From "Radiation Bio-Effects" Glaser
89
by Mills, W.A. Report
Hodge, D.M. (ed) (1965) p. 89

PHYSIOLOGY AND BIOPHYSICS LABORATORY

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In evaluating the hazards of ionizing radiation, a number of approaches have been taken by the Physiology and Biophysics Laboratory. One approach is based on the hypothesis that ionizing radiation produces its most significant long-term effects through alterations in DNA, the structural integrity of which is essential to the maintenance of all normal cellular activity and thus the functional viability of any living organism. Any radiation-induced alteration, produced directly or indirectly in the normal base sequence of DNA, constitutes a mutation. Such mutations are generally considered to be deleterious, and even if not, constitute damage produced by ionizing radiation. Mutations may be expressed in such obvious ways as birth abnormalities, decreased lifespan, tumor induction, or cataract formation. Mutations may also find more subtle expression as decreased functional capacity of specific organ systems, alterations in immunological competency that may decrease resistance to bacterial or viral infection, or alterations in the synthesis of proteins whose synthesis is directly dependent on the integrity of DNA, Messenger RNA, and the translation process.

Other studies have examined the effects caused by radiation delivered in utero. These studies have dealt with protein synthesis in general, specific enzyme systems, possible effects on immunological processes, and effects on components of the peripheral blood.

This past year, the Physiology and Biophysics Laboratory integrated studies related to the effects of microwave radiation into many of its projects. The first study, summarized in the following section, relates to the biological effects of microwaves. Additional emphasis on nonionizing radiation studies and low-energy X-ray studies are planned for next year.

A. EFFECTS OF MICROWAVE RADIATION ON CHINESE HAMSTERS

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Increased concern has developed recently over the possible biological hazard of electromagnetic radiation in the microwave frequencies. The scientific literature indicates that microwave radiation produces significant effects on a number of biological systems, including the nervous system, the eye, the circulatory system, and the reproductive system. At the cellular level, observations have shown that microwaves (1) produce lenticular opacities, (2) can arrest the process of differentiation in embryos without necessarily arresting cellular proliferation, (3) can produce testicular damage (even when such irradiation is effected below temperatures necessary to cause injury with infrared exposures), and (4) can produce irregularities in the mitotic process.

The present study, using Chinese hamsters, investigates the biological effects of microwaves on the mitotic process of bone marrow cells, and on the in vivo incorporation of ^{14}C-labeled phenylalanine into protein of liver and testis. The cytogenetic studies provided suggestive evidence for increased chromosomal stickiness after microwave radiation. However, no chromosomal or chromatid aberrations were observed. Studies on protein synthesis indicate that microwave radiation causes a marked decrease in the in vivo incorporation of labeled amino acid into protein both in liver and testis.

Unanesthetized Chinese hamsters weighing 25-35 grams were used in this study. Experimental animals were exposed to radiation produced by a microwave oven operating with the door open at a frequency of 2450 MHz (12.25 cm wavelength).

The irradiation was not performed under unequivocal far field conditions and the average power density of the microwave field at the point of exposure could not be effectively determined with instruments available for the measurement of average power density. Thus two approaches were employed for the relative characterization of the field; one, biological, employed lethality as an endpoint, the other, physical, employed calorimetry techniques using spherical water loads.

For the lethality studies, animals were irradiated for 3 minutes followed by no radiation for 1 minute until the time of expiration. The duration of microwave exposure which resulted in deaths of the hamsters ranged from 9 to 36 minutes. Rectal body temperatures in hamsters at the end of the irradiation period during which mortality occurred ranged from 37.9° C to 47° C with a mean of 43.6° C.

Data were obtained on the absorption of microwave energy in spherical phantoms filled with distilled water. The phantoms had volumes of 500 ml and 200 ml and radii of 4.92 and 3.63 cm respectively. The pyrex containers used for the phantoms were assumed to be in thermal equilibrium with the water mass of the phantom. The calorimetric technique showed an average temperature rise per minute in the 500 ml water phantom (4.92 cm radius) to be 0.178 ± 0.002° C per minute; in the similar 200 ml (3.63 cm radius) phantom, 0.198 ± 0.001° C per minute. The average rates of energy absorption, expressed per unit of cross sectional area of the phantom were 87.1×10^{-3} (500 ml) and 71.3×10^{-3} (200 ml) joules sec^{-1} cm^{-2}. These values are useful as a point of reference, but cannot readily be extrapolated to measures of the average power density of the microwave field since the absorption cross section for these phantoms is not known.

1. Chromosomal Effects

Cytogenetic studies were performed in animals exposed to the microwave field for a total of 12 minutes. Two chromatid breaks were

observed in a total of 50 analyzable cells. In the control animals, one chromatid break was observed in 100 cells that were examined. Five of the 100 control cells were aneuploid; four had a small acrocentric chromosome missing, the other cell contained two extra submetacentric chromosomes. Aneuploidy was not observed in the irradiated cells.

A substantial proportion of metaphase cells from irradiated animals showed gross chromosomal anomalies, similar in appearance to the "early physiological" or "stickiness" effects observed in dividing cells after X irradiation. Because of the complexity in stickiness effects after X radiation, it may be of questionable value to attempt, at the present, a quantitation of stickiness in cells exposed to microwaves. Stickiness phenomena may be observed either between parts of one chromosome (chromatid stickiness) or between two or more chromosomes (chromosome stickiness). In the present study only metaphase cells were evaluated for chromosome stickiness and chromosomal aberrations. In the controls up to 20 percent of the metaphase spreads contained what appeared to be chromatid stickiness, but no chromosome stickiness. In the cells from animals irradiated two hours after Colcemid injection, over half of the metaphases showed chromatid stickiness. Chromosome stickiness varied from preparation to preparation in a range from about 25 percent to 67 percent of the metaphase cells. The cells from animals injected after irradiation were essentially similar to control cells. Almost 50 percent of cells from animals injected just prior to irradiation showed chromatid stickiness, but chromosome stickiness was not observed.

In preparations obtained from animals irradiated two hours after injection, we found evidence that cells were escaping from the Colcemid block, and attempting to complete mitosis and cytokinesis. Detectable cytokinesing cells were those in which stickiness interfered with daughter chromosome separation. Long chromosomal bridges connected the daughter nuclei in about 10-15 percent of the dividing cell population. Similar bridging was observed in cells from animals exposed immediately after injection (4-5 percent), as well as cells from animals injected after exposure or control cells (less than 1 percent in either case). It is apparent that stickiness may interfere with the completion of cytokinesis. Since chromosomal material is in the bridge between the daughter nuclei, it is possible that chromosome rearrangements may be detected in subsequent cell divisions. Experiments to examine this possibility are in progress.

2. Effects on Amino Acid Incorporation

The in vivo incorporation of ^{14}C-labeled phenylalanine (^{14}C Phe) into liver and testis protein was examined in Chinese hamsters after microwave irradiation. Since animals were injected with ^{14}C Phe intraperitoneally, variable amounts of the labeled amino acid may have been available within the tissues of interest for incorporation into protein. Thus both acid soluble ^{14}C Phe in liver and testis as well as acid insoluble ^{14}C Phe were determined. The data on incorporation are expressed

as relative activity -- % injected dose ^{14}C Phe in tissue protein/% injected dose ^{14}C Phe in tissue acid soluble fraction.

Animals received a total microwave exposure of 12 minutes as previously described. Controls were sham irradiated. Rectal temperature was obtained with a thermistor probe on completion of exposure or sham exposure. The mean increase in rectal temperature as a result of microwave irradiation was $4.2 \pm 0.6^{\circ}$ C.

Table 19 presents the average relative activity in liver and testis at 80 minutes and 20 hours after the midpoint of exposure. Since liver value for irradiated animals is not significantly different from zero at 80 minutes, it appears that labeled amino acid incorporation into liver protein was essentially abolished by 80 minutes after microwave exposure. A similar effect, though not as pronounced was seen in testis 80 minutes after exposure. The value of relative activity in testis of irradiated vs. control animals was decreased by approximately 45 percent. The difference between sham controls and irradiated animals was significant ($P < .05$).

TABLE 19. RELATIVE ACTIVITY[a] OF AMINO ACID INCORPORATION AT VARIOUS TIMES AFTER MICROWAVE IRRADIATION

Time After Irradiation	Liver		Testis	
	Irradiated	Control	Irradiated	Control
80 minutes[b]	0.564 ± 0.334	4.73 ± 0.328	0.331 ± 0.065	0.609 ± 0.093
20 hours[c]	5.368 ± 0.603	5.689 ± 0.455	0.539 ± 0.082	1.092 ± 0.231

a. $\frac{\text{\% injected dose } ^{14}C \text{ Phe in tissue acid insoluble fraction}}{\text{\% injected dose } ^{14}C \text{ Phe in tissue acid soluble fraction}}$

b. Average of 11 animals.

c. Average of 12 animals.

Table 19 also shows that incorporation of amino acid into protein, which was essentially abolished 80 minutes after exposure was restored to normal values 20 hours later. The relative activity of sham irradiated controls was not significantly different in either the liver or the testis group although a tendency toward lower values is suggested 80 minutes after sham irradiation relative to the 20 hour controls.

In contrast to the recovery of amino acid incorporation into liver, the value for testis remained depressed relative to sham controls 20 hours after exposure to microwaves. The factors underlying this difference are not readily apparent, but hormonal stresses associated with the sham irradiation may be involved. It is apparent, however, that in all cases exposure to microwave radiation did decrease the incorporation of labeled amino acid into testicular protein. This effect was apparent

as early as 80 minutes after irradiation and lasted 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 causes an increase in the amino acid pool in tissue, then the specific activity of the amino acid precursor (labelled Phe/total Phe) will be lower in irradiated relative to sham control animals. Under this condition the incorporation of equivalent amounts of phenylalanine in both groups of animals will lead to a decreased incorporation of ^{14}C Phe in the irradiated animals. The degree to which incorporation of label was reduced in liver 80 minutes after irradiation, as well as the persistent effect in testis 20 hours after irradiation, however, 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 either thermal or nonthermal. If thermal, however, it is probably an indirect rather than a direct effect, since temperature rises such as those observed in this study are not normally associated with protein denaturation. Thus, a molecular effect is implied which may be mediated by hormonal or other factors.