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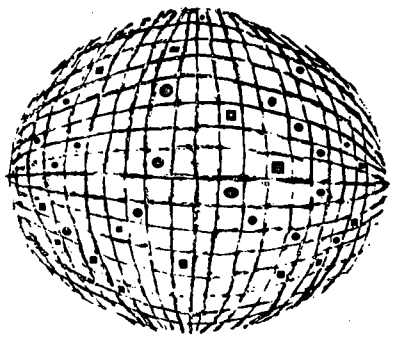

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Effects of 2450MHz microwaves on protein synthesis and on chromosomes in Chinese hamsters

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MICROWAVE RADIATION produces significant effects on a number of biological systems including the nervous system, the eye, the circulatory system, and the reproductive system.[1] These effects include lenticular opacities,[2] testicular degeneration and reduced sperm production,[3] arrested embryonic differentiation without an effect on cellular proliferation,[4] and irregularities among chromosomes during mitosis.[5] Subcellular effects observed after microwave radiation *in vitro* include changes in the electrophoretic pattern and antigenic reactivity of human gamma globulin[6] and reduced activity of alpha amylase.[7]

The present study with male Chinese hamsters investigates the effects of whole body microwave irradiation on the *in vivo* incorporation of ^{14}C-labeled phenylalanine (Phe) into protein of liver and testis, and the chromosomes of mitotic bone marrow cells. Incorporation of labeled amino acid into protein was decreased in both liver and testis. Chromosome stickiness phenomena were increased. Chromatid aberrations were not seen in the first division following microwave exposure.

Materials and methods

Unanesthetized male Chinese hamsters (*Cricetulus griseus*), weighing 25 – 35gm, 90 – 120days old, were used in this study. The exposure source was a microwave oven (2450MHz, 12.25cm wavelength), operated with the door open. Operation of the radiation source was cyclical, three minutes on and one minute off. Hamsters were irradiated individually in a perforated screw-cap plastic cylinder (radius 2cm, length 8cm) placed horizontally 50cm in front of the center of the oven cavity. The cylinder axis was parallel to the plane of the oven aperture so that the side of the animal faced the radiation source. Within narrow limits the animal could move freely. Total duration of microwave exposure of animals for both biochemical and cytogenetic studies was 12min, delivered in a total period of 15min.

Because irradiation was not performed under far-field conditions the average power density could not be determined with available instruments. Thus, two approaches were taken to provide relative characterization of the radiation fields. One employed hamster lethality; the other, calorimetry with spherical water loads.

Hamster lethality. Hamsters were exposed according to the cycle described above for operation of the oven, and examined after each three-minute-on exposure. At the end of the radiation cycle in which death occurred, the animal was rapidly removed and its rectal temperature measured with a thermistor probe encased in a flexible plastic sheath. The log probit plot of cumulative mortality of 30

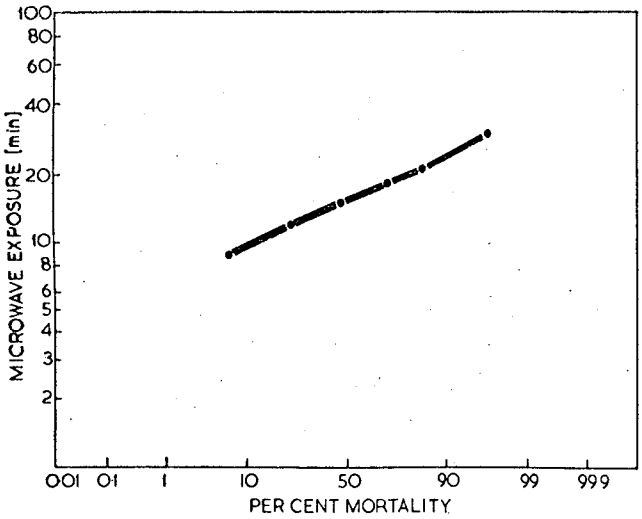

Fig. 1 Cumulative percent mortality of Chinese hamsters during exposure to 2450MHz microwaves. Animals were irradiated in a cyclical 3 minutes on, 1 minute off, pattern until they died in the microwave field.

hamsters is presented in Fig 1. Median time to death was 15.4min. Ninety-five percent confidence limits on the median, according to the method of Litchfield [8] was 13.6 to 17.5 minutes. Rectal temperatures at the end of the irradiation period during which death occurred ranged from 37.9 – 47°C, with a mean of 43.6°C.

Calorimetry For calorimetry, spherical pyrex glass containers having a volume of 200 and 500ml (radius 3.63 and 4.92cm) were filled with distilled water. The containers were insulated in styrofoam blocks. Pyrex beads (8gm in 200ml, 20gm in 500ml) were placed in the phantoms to facilitate mixing. The glass of the containers was assumed to be in thermal equilibrium with the water. The temperature of the water was measured with the thermistor probe before irradiation, and after 5 or 10min periods of microwave exposure. The containers were placed in front of the oven so that their centres corresponded to the position of the centre of the animal irradiation chamber.

The average temperature rise per minute, ± one standard error, was 0.198 ± 0.001°C in the 200ml water load and 0.178 ± 0.002°C in the 500ml water load. Temperature rise was independent of the pre-irradiation water temperature in the range tested, i.e., 10 – 45°C. The average rate of energy absorption per unit of cross-sectional area of the spheres was 71.3×10^{-3} (200ml) and 87.1×10^{-3} (500ml) J sec^{-1} cm^{-2}. These values are useful reference points; however, they cannot be extrapolated to measures of the average power density of the microwave field because the absorption cross sections of these phantoms are not known. Current work with phantoms for which the absorption cross section is known may permit characterization of the power density of the microwave field.[9]

^{14}C-phenylalanine (^{14}C-Phe) incorporation into protein. Chinese hamsters were exposed to microwave radiation. Either at one hour or at 20 minutes after the midpoint of the irradiation or sham irradiation period, experimental and control animals were injected intraperitoneally with 12.5μCi ^{14}C-Phe (12.5μCi per 5.05μg Phe). Twenty minutes after injection, the animals were sacrificed by decapitation and the liver and testes removed for analysis. Tissues from each animal were analyzed individually. Excised tissues were cooled to 0 – 4°C in an ice bath and weighed. The remaining procedures were carried out at this temperature with cold reagents unless otherwise noted. Tissues were homogenized in 1.5 times their wet weight in Hoagland's medium A (reference 10) using a Dounce homogenizer. (Medium A: 0.05 M Tris, 0.025 M KCl, 0.005 M $MgCl_2$, 0.25 M Sucrose, pH 7.5).

Two hundred and fifty μl of each homogenate were pipetted into individual test tubes and precipitated with 4ml of 10% trichloracetic acid (TCA). Tubes were allowed to stand for 15min and then centrifuged at 5°C. The supernatant was decanted and saved. The pellet was resuspended with 4ml 10% TCA, the suspension centrifuged and the supernatant decanted. The pellet was resuspended in 4ml of 10% TCA, put in a 90°C waterbath for 15min, cooled to 0 – 4°C, centrifuged as before and the supernatant decanted.

All of the supernatants were pooled for analysis of ^{14}C– acid soluble phenylalanine (^{14}C–Phe A.S.). The pellet was successively suspended in 4ml of a 3 : 1 alcohol : ether mixture, the suspension centrifuged and the supernatant discarded. Finally the pellets were suspended in 4ml of anhydrous ethyl ether, suspensions centrifuged, and supernatants discarded. The pellets were air-dried and used for the analysis of ^{14}C-Phe incorporated into the acid insoluble fraction (^{14}C-Phe A.I.). The pellets were dissolved in 1ml concentrated formic acid. Aliquots of the dissolved pellets, and ^{14}C-Phe A.S. were pipetted into 10ml of Bray's scintillant.[11] Samples were counted in a liquid scintillation spectrometer and were corrected for variable counting efficiency.

Cytogenetic studies. Hamsters were irradiated either 15min before or after, and 2 hours after, injection of 0.5ml of a 0.1% solution of Colcemid (Ciba) used to arrest dividing cells at metaphase. Injected sham irradiated animals served as controls. Five hours after the injection of the Colcemid the animals were sacrificed with sodium pento-barbital. Bone marrow from the femurs was removed and placed in hypotonic Hank's basal salt solution. Bone marrow cells were concentrated by centrifuge, fixed in 1 : 3 acetic acid-methanol and spread on slides. These were evaluated for mitotic and chromosomal aberrations.

Results and discussion

Incorporation of ^{14}C-labelled phenylalanine into protein. To examine effects of microwave exposure on *in vivo* incorporation of ^{14}C-Phe in liver and testes, it was necessary to determine both acid soluble and acid insoluble ^{14}C-Phe. The animals were injected intraperitoneally after irradiation, so variable amounts of ^{14}C-Phe may have been available for incorporation in the tissues

of interest. The data on incorporation is expressed as relative activity which is the percentage injected dose in ^{14}C-Phe A.I. per percentage injected dose in ^{14}C-Phe A.S.

Incorporation in irradiated hamsters was compared with the incorporation in sham-irradiated controls at 1.33 and 20h post-irradiation. The ^{14}C-Phe was available for incorporation for 20min before sacrifice. Tables 1 & 2 show relative activity ± one standard error in liver and testis 80min after midpoint of exposure. In liver the value for sham controls was 4.73 ± 0.33; for irradiated animals, 0.564 ± 0.334. The difference between sham and irradiated animals is significant ($P < 0.001$). The value for irradiated animals is not significantly different from zero. Thus, at 80min after microwave exposure labeled amino acid incorporation into liver protein was essentially abolished. A similar depression, though not as pronounced, was seen in testis 80min after exposure. The value of relative activity in sham controls was 0.609 ± 0.093; in irradiated animals, 0.331 ± 0.065. Thus incorporation of labeled amino acid into protein was decreased by approximately 45%. The difference between sham controls and irradiated animals is significant ($P < .05$).

Table 3 presents data on amino acid incorporation in liver 20 hours after the midpoint of irradiation or sham exposure. The sham control value for relative activity in liver was 5.69 ± 0.46; for irradiated animals it was 5.37 ± 0.60. Incorporation of amino acid into protein, which was essentially abolished 80min after exposure, was restored to normal values 20h later. It should be noted that the average relative activities of livers of both groups of sham irradiated controls (Tables 1 & 3) are not significantly different from one another. However, the variance of the 20-hour group is greater than the 80-minute group, and the 80-minute average relative

Table 1 Relative activity in liver

$$\text{Rel. act.} = \frac{\%\ I\ D\ ^{14}\text{C-Phe in liver protein}}{\%\ I\ D\ ^{14}\text{C-Phe in liver acid soluble}}$$

	relative activity*	
	sham control	irradiated
1	3.655	0.043
2	4.868	0.831
3	4.469	0.049
4	4.409	3.735
5	4.268	0.024
6	–	1.010
7	4.616	0.073
8	5.095	0.063
9	7.457	0.032
10	4.080	0.331
11	4.361	0.016
X	4.73	0.564
S. E.	0.328	0.334
$P < 0.001$		

*Animals killed 80min post midpoint of exposure

Table 2 Relative activity in testis

$$\text{Rel. act.} = \frac{\%\ I\ D\ ^{14}\text{C-Phe in testicular protein}}{\%\ I\ D\ ^{14}\text{C-Phe in testicular acid soluble}}$$

	relative activity*	
	sham control	irradiated
1	0.481	0.146
2	0.454	0.638
3	0.424	0.455
4	0.475	0.323
5	0.645	0.018
6	–	0.470
7	0.615	0.107
8	0.359	0.400
9	1.368	0.376
10	0.742	0.633
11	0.510	0.080
X	0.609	0.331
S. E.	0.093	0.065
$P < 0.025$		

*Animals killed 80min post midpoint of exposure

Table 3 Relative activity in liver

$$\text{Rel. act.} = \frac{\%\ I\ D\ ^{14}\text{C-Phe in liver protein}}{\%\ I\ D\ ^{14}\text{C-Phe in liver acid soluble}}$$

	relative activity*	
	sham control	irradiated
1	7.218	5.021
2	2.364	5.004
3	4.096	4.286
4	5.252	5.615
5	5.943	4.124
6	7.681	4.720
7	6.362	8.807
8	6.160	–
9	5.032	–
10	4.101	–
11	7.122	–
12	6.934	–
X	5.689	5.368
S. E.	0.455	0.603

*Animals killed 20h post midpoint of exposure

activity appears slightly lower than that of the 20-hour average. This is discussed in the next paragraph.

In contrast to the recovery of amino acid incorporation into liver, it can be seen that testis remained depressed relative to sham controls 20h after exposure to microwaves (Table 4). The value of relative activity for sham control animals was 1.09 ± 0.23; for irradiated animals it was 0.539 ± 0.082. This represents a depression of approximately 50% in amino acid incorporation into protein in irradiated animals. The difference between sham controls and irradiated animals was statistically significant ($P < .05$).

The tendency towards lower incorporation 80min after exposure (relative to 20-hour values) was more marked in testis than in liver. The relative activity for sham irradiated controls 80min after sham exposure was 0.609 ± 0.093; 20h after sham exposure 1.09 ± 0.23. The mean values are significantly different ($P < 0.5$). The factors underlying this difference are not readily apparent. It is possible that hormonal stresses associated with the sham irradiation are involved. However, in each case exposure to microwave radiation did decrease the incorporation of labeled amino acid into testicular protein. This effect was observed as early as 80min after irradiation and maintained at least through the 20th hour after exposure.

THE FINDING of decreased amino acid incorporation into protein in liver and testis after microwave exposure cannot be equated to decreased protein synthesis without qualification. For instance, if microwave radiation results in an increase in the Phe pool in tissue (Phe A.S.), then the specific activity of the amino acid precursor (^{14}C-Phe A.S./Phe A.S.) will be lower in irradiated than in sham control animals. Under this condition the incorporation of equivalent amounts of Phe into protein in both groups of animals will lead to a decreased incorporation of ^{14}C-Phe in the irradiated animals. However, the degree to which incorporation of label was reduced in liver 80min after irradiation, as well as the persistent effect in testis 20h after irradiation, suggest that increase in the size of the precursor amino acid pool may not be the mechanism underlying this effect.

Further experiments will have to be performed to establish the mechanisms underlying altered amino acid incorporation into proteins in tissues of microwave irradiated animals. The mechanism may be mediated through rise in body temperature, or may be an otherwise indirect or direct effect on protein synthesis.

It has been suggested that hyperthermia results in decreased protein synthesis and increased protein catabolism in tranquilized rabbits whose body temperatures were maintained at 41 ± 0.3°C for 24 – 26 hours.[12] However, possible alteration in amino acid pools was not investigated in these studies. Thus, a definitive statement on the mechanism for the altered protein metabolism after microwaves or hyperthermia is not possible at this time.

In the hyperthermia experiments in rabbits, rectal temperatures of 41.2°C were maintained for 24 – 26 hours.[12] Hamster exposures to microwaves were accomplished in the present study within 0.25h, and resulted in a mean rectal temperature of 39.7°C at the end of irradiation. The mean increase in rectal temperature of the irradiated hamsters was 4.2 ± 0.6°C above that of the sham irradiated controls.

Table 4 Relative activity in testis

$$\text{Rel. act.} = \frac{\%\ \text{I D}\ ^{14}\text{C-Phe in testicular protein}}{\%\ \text{I D}\ ^{14}\text{C-Phe in testicular acid soluble}}$$

	relative activity*	
	sham control	irradiated
1	0.892	0.332
2	3.358	0.335
3	0.752	0.478
4	0.336	0.708
5	1.175	0.425
6	1.684	0.562
7	1.064	0.932
8	0.599	–
9	0.570	–
10	1.243	–
11	0.677	–
12	0.794	–
X	5.689	5.368
S. E.	0.455	0.603

*Animals killed 20h post midpoint of exposure

Cytogenetic studies. To confirm the effects of microwave irradiation on chromosomes previously observed with plant material,[5] Colcemid-metaphase cells were examined in direct preparations of bone marrow from irradiated hamsters. Table 5 is a summary of aberrations. Irradiation did not increase the incidence of aberrations in the animals. The interval between irradiation and fixation permits examination of cells that were irradiated either during or after DNA synthesis. Therefore only chromatid aberrations would be expected.

A substantial proportion of Colcemid-metaphase cells from some irradiated animals showed gross chromosomal anomalies which prevented cytogenetic analysis. The chromosomal appearance was similar to the 'early physiological' or 'stickiness' effects observed in dividing cells after X-irradiation.[13] Because of the complexity in describing and understanding stickiness effects after X-rays,[14] the stickiness in cells exposed to microwaves can be described only qualitatively at the present time. Stickiness may be seen either between parts of one chromosome (chromatid stickiness) or between two or

Table 5 Chromatid aberrations and aneuploidy in bone marrow cells of microwave-irradiated Chinese hamsters

treatment schedule*	mitotic index	number of cells scored	chromosome aberrations	chromatid aberrations		cells with aneuploidy (2N = 22)	
				gaps	breaks	< 2nN	> 2N
MHz —0.25→ C —4.75→ Fix	11.0	50	0	0	0	0	2
C —0.25→ MHz —4.75→ Fix	6.1	50	0	2	0	2	0
C —2→ MHz —3→ Fix	5.0	49	0	0	0	0	0
C —5→ Fix	8.5	50	0	1	0	4	0

*C is Colcemid injection, MHz represents microwave irradiation, and numbers the intervals (in hours) between treatments.

more chromosomes (chromosome stickiness). The stickiness phenomena observed in metaphase cells are presented in Table 6. More chromatid stickiness was observed in cells from hamsters injected before microwave exposure than in cells from animals injected after irradiation. Substantial chromosome stickiness was observed only in the experiment in which cells were blocked in metaphase for two hours before irradiation.

Although chromosomal material is affected in stickiness phenomena, the effect can be considered as an irregularity in the mitotic process. Separation of adhering chromosomes is inhibited and cytokinesis may be incomplete, so that the cells may die.[1 3] In preparations from animals irradiated two hours after injection, we found evidence that cells were escaping from the Colcemid block and attempting to complete cytokinesis. Chromatin bridges connected daughter nuclei in about 10 – 15% of the dividing cell population. Such effects have been previously described.[5] If such cells survive to a second division after microwave irradiation, they may contain chromosome structural aberrations. Experiments to test this possibility are in progress.

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Table 6 Stickiness in colcemid-metaphase bone marrow cells of Chinese hamsters

treatment schedule*	number of cells scored	% of cells with stickiness	type of stickiness effects: % of cells	
			chromatid	chromosome
MHz —0.25→ C —4.75→ Fix	120	16.7	15.0	1.7
C —0.25→ MHz —4.75→ Fix	120	48.3	45.8	4.2
C —2→ MHz —3→ Fix	90	77.8	67.8	53.3
C —5→ Fix	120	23.3	19.2	5.0

*Symbols as in Table 5.

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