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MECHANISMS OF MICROWAVE NEURAL INTERACTION.(U)
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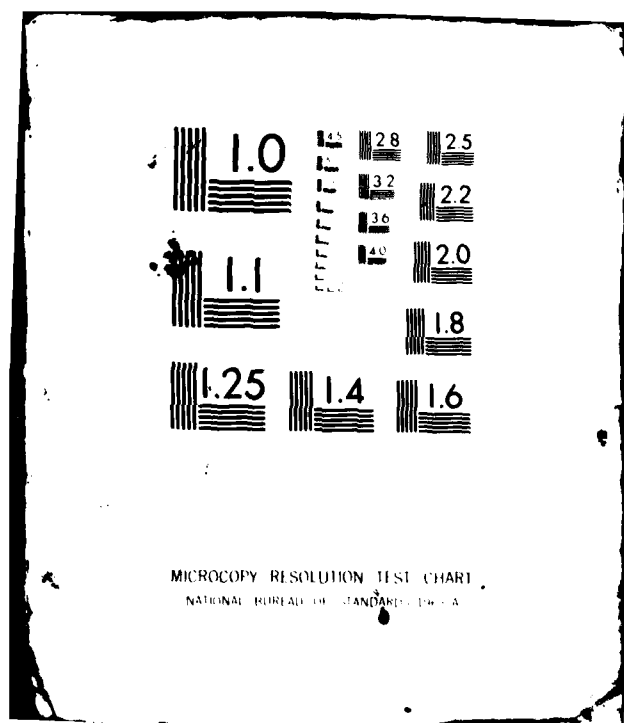
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MECHANISMS OF MICROWAVE NEURAL INTERACTION

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Summary of Work Done in Preceding Years

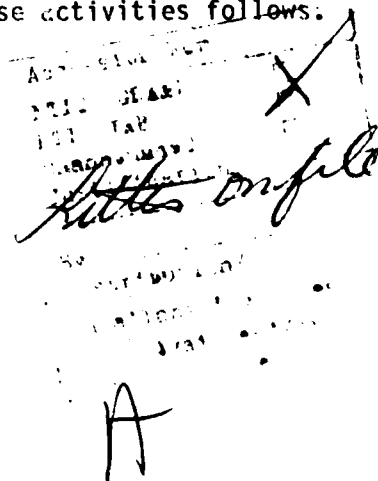
During the initial years of this contract, much of our effort was directed toward accurately quantifying the amount of energy absorbed by a test system from a defined incident field, since such information was necessary in studying electromagnetic biological effects. The most common method of measuring SAR (specific absorption rate) requires measurement of a temperature change over time with at least $\pm 0.1^{\circ}\text{C}$ accuracy. However, during this period no instrument was available to accomplish this that did not perturb the field or was not strongly influenced by the EM energy. Consequently, we developed a LCOF (Liquid Crystal Optical Fiber) nonperturbing temperature probe that could be used during irradiation. Patent #4,016,761 was granted for this system on 12 April 1977(1). This instrument, which is commercially available, has some minor technical difficulties, but remains one of the few systems that can be used to measure temperature in a microwave field. Since the liquid crystal mixtures used in this system appear to degrade slightly with time, a ready source for calibration is essential. We did develop a prototype of an automatic calibrator, but it was not included in the commercial unit. The details of the LCOF and the calibrator system have been reported in previous Annual Reports (ONR) as well as the open literature (see ref. #2, 3, 4, 5).

Simultaneously with the development of the LCOF temperature probe, the study of the turtle heart, rat heart and rat gut were actively pursued. The initial studies indicated that 960 MHz CW irradiation of the isolated heart produced a change in heart rate that appeared to be SAR dependent. Between 1.5 and 2.5 mW/g, (1mW/g = 1W/Kg) bradycardia was seen, while at 8.0 mW/g tachycardia was the response. (See ref. 6, 7, 8, 9, 10 and Annual Reports, ONR). This effect in isolated hearts could not be duplicated in intact animals. When the turtle heart was exposed, but not removed from the animal

the effects were not observed. In the anesthetized rat, the effects were not observed. Thus the homeostatic mechanisms in the intact animal are capable of masking any effects. These changes may be unimportant as a hazard from microwave exposure but could be a component of mild stress. Further work in isolated hearts established that drugs active at the synapse could modify the response seen at 1.5 to 2.5 mW/g (see ref. 10, 11, 12). With these data we formulated a hypothesis that absorbed microwave energy was interacting with our system at the synapse -- not at the muscle fiber or at nerve remnants in the system. In order to assess the drug - synapse interaction, we also studied an isolated rat gut preparation. The isolated rat gut responded in a fashion that would also imply a synaptic site of action. These studies strengthened the hypothesis suggested above (see ref. 13).

Based upon the studies of isolated organs, we began an investigation of isolated synaptosomes and receptor molecules from rat brain preparations to further define the response as a neural one. Whole brain preparations of synaptosomes were shown not to increase membrane recycling under treatment with SAR of 1.5 to 2.5 mW/g. These data suggest that isolated rat brain synaptosomes cannot be used to define the effect seen in the heart and gut studies (see ref. 14, 15, 16).

The effects seen in the synaptosome study, while discouraging, led us to concentrate on the receptor-transmitter interaction -- possible site of action (see ref. 17, 18, 19). The final report of these activities follows.



Binding of Receptor Molecules Under Microwave Irradiation

SUMMARY

Examination of the binding characteristics of acetylcholine receptor (AChR) and either tritium labeled acetylcholine ($[^3\text{H}]\text{-ACh}$) or calcium 45 (^{45}Ca) via a modified Hill plot revealed a decrease in synergistic binding for both acetylcholine (ACh) and calcium (Ca^{++}) in low concentrations. This decrease was observed at a SAR of 1.5 - 2.5 mW/g ($1 \frac{\text{mW}}{\text{g}} = 1 \text{ W/Kg}$), 960 MHz CW, or 16 Hz square wave modulated ($P < 0.01$ in both cases). Cooperative binding of Ca^{++} was not changed at physiological concentrations ($P > 0.25$).

Experiments performed at 5°C could not be distinguished from those at 4°C.

I. Introduction

The emphasis of much of the work reported in the literature in the microwave bioeffects area has been on cataloging of effects rather than in defining a mechanism. In the work described here, we have attempted to define a definite site of action for the observed effects. An integral part of this effort was to design a system that does not have the feedback mechanisms of an intact animal. With the negative results of the synaptosome studies, the receptor site seemed more likely to be a site of interaction.

II. Receptor Study

The details of our system for the study of the neural receptors in rat brain have been detailed previously (17, 18). Briefly we begin by separating an enriched fraction of receptor molecules tested as acetylcholine receptors, from homogenized whole rat brain. Whole rat brain is homogenized in 0.32 M sucrose and then centrifuged at 2500 x g for 5 minutes. The supernatant is decanted and centrifuged at 20,000 x g for 25 minutes. The pellet from this high speed centrifugation is discarded and the supernatant is dialyzed to equilibrium (approximately 22 hours) in Ringer's solution that contains the radioactive ligand. This procedure is conducted in tandem with control and treated, either with or without (sham) microwave irradiation. Every effort possible is made to be certain all variables except microwave power are the same between the treated or sham system and the control. With this system we are able to ascertain the binding characteristics for both transmitter and antagonistic substances of the receptor molecule.

Initially a great deal of time was spent refining the acetylcholine receptor interaction with atropine as the ligand. The rationale was that atropine is not destroyed by acetylcholinesterase and should therefore result in a stable system. Inconsistent data were obtained from the [^3H]-atropine

binding studies. Trends were seen but the variance in the system was very high. Initial studies using the natural transmitter [^3H]-acetylcholine ($^3\text{H-Ach}$) utilizing physostigmine to inhibit acetylcholinesterase were also not productive as Physostigmine activity was lost in a few hours. From these experiments it became apparent that a very stable inhibitor of acetylcholinesterase was required. CYTHION^R, the premium grade of the commercial insecticide MALATHION^R, was employed to inhibit the action of acetylcholinesterase. (The CYTHION^R was furnished by American Cyanamid Co.)

As with most experiments with low SAR's consistency was difficult to obtain. Early experiments were attempted in the parallel-plate irradiation system with very poor results. The system did not provide a uniform field over the entire dialysis system, making dosimetry inconsistent. As a result of these preliminary findings, we redesigned our system to place the irradiated sample in the center of the wave-guide. The system gave consistent and predictable SAR's.

The results of the acetylcholine binding studies are presented in Table 1 and Figure 1. In all experiments there was a change in the binding of the ligand to the receptor when the microwave treated group was compared to controls or shams. When the activity (CPM) inside the dialysis bag was plotted as a function of the activity outside the bag, a least squares linear regression line could be obtained. These data revealed a slope of 1.049 with a correlation coefficient of 0.988 for 26 controls and a slope of 0.848 for 10 irradiated samples with a correlation coefficient of 0.992. Sham irradiated samples (10) had a slope of 1.002 with a correlation coefficient of 0.914. Analysis of covariance reveals that these data are significant at the $P < 0.01$ level.

Repetition of these experiments under exposure conditions of 960 MHz,

16 Hz square wave modulation, showed that for the system described, synergistic binding of Ach is still decreased by exposure to microwave energy at a rate of 1.5 - 2.5 mW/g ($P < 0.01$) (Table 2, Figure 2).

The demonstration of calcium efflux from cat brain and the importance of Ca^{++} to normal nerve function enticed us to look at the binding of ^{45}Ca and AchR.

With the Ca^{++} concentration in the Ringer's dialysate at physiological values, no change was observed in synergistic binding ($P > 0.025$) (Tables 3, 4) (Figs. 3, 4). However, when the concentration of Ca^{++} was reduced to 10% of the normal value, a definite decrease in synergistic binding was observed ($P < 0.01$) (Table 5) (Fig. 5). We believe that the negative results at physiological concentrations are due to masking by the variance inherent in the system. Exposure conditions for these Ca^{++} experiments were 960 MHz CW, SAR = 1.5 - 2.5 mW/g.

III. Conclusions and Discussion

The Hill plot referred to in the preceding text is a graph of $\text{Log } (\theta/1-\theta)$ as a function of $\text{Log } [A]$, where θ is the fraction of sites occupied by Ligand A ($1-\theta$ is then the fraction of sites not bound), and $[A]$ is the concentration of the ligand A. The slope of this plot is an indication of the synergism between binding sites. In order to determine the number of sites there are in a sample, (equivalent to finding the concentration of binding sites), it is necessary to somehow label the sites with an irreversible radioactive tag. The only tag available is $[^3\text{H}]\text{-}\alpha\text{-Bungarotoxin}$. Since Bungarotoxin is fatal in μg doses, we decided that the risk/benefit ratio did not warrant its use. We chose instead to plot activity of bound and free ligand as a function of free ligand activity. This approach worked very well in that the linear regression for each experimental series correlated in excess of 90%.

The series of experiments described strongly indicate that a probable site of interaction is the transmitter receptor system. Although a definite mechanism has not been defined, speculative mechanisms include:

- 1) Conformational changes of membrane proteins.
- 2) Alteration of the relative receptor-calcium distribution
- 3) Hydration changes of calcium, transmitter, and/or receptor
- 4) Alteration of the allowed quantum states of the receptor ("potential well" problem).

Heating, although not eliminated, is probably not a mechanism for the observed effect for the following reasons:

- 1) Identical experiments run at 5°C could not be distinguished from those at 4°C.
- 2) Proposed mechanisms for micro-heating (temperature variation over a very small distance) require an intact membrane or a very "sharp" edge. Neither exists in our system.

It is our opinion that further research in the area of this neural-microwave interaction is needed in order to further determine the mechanism and any useful (diagnostically or therapeutically) or deleterious implications.

The educational benefits from this contract are as follows:

3 M.S. degrees (2 in Bioengineering and 1 in Biology) and 2 Doctorates, both in Electrical Engineering. In addition, one Doctorate in Bioengineering is expected to be completed in this academic year.

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TABLE 1

Ach 960 MHz CW SAR = 1.5-2.5 mW/g

<u>CONTROL</u>		<u>IRRADIATED (SHAM)</u>	
CPM outside	CPM inside	CPM outside	CPM inside
2938.5	3081.8	3108.5	2842.8
2183.9	1987.4	(3111.5)	(2907.7)
3336.6	3253.6	3338.1	2785.7
1258.4	1124.5	(2812.7)	(2528.4)
2944.0	2857.0	3109.5	2936.5
3042.5	2905.0	(2775.0)	(2618.5)
2293.0	2194.5	(2235.5)	(2018.0)
2107.0	2021.6	(2885.3)	(2791.3)
3151.0	3063.0	(3020.0)	(3013.7)
2898.7	3318.3	(2778.7)	(3314.7)
3101.0	3018.0	(3485.7)	(3355.3)
3020.3	2900.7	(3090.0)	(2938.3)
1619.3	1501.5	(1666.3)	(1546.7)
1195.9	1109.2	1525.5	1563.4
1378.8	1291.3	1493.7	1445.2
1792.6	1700.5	1708.9	1605.3
1983.2	1831.0	1683.2	1547.9
1885.2	1664.2	2093.7	1879.3
2029.4	1879.5	1897.9	1730.3
1439.6	1423.6	1230.3	1151.0

FIGURE 1

Ach

960MHz CW

SAR = 1.5-2.5 mW/g

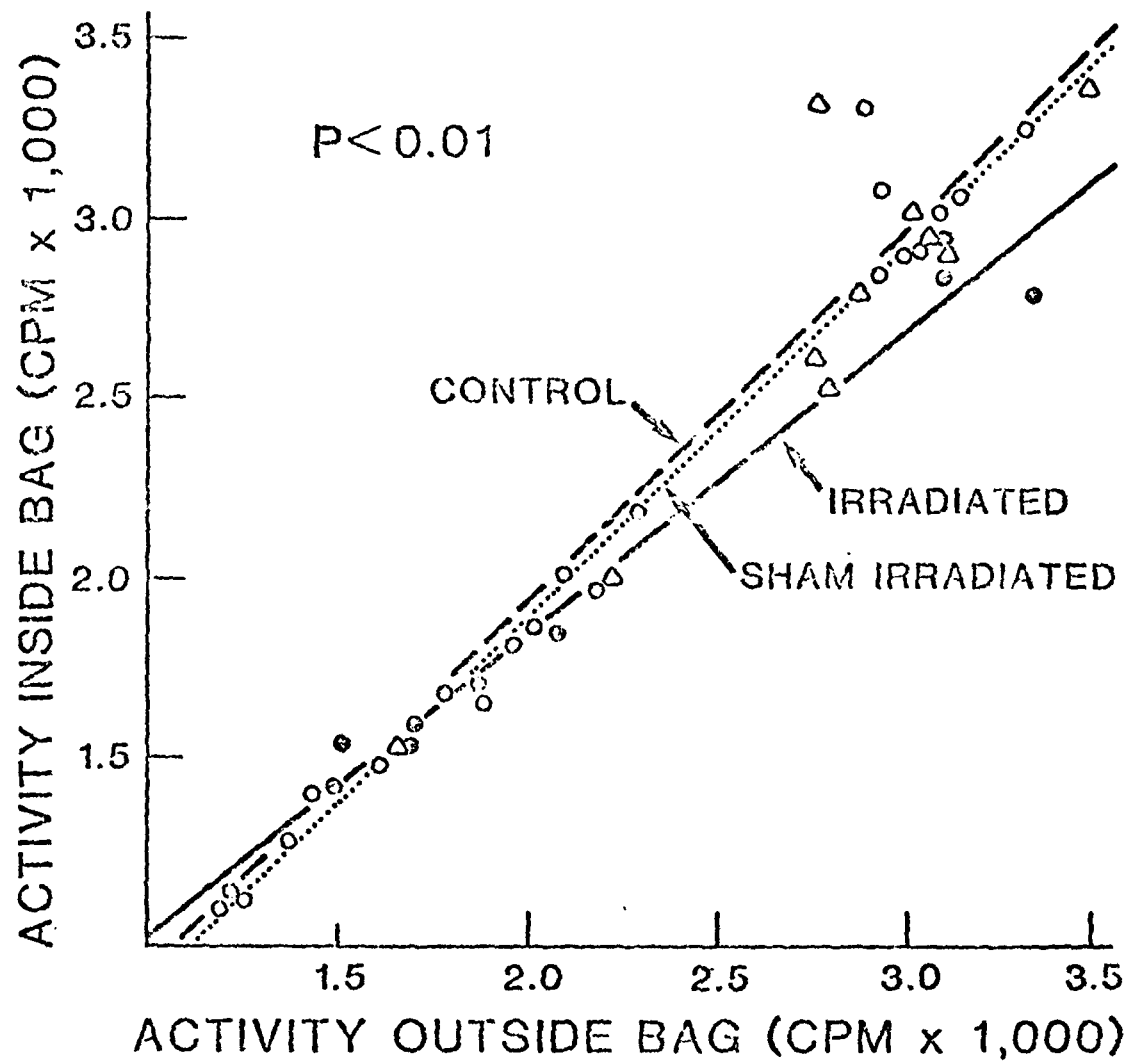


TABLE 2

Ach 960 MHz 50% duty cycle square wave at 16Hz SAR = 1.5-2.5 mW/g

<u>CONTROL</u>		<u>IRRADIATED</u>	
CPM outside	CPM inside	CPM outside	CPM inside
1879.8	1766.0	1467.5	1428.5
1971.7	1902.4	1628.7	1555.8
1689.7	1570.2	1778.7	1641.1
1691.2	1447.0	1584.7	1398.3
1759.9	1661.5	1594.0	1483.9
1604.0	1514.6	1723.1	1608.8
1769.5	1721.7	1705.2	1617.2
1543.8	1482.3	1706.7	1595.1

FIGURE 2

Ach

960MHz 50% duty cycle square wave at 16Hz

SAR=1.5-2.5 mW/g

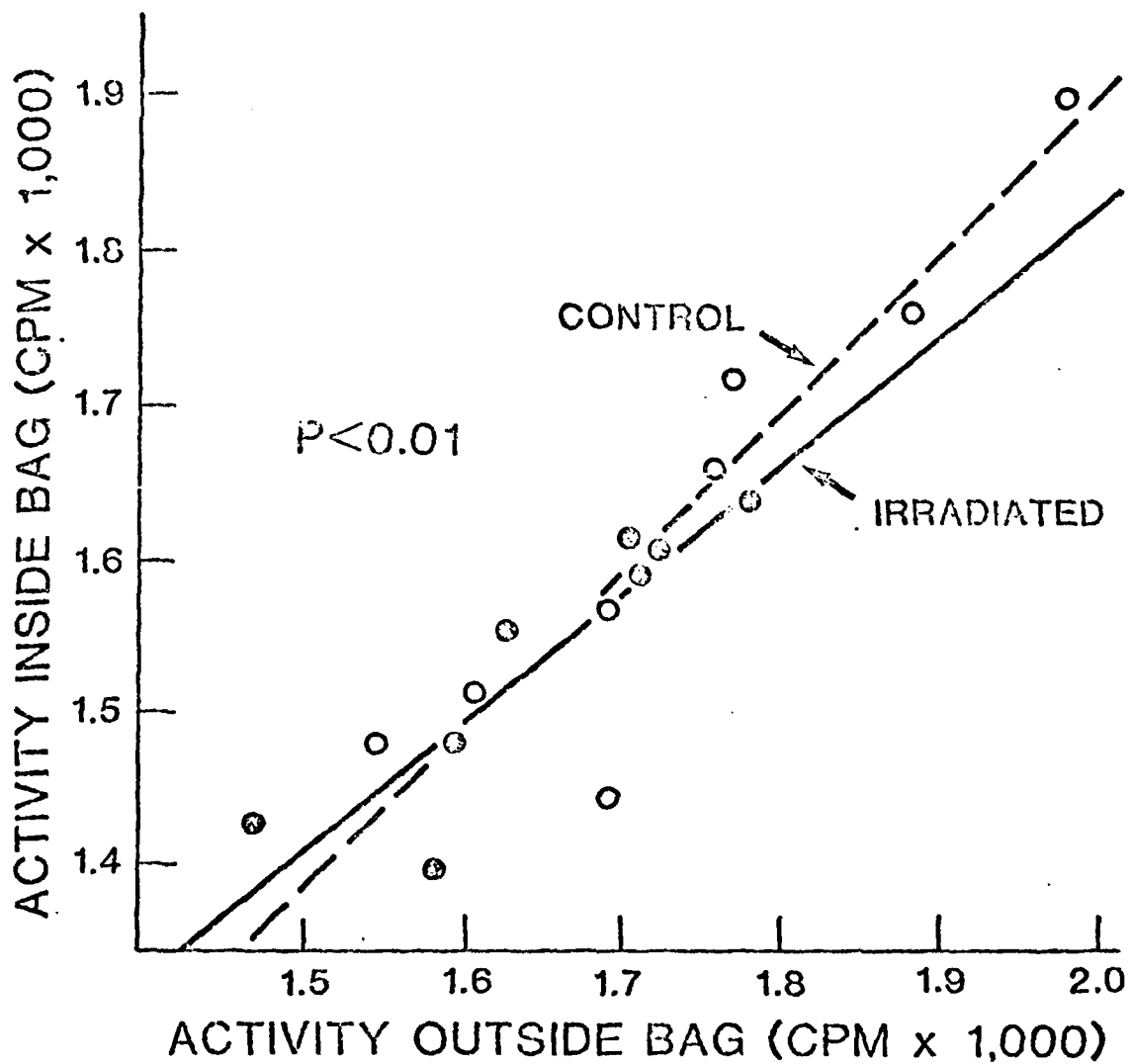


TABLE 3

Calcium x 1 960 MHz CW SAR = 1.5-2.5 mW/g

<u>CONTROL</u>		<u>IRRADIATED</u>	
CPM outside	CPM inside	CPM outside	CPM inside
21114.2	22204.6	22017.4	23116.4
19473.0	20808.0	19010.0	20530.0
17617.0	18107.9	18115.7	18287.4
17059.7	18195.5	17576.4	18673.0
17052.5	17851.1	16681.3	18560.5
15845.3	16887.7	16391.8	17479.0
16353.3	17334.1	16344.1	17068.4

FIGURE 3

Calcium x 1

960MHz CW

SAR=1.5-2.5 mW/g

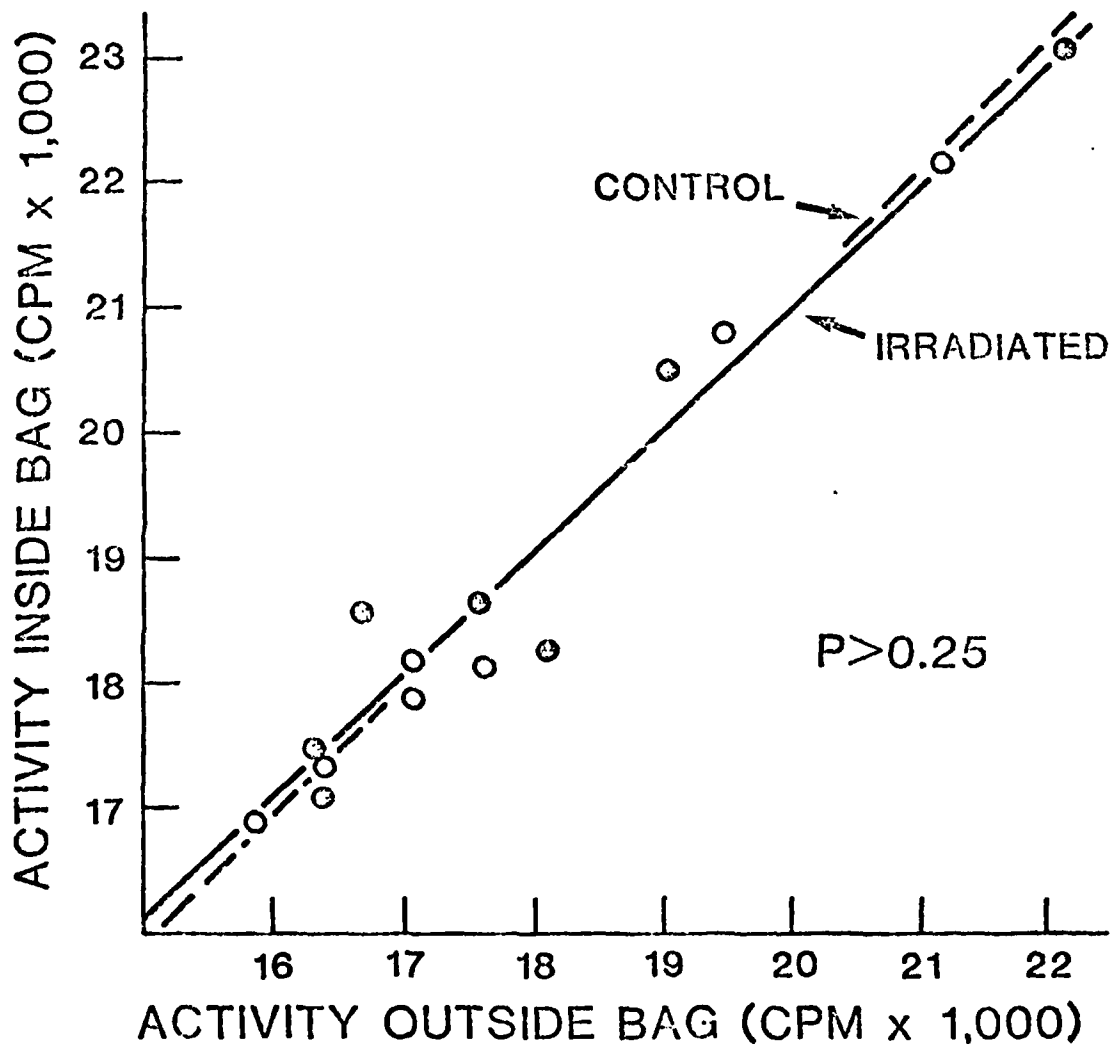


TABLE 4

Calcium x 1 960 MHz 50% duty cycle square wave at 16Hz SAR = 1.5-2.5 mW/g

<u>CONTROL</u>		<u>IRRADIATED</u>	
CPM outside	CPM inside	CPM outside	CPM inside
7125.0	7658.0	7001.0	7453.0
7897.4	8550.6	7666.8	8299.0
7362.4	7820.3	7153.5	7498.9
7443.7	7927.6	7733.5	8202.1
7460.7	8074.9	7664.5	8000.0
7627.4	8053.7	7460.5	8029.4
7771.9	8184.4	8034.0	8679.2
7590.5	7983.5	7648.2	8182.8

FIGURE 4

Calcium x 1

960MHz 50% duty cycle square wave at 16Hz

SAR=1.5-2.5 mW/g

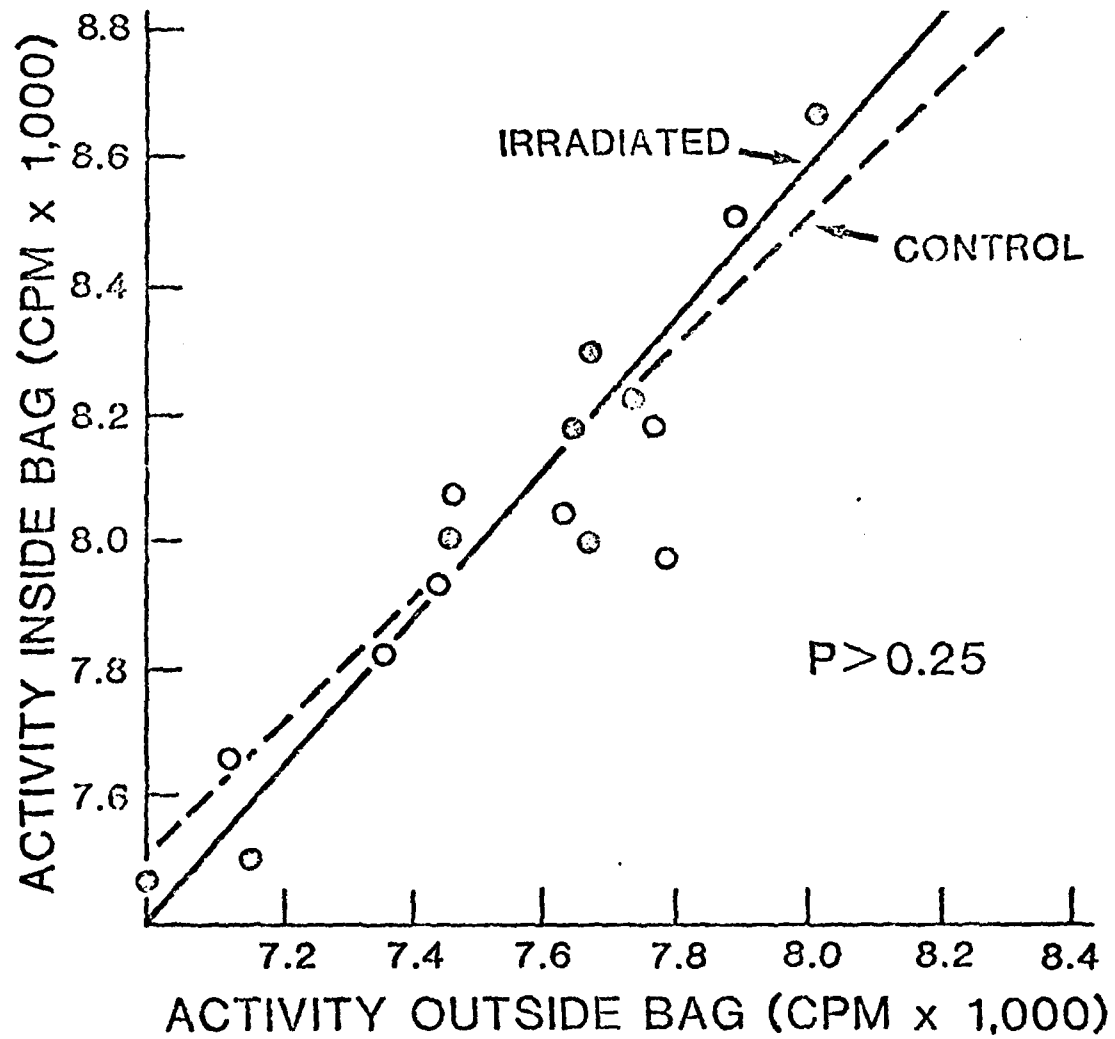


TABLE 5

Calcium x 0.1 960 MHz CW SAR = 1.5-2.5 mW/g

<u>CONTROL</u>		<u>IRRADIATED</u>	
CPM outside	CPM inside	CPM outside	CPM inside
12103.2	14234.6	11443.2	14727.0
12098.6	14214.0	11403.2	14709.6
12014.4	14210.2	11470.2	14762.0
12417.6	14487.5	12488.7	14968.1
12414.9	14570.9	12466.9	14939.9
12620.1	14647.0	12826.4	14730.1
12657.0	14526.9	12824.1	14684.5

FIGURE 5

Calcium x 0.1

960MHz CW

SAR=1.5-2.5 mW/g

