may, in fact, reflect a dependancy of sleeping time on age. In order to take such effects into account and in view of the constraints involved in limitations on the number of animals that can be safely transported from our laboratory to Dahlgren, a number of sleeping time experiments involving 8 to 10 litter-mate rabbits were conducted over a nine month period instead of one experiment involving larger numbers of animals. The effects of EMP exposure were determined by a statistical comparison of the EMP treatment mean sleeping time with the corresponding age (or weight) matched control group.

The results of the pentobarbital-induced sleeping time experiments are summarized in Table I. Upon reviewing the data obtained for groups 8 and 10 it appeared in both cases that observations of sleeping time and rectal temperature changes for one animal were widely divergent from the other treated animals. In

both instances the animal slept significantly longer than other animals in its treatment group. The treatment consisted of exposure to the EMP field either at a plate voltage of 26KV and a PRF of 10 Hz or at a voltage of 36KV and a PRF of 24 Hz. Since both of the atypical responses were encountered during EMP exposure the data is reported for the mean response variable with and without the outlying observations included. Group 9 is thus the same as group 8 with the exclusion of the outlier as is group 11 which is identical in composition to group 10 with the exclusion of the data for the one atypical animal. The significance of these atypical responses is in doubt due to the low frequency of occurrence, but such responses were not encountered in any of the sham irradiations or the baseline control studies. Additional observations will be necessary to consider the possibility that this effect may be related to EMP exposure. Data for groups 12 and 13 were obtained in a separate study of the effects of 1.7 GHz microwave irradiation on drug-induced sleeping time in different groups of Dutch rabbits. These data have been included in Table I to enable a comparison to be made of microwave radiation effects versus EMP radiation.

The use of drug-induced sleeping time to detect the effects of physiological stress is based upon the successful application of this technique in pharmacological and toxicological investigations in which it has been determined that this response variable is a relatively sensitive indicator of certain types of stress. This method has been extensively used in our laboratory to investigate the effects of 1.7 GHz and 2.45 GHz continuous wave and pulse modulated microwave radiation on the Dutch rabbit (2). In these studies it has been established that microwave exposure results in a statistically significant, dose-dependent decrease in pentobarbital-induced sleeping time in the intensity range of from 5 to 50 mW/cm² at 2.45 GHz and in the range of from 10 to 50 mW/cm² at a microwave frequency of 1.7 GHz. Although the mechanisms for this dose-dependent decrease in sleeping time are still being investigated, it appears likely that the response is related to the effect of thermal

stress due to microwave heating. Since there is evidence that pentobarbital sleeping time is related to thermal stress, rectal temperatures were measured for the animals exposed to EMP fields.

Rectal (deep colonic) temperatures were measured with a thermistor and a digital thermometer with a time constant of 8 seconds. The rectal temperature was measured immediately prior to anesthetization and immediately following regaining of the righting reflex. The mean rectal temperature change for each group of treated and control animals is included in Table I.

The statistical significance of the differences in mean sleeping time for the treated and control animals is determined by a student's "t" test for the appropriate group contrasts. The results of the statistical analyses are summarized in Table II in addition to the percentage alteration in sleeping time. None of the group contrasts resulted in statistically significant differences at the 0.05 level of significance (ie $p < 0.05$) for a one-tailed t-distribution criterion. Comparison of the sham irradiated control group 2 mean sleeping time with group 6, exposed to 26KV, 10 Hz EMP radiation, resulted in a level of significance of 0.06 which is of borderline statistical significance. For comparison with the effects of 1.7 GHz microwave exposure at $10\text{mW}/\text{cm}^2$ the results for groups 12 and 13 are included in the table. In this case there was a highly statistically significant decrease in the mean sleeping time as a result of microwave exposure.

Although none of the group contrasts for the EMP exposures were statistically significant at the 0.05 level of significance, a trend is suggested by these data. With the exception of the contrasts of group 1 to groups 3 and 4, EMP exposure either at 10 Hz or 24 Hz resulted in an increase in the mean duration of sleep with the percentage increase ranging from 5 to 51%. Exposure to a 10 Hz EMP field appears to result in a greater increase in sleeping time than exposure at 24 Hz, even though the field strength is 28% lower at the lower PRF. Comparison of the data for the EMP exposed group 1 with the pre-and post-exposure baseline data from groups 3 and 4

indicates a reduction of sleeping time for the treated group. These results are attributable to the fact that whereas the data for groups 3 and 4 were obtained from experiments conducted in our laboratory at the Medical College of Virginia, the data for groups 1 and 2 were collected from animals either exposed to EMP or sham-EMP in the exposure facility at the Electromagnetic Radiation - Bio Effects Laboratory at Dahlgren, Virginia. Subsequent to the completion of these experiments it was determined by the staff of the Dahlgren laboratory that there was significantly increased concentrations of ozone in the EMP facility due to corona discharge of the pulser. This condition, which was corrected before additional experiments were performed, most probably caused the decrease in sleeping time during EMP since it is well known that elevated ozone concentrations can result in sleep disturbance in mammals.

The effect of EMP exposure on rabbit rectal temperatures is summarized in Table I and the statistical analysis of this dependent variable is summarized in Table III for appropriate group contrasts. In general EMP exposure tends to result in a depression of rectal temperatures relative to sham-irradiated controls, although conflicting results were obtained. Whereas exposure at 10 Hz led to a statistically significant ($p=0.02$) 99% reduction in rectal temperature relative to the sham-irradiated animals in the case of group 6 versus group 9, a similar comparison of group 7 to group 9 indicated a 98% increase following EMP exposure at 10 Hz. Studies conducted with sham-irradiated or control animals have indicated that in the rabbit and other mammals, Na pentobarbital anesthesia produces a linear time-dependent decrease in body temperature (2). In view of the trend which suggests that EMP exposure leads to an increase in sleeping time, the depression in rectal temperature of EMP exposed animals would be anticipated on the basis of the time-dependent anesthesia-induced rectal temperature depression.

Based upon the results of this study it is tentatively concluded that EMP exposures, as employed in this investigation, do not result in a statistically

significant alteration in pentobarbital-induced sleeping time. The trend toward longer sleeping times during EMP exposures is the reverse of the effect of microwave irradiation on this response variable. Since there is definite evidence that the reduction in sleeping time induced by microwave exposure is related to thermal stress, it is suggested that EMP exposure does not thermally stress the Dutch rabbit when exposures are limited to the types used in this phase of the investigation. This suggestion is supported both by theoretical considerations and by the rectal temperature changes measured in the treated and control animals. The significance of the small prolongation of sleeping time during EMP exposure will be further investigated. The fact that the prolongation was somewhat greater at 10 Hz at an electrical field strength of 1.37 KV/cm than at 24 Hz and 1.9KV/cm indicates the effect may be frequency dependent rather than related to field strength. Additional studies of the effects of variation in the EMP pulse repetition frequency are thus indicated.

III In Vitro Effects of Pulsed Electrical Fields on Membranes

This phase of the study has involved the development of experimental and theoretical models to be used to investigate the basic modes of interaction of pulsed electrical fields with membranes. Although there is a growing body of information which suggests that membrane alterations by various modalities of electromagnetic radiation are responsible for physiological changes in mammalian and sub-mammalian species, the basic mechanisms are unknown. An extensive review of the literature has thus been undertaken during the past nine months to obtain information regarding the types of alterations induced in membranes and molecular systems by exposure to electrical fields of various types. Attention has also been given to the theoretical explanations of these effects as provided by various authors to provide a basis for the development of a model for the interaction of EMP radiation at the level of the biological membrane.

With the exception of the paper by Wilson et. al. (3) the papers reviewed have dealt primarily with the effects of steady or pulsed electric fields on biological systems. The effects described appeared to be largely or totally non-thermal in nature. The results of several experiments on intact organisms were reviewed as well as reported effects of electric fields on macromolecules in solution and on membranes.

Wilson and co-workers (3), observed that pulsed microwave radiation (Diapulse) was more effective than CW radiation in increasing the rate of healing of various injuries. In a controlled experiment it was found that Diapulse accelerated the regeneration of severed nerves. Hamilton and Sale (4,5) found that bacteria and yeast were killed with electric fields of about 25KV/cm. They observed that the membranes of the dead organisms appeared intact and they proposed that the electric field caused an irreversible alteration in the semipermeable barrier function of the cellular membrane. Levy (6) found that the regeneration of bone was faster if stimulated by electrical pulses at a frequency of 0.7 Hz as compared to bone stimulated by a D.C. field or not electrically stimulated. Marino et. al. (7) exposed rats continuously to either vertical or horizontal electric fields of 6-197 V/cm for a period of 30 days. Changes in the distribution of serum proteins were observed, and a significant number of animals in the vertical field developed secondary glaucoma.

Neumann and Katchalsky (8) exposed solutions of synthetic polynucleotides to short pulses of 20KV/cm fields. Long-lived conformational changes were observed which were attributed to base pair dissociation resulting from displacement of the counter-ion sheath which surrounds a polyvalent macromolecule. In a similar experiment Revsin and Neumann (9) found evidence that 44KV/cm pulses produced helix-coil transitions in ribosomal RNA.

The fact that membranes and membrane associated components may be the cell components most affected by electric fields is suggested by a number of authors.

Helfrich (10) presents theoretical calculations of the deformation of lipid bilayer spheres (vesicles) in a uniform external electrical field. Bernhardt and Pauly (11) have calculated the potential generated across ellipsoidal cells in a uniform external electric field. In an external field of only 1V/cm a cell under physiological conditions can theoretically have a field strength of 10^3 to 10^5 V/cm (1 - 100mV) across a portion of the cell membrane. This "amplification" of the field in the membrane is most pronounced in long, thin cells, such as nerve and muscle, when the long axis of the cell is parallel to the applied external field.

The importance of membranes in the generation of action potentials in excitable cells and the effect of alterations in membrane voltage on these cells is well known. To gain insight into the mechanism underlying excitability, several proteins and polypeptides which form voltage dependent conducting channels across bilayer lipid membranes have been studied as models of the nerve axon membrane (12-17). It is apparent that under the proper conditions externally applied fields can influence the transmembrane potential of excitable cells thus affecting the conductance and the generation of action potentials. The importance of the effects of electric fields on synaptic transmission of nerve signals is suggested by the study of Neumann and Rosenheck (18), who found that the release of catecholamines from storage vesicles of bovine neurones could be induced by pulses of 20KV/cm. The release was thought to be due to the interaction of the field with charged and/or dipole structures associated with the vesicle membrane and were not due to breakdown of the membrane.

Another effect that has been investigated is the induction of holes or pores in cell membranes by the application of electric fields. Some authors term this effect "dielectric breakdown". Yafuso et. al. (19) observed step changes in the conductance of artificial bilayer lipid membranes and attributed this to the voltage dependent formation of pores. Coster (20) in a theoretical study of fixed charge membranes showed that under certain conditions an electrical breakdown phenomenon called "punch through" could occur. A different approach was taken by Crowley (21)

who analyzed the breakdown of a membrane as a result of electrostatic compression of an elastic film and obtained reasonable agreement with experimental studies on lipid bilayers. Electrostatic compression of lipid bilayers as observed by White (22,23) and other investigators (24), results in an increase in membrane capacitance. Zimmerman and colleagues (25,26) reported the breakdown of red cell membranes by external electric fields of about 5KV/cm. Coster and Zimmerman (27) observed electrical breakdown of algae membranes by current pulses. They found that breakdown, which occurs at a critical voltage of 0.85 V, is independent of pulse duration and, within limits, is independent of the pulse repetition rate. Heating of the membrane was not thought to be important, and the most likely mechanism for breakdown was thought to be that proposed by Crowley (21).

It may be concluded from this review that the effect of electrical and electromagnetic fields on membrane systems is variable, the results depending upon the type of stress and the biological-end point. It is suggested that regardless of the systems under investigation, a threshold field strength for the irreversible alteration of membranes exists. Field strengths on the order of 10KV/cm, at the cellular level, appears to result in dielectric breakdown of such systems although lower field strengths have been reported to cause alterations in membrane systems. The theoretical mechanism of field induced polarization of the molecular counter-ion layer leading to disruption of cooperative intramolecular bonding, as proposed by Neumann and Katchalsky (8), suggests that this effect, is dependent upon the pulse duration as well as the field strength. It is evident from this review and from the results of the study of EMP effects upon sodium pentobarbital-induced sleeping time in the rabbit, that the independent variables that must be considered in the investigation of the effects of electromagnetic pulsed fields on biological systems must include field strength, pulse repetition rate and pulse duration.

In order to investigate the effects of these variables on membrane systems, chambers for the formation of bilayer lipid membranes have been constructed in our

laboratory. The chambers are constructed with 5 ml teflon beakers which are supported in the center of glass crystallizing dishes. A 1mm circular aperture is bored in a thinned section of the teflon beaker, and when the chamber is filled with aqueous buffer solution, a bilayer lipid membrane is formed across the aperture with a fine brush or a micropipet containing the lipid solution. The solution in the chamber is stirred by magnetic bars driven by a magnetic stirrer located beneath the chamber. The chamber can be heated with a small resistive heater, and the temperature is detected with a thermistor. A temperature regulating circuit is being built to maintain constant temperature in the chamber. The chamber is mounted on a frame of lead blocks and rubber pads to minimize the effects of vibrations. Electrical contact with the solution in the chamber will be made with calomel electrodes. Visual observation of the membrane will be accomplished by the use of a 40x microscope and microscope illuminator.

A Keithley 610 A electrometer was to be used to measure the voltage across the membrane, but since it has proven unsuitable for measuring transient membrane currents, a simple current amplifier will be constructed from an electrometer operational amplifier. A pulse generator (H.P. Model 214A) will be used to provide pulses of up to 10msec duration. A pulse generator will be constructed for longer duration pulses.

Upon completion of the apparatus the initial experiments will be to determine the stability of the membrane to electrical stress. It is known that when the voltage across a membrane is increased beyond a certain point the membrane will break. It has been determined that if short voltage pulses are used, higher voltages can be applied before membrane breakage occurs. To our knowledge, however, there has been no systematic quantitative study of the relation between pulse duration and breakdown voltage, and our first goal will be to establish this relationship. Other variables affecting membrane stability will be studied concurrently. Initially we will use a simple lipid, glycerol-monooleate, in a hydrocarbon solvent as the

membrane forming material. Other lipids will also be used in future studies.

After stability criteria have been established subcritical voltage pulses will be applied to the membrane to detect reversible effects such as transient pore formation and alteration in ion selectivity. The approach to be tried first will be to produce an ion gradient across the membrane, followed by the application of a voltage pulse. The open circuit membrane voltage will then be observed as a function of time to determine transient conductance changes of the artificial bilayer lipid membrane system as a function of electrical field strength, pulse duration and temperature.

TABLE I
Effect of EMP Exposure on Sodium Pentobarbital-
Induced Sleeping Time (Dosage 22 mg/kg)

Group No.	Treatment	Mean Sleeping Time (\pm S.E.) min.	Rectal Temp. $^{\circ}$ Change (\pm S.E.) C	Mean Whole Body Weight (\pm S.E.) lbs	No. of Animals
1	EMP; 36KV; 24Hz	38.7 \pm 6.1	-0.7 \pm 0.24	3.05 \pm 0.19	5
2	EMPsham exposure	30.8 \pm 5.7	-0.58 \pm 0.25	3.03 \pm 0.18	4
3	EMP pre-exposure baseline	43.8 \pm 5.3	*	2.71 \pm 0.10	10
4	EMP post-exposure baseline	40.4 \pm 4.2	*	5.1 \pm 0.30	10
5	EMP; 36KV; 24Hz	47.1 \pm 7.5	-0.42 \pm 0.10	5.3 \pm 0.50	5
6	EMP; 26KV; 10Hz	62.0 \pm 9.0	-1.1 \pm 0.50	5.1 \pm 0.33	4
7	EMP sham exposure	51.2 \pm 6.0	-0.58 \pm 0.20	3.1 \pm 0.34	6
8	EMP; 26KV; 10Hz	61.8 \pm 9.0	-0.44 \pm 0.10	2.8 \pm 0.10	6
9**	EMP; 26KV; 10Hz	53.8 \pm 4.0	-0.01 \pm 0.07	2.9 \pm 0.12	5
10	EMP; 36KV; 24Hz	63.5 \pm 9.4	-0.40 \pm 0.07	3.0 \pm 0.13	6
11***	EMP; 36KV; 24Hz	55.6 \pm 6.2	-0.40 \pm 0.08	3.0 \pm 0.15	5
12	1.7 GHz, CW microwaves, 10mW/cm ²	34.9 \pm 1.6	1.16 \pm 0.22	4.2 \pm 0.44	5
13	microwave sham-irradiation	53.3 \pm 8	*	3.8 \pm 0.23	4

* Rectal temperature changes not determined

** Group 9 identical to Group 8 with the exclusion of one outlying observation (for details see text)

*** Group 11 identical to Group 10 with the exclusion of one outlying observation (for details see text)

TABLE II

Summary of the Statistical Analysis of Sleeping
Time Alterations

<u>Group Contrasts</u>	<u>% Alteration in Sleeping Time</u> *	<u>t-statistic</u>	<u>Level of ** Significance</u>
1 vs 2	20.5	0.93	0.20
1 vs 3	-11.6	0.59	0.28
1 vs 4	- 4.2	0.23	0.43
1 vs 5	17.8	0.87	0.21
2 vs 6	50.8	1.82	0.06
5 vs 6	-24.0	1.56	0.12
7 vs 8	20.7	1.00	0.17
7 vs 9	5.1	0.34	0.37
7 vs 10	24.0	1.10	0.15
7 vs 11	8.6	0.50	0.31
12 vs 13	-34.5	2.60	0.02

$$* \% \text{ Alteration in Sleeping Time} = \frac{\bar{t}_t - \bar{t}_c}{\bar{t}_c} \times 100$$

where \bar{t}_t = treatment mean sleeping time

\bar{t}_c = control mean sleeping time

** one-tailed t-distribution

TABLE III
 Summary of the Statistical Analysis
 of Rectal Temperature Changes

<u>Group Contrast</u>	<u>% Alteration in Rectal Temp.</u> *	<u>t-statistic</u>	<u>Level of Significance</u> **
1 vs 2	-20.69	0.36	0.37
2 vs 6	-89.66	0.93	0.20
5 vs 6	-61.81	1.56	0.08
6 vs 9	-99.00	2.44	0.02
7 vs 9	98.3	2.77	0.01
7 vs 11	31.3	0.96	0.18

$$* \% \text{ Alteration in Rectal Temp. Change} = \frac{\overline{\Delta T}_t - \overline{\Delta T}_c}{\overline{\Delta T}_c} \times 100$$

where $\overline{\Delta T}_t$ = treatment mean rectal temperature change ($^{\circ}\text{C}$)

$\overline{\Delta T}_c$ = control mean rectal temperature change ($^{\circ}\text{C}$)

** one-tailed t-distribution

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