Effects of Low Intensity Microwave Radiation on Mammalian Serum Proteins

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EFFECTS OF LOW INTENSITY MICROWAVE RADIATION ON MAMMALIAN SERUM PROTEINS

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SUMMARY

The effect of 2.45 GHz continuous wave and pulsed wave radiation on serum proteins, blood chemistry, and tissue pathology have been investigated during the past year. Changes in serum proteins were detected by the use of acrylamide gel electrophoresis which appear to be related to microwave exposure. Microwave irradiation at an intensity of 25 mW/cm^2 for 2 hours resulted in significant increases in blood urea nitrogen (BUN), glucose, uric acid, cholesterol, and SGOT. At an intensity of 10 mW/cm^2 glucose was again increased but not to as great an extent as at 25 mW/cm^2. BUN was increased following CW exposure and non-statistically significant increases were also detected in serum cholesterol and uric acid. Inorganic phosphate was decreased but not to statistically significant levels. A dose response relationship was found for the effect of 2.45 GHz microwave exposure on pentobarbital-induced sleeping times in the Dutch rabbit and the maximally effective intensity was 15 mW/cm^2 which produced an 80% reduction in sleeping times. Tissue pathology and histopathology of rabbits irradiated at 25 mW/cm^2 suggested that the primary effect of such exposure was nephrosis. Studies were also undertaken of the effects of 2.45 GHz microwave on the Chinese hamster retina and on solutions of serum proteins but these studies are incomplete at this time.

FORWARD

In conducting the research described in this report, the investigator (s) adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

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SUMMARY REPORT

Effects of Low Intensity Microwave Radiation on Mammalian Serum Proteins

The effects of 2.45 GHz microwave radiation on serum proteins, blood chemistry, sleeping times and radiation-induced tissue pathology have been under investigation during the past year using the Dutch rabbit as the experimental subject. Exposures were at average power densities of 25, 10, or 5 mW/cm^2, either as continuous or pulsed wave radiation. A limited number of exposures were performed at 5 mW/cm^2 and before the end of the contract year (July 1, 1973) additional data will be obtained at this intensity which will be used to determine dose response relationships for the indicated dependent variables. The methods of analysis used in this study include agar and acrylamide gel electrophoresis, standard methods of blood biochemical analysis, gross and histopathology, spectrofluorimetry, 90° light scattering, and ultra violet absorption spectroscopy.

The results of these studies are currently being analysed and it is therefore not possible, in most cases, to arrive at final conclusions at this time. This report will therefore be devoted to a summary of the findings to date with respect to the various aspects of the study. A detailed report, which is presently being prepared, will be submitted at the end of the first contract year.

ELECTROPHORESIS OF SERUM PROTEINS

Microwave-induced alterations in rabbit serum proteins have been studied by electrophoresis. Preliminary studies were performed by the

technique of agar gel electrophoresis but due to poor reproducibility and resolution this technique was abandoned in favor of acrylamide gel electrophoresis. Apparatus for flat preparation acrylamide gel electrophoresis was designed and constructed for this study thus permitting the electrophoretic separation and densitometric analysis of 10 ul samples of sera obtained from the ear veins of microwave exposed, sham irradiated, or non-exposed, non-sham irradiated rabbits. In this way it was possible to study the effects on serum proteins of microwave exposure, animal constraint during exposure, and the effect of transporting the animals from Richmond, Virginia to the Forest Glen Microwave Facility (i.e. the "trip" effect). A schematic representation of the characteristic densitometric tracing obtained from the acrylamide gel electrophoresis of rabbit serum proteins (i.e. the pherogram) is shown in Figure 1 in which the components or zones of the serum proteins are indicated. Due to the high concentration of albumin with respect to the other serum proteins, the density of the albumin zone was too great to be measured densitometrically and it was thus necessary to resort to an alternate method of analysis for this component.

The sampling procedure used for the study of serum proteins involved obtaining a baseline sample for each animal 1 - 2 weeks prior to exposure followed by an immediate pre-exposure sample, immediate post-exposure (or post-sham) sample and then samples taken at 3 or 4 days post exposure and at 1, 2, 3, and 4 weeks post exposure. In this way it was possible to obtain intra- and inter- animal treatment effects. Table 1 is a summary of the duration of exposure and the microwave intensity (2.45 GHz) adminis-

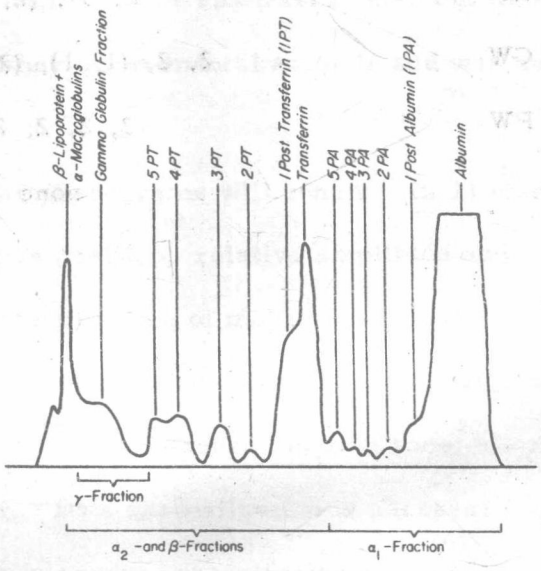

FIGURE 1

Densitometric tracing of serum protein zones as obtained from flat-preparation acrylamide gel electrophoresis of rabbit sera.

Table I

SUMMARY OF MICROWAVE EXPOSURES FOR ELECTROPHORESIS STUDY

Treatment	Duration of Exposure (hrs)
Sham irradiation	2, 3*, (2, 2)
25 mW/cm^2, CW	2, 2, (1, 1), (2.5, 1), 3*, 1, (2, 2)
25 mW/cm^2, PW	3, 2, 2, 2
control	none

*Animal sham irradiated for 3 hrs and subsequently exposed for 3 hrs to 25 mW/cm^2, CW microwaves

tered to the animals whose serum proteins were assayed by acrylamide gel electrophoresis. In each case the indicated duration denotes the exposure of a single animal for that length of time and the times in brackets refer to the duration of each exposure for an animal exposed more than once. Fourteen animals were included in this phase of the study. The animal denoted as control was not irradiated or sham irradiated but was transported back and forth with the other experimental animals and was used to study the trip effect.

Analysis of the pherograms will consist of: 1) visual inspection for alterations in relative position, relative amplitude and shape of the serum protein zones; 2) determination of mobilities relative to albumin and to transferrin peak of pre-exposure serum sample; 4) analysis of peak heights normalized to the width of pre-exposure albumin zone; 5) area normalized to baseline pherogram. Data normalization is necessary to correct for sample to sample variation in serum concentration.

Table II is a summary of the changes noted in the irradiated and sham irradiated serum protein samples. The most significant changes were noted in the haptoglobin (post transferrin 3) zone. In most cases the amplitude and/or position of this peak were altered following irradiation. The general trend appeared to be a transient decrease in amplitude following irradiation followed by an increase to greater than pre-exposure values at 1 - 3 weeks post irradiation. Transient changes of this type were also noted in the glycoprotein (post transferrin 2) zone. The significance of these changes is, however, in doubt since similar changes were found in the serum pro-

Table II

ELECTROPHORETIC SERUM PROTEIN CHANGES

Animal Number and Treatment	Serum Protein Alterations
No. 20, sham irradiated on 10/20; 25 mW/cm^2 11/15/72 3 hours, (cw)	Normal patterns seen pre and post sham and at 2 days post sham. Large haptoglobin (post transferrin 3) peak 1 week post sham exposure. Post transferrin 2 peak absent. Two week data the same. At 3 weeks the post transferrin 3 peak is still elevated but post transferrin 2 peak reappears. On 11/15 (pre exposure) the picture remains the same. Immediately post exposure post transferrin 2 and 3 are decreased. The animal died 104 minutes post exposure. Post transferrin 3 component changes may be related to cause of death. No changes were noted in the amplitude of the α_1 region with respect to the post transferrin region following sham irradiation. No data available following irradiation due to the death of the animal.
No. 14, 2 hour exposure at 25 mW/cm^2 11/14/72 (cw)	Mobility of post transferrin 2 peak decreased immediately post exposure and the amplitude is increased. Post transferrin 2 absent at 3 days post, post transferrin 3 still elevated and transferrin is decreased. Pherogram appears about normal at 7 days post but no post transferrin 2 peak is noted. No change noted in α_1 region baseline.
No. 14, 2 hour exposure at 25 mW/cm^2 10/17/72 (cw)	Small post transferrin 2 peak disappears immediately post exposure. Same at 3 and 8 days. At 2 weeks post, post transferrin 2 reappears but is small as is post transferrin 3. A new peak appears between albumin and β fraction. This new peak remains at 3 weeks post exposure at which time the post transferrin 2 peak is not seen and the post transferrin 3 peak is large. No change noted in the α_1 region baseline.
No. 18, 25 mW/cm^2 2.5 hour irradiated 10/19/72 (cw)	Post transferrin 2 peak disappears and post transferrin 3 is decreased in amplitude immediately post exposure. Post transferrin 2 reappears and looks normal (i.e. pre exposure value) and post transferrin 3 is still low at 3 days post. At 8 days post small post transferrin 2 peak but post transferrin 3 reappears at about pre exposure amplitude. Post transferrin 2 is absent in 2 week sample and post transferrin 3 increased over pre exposure value. At 3 weeks post the post transferrin 2 fraction is still absent, post transferrin 3 is larger than pre exposure value and another component is seen in small magnitude in the α_1 region. An increase in the α_1 region amplitude relative to post transferrin is seen at 3 days post exposure. The amplitude returns to normal by day 8 post exposure.

Table II (continued)

Animal Number and Treatment	Serum Protein Alterations
No. 18, 25 mW/cm^2 1 hour irradiated 11/13/72 (cw)	In pre exposure sample the post transferrin 2 component has reappeared and the post transferrin 3 component is still of somewhat greater amplitude than the pre exposure value of 10/19/72. In the post exposure sample the post transferrin 2 component is missing. At 5 days post exposure there is a decrease in the post transferrin 3 fraction and some slight hint of a post transferrin 2 fraction although this is difficult to determine with any certainty. A slight increase in the baseline in the α_1 region is seen in the 5 day post exposure sample. This change is not nearly as great as that at 3 day post 2 1/2 hours of exposure noted above.
No. G 215, 25 mW/cm^2 2 hour irradiation 12/4/73 (cw)	Only difference in pre and post exposure pherogram is an increase in the post transferrin 3 fraction and possibly some alteration in leading edge of albumin fraction. No other data available.
No. G 335, 25 mW/cm^2 2 hours irradiated 12/4/72 (cw)	Again there is an alteration in the leading edge of the albumin peak with no other noticeable changes. No other data available.
No. 11, 25 mW/cm^2 1 hour irradiated 10/16/72 (cw)	The immediate post exposure sample shows a significant reduction in the post transferrin 3 peak and the appearance of a small peak not seen in the pre exposure sample. At 3 days post exposure there is a definite post transferrin 2 peak and a post transferrin 3 peak of decreased amplitude. At 8 days post exposure the amplitude of the post transferrin 3 has increased somewhat. The pherogram appears to have returned to the pre exposure baseline value run on 10/9/73 before the trip. A significant increase is noted in the α_1 baseline in the 3 day post exposure sample with a return to normal by 8 days post exposure.
No. 12, control, no sham, no irradiation; trip to WRAIR on 10/11/72 11/13/72	Some alterations noted in pre and post trip samples for trip No. 1, i.e. post transferrin 3 is decreased after the trip. On 10/19/72, 8 days post trip there is a slight decrease in the post transferrin 1 component or a change in mobility. The post transferrin 3 fraction has increased significantly w.r.t. post transferrin 2 and 4. On 11/6/72 the sample looks quite similar to the 10/9/72 baseline sample except for the post transferrin 4 region which is less peaked. The 11/13/72 sample taken after the second trip shows little variation except for the absence of a small peak in the pre β fraction which may have been decreased in mobility and merged with the β peak. On 11/18/72, 5 days post trip No. 2 the post transferrin 2 fraction has disappeared and a large post trans-

Table II (continued)

Animal Number and Treatment	Serum Protein Alterations
No. 12 (continued)	ferrin 3 fraction is seen. On 11/21/72, the post transferrin 2 and 3 fractions have both reappeared but at somewhat smaller magnitude than the baseline values of 10/9/72. No alterations are noted in the α_1 region baseline values at any time.
No. 10, 25 mW/cm^2 for 1 hour on 10/16/72 (cw)	No changes are noted in the post exposure samples or on those for day 3 or 8 or 2 or 3 weeks except for a change in the amplitude and shape of the post transferrin 4 fraction between 8 days and 2 weeks. A significant increase in the baseline is seen in the α_1 region in the 3 day post exposure sample. The baseline returns to normal by 8 days post exposure.
No. 10, 25 mW/cm^2 for 1 hour on 11/13/72 (cw)	The post transferrin 3 amplitude is decreased in the immediate post exposure sample and the post transferrin 2 is shifted toward the post transferrin 2 fraction. At 4 days post exposure the post transferrin 3 peak is significantly increased but by 8 days post exposure it is diminished to a level below the pre-exposure value. A slight increase in the baseline in the α_1 region is seen in the 4 day post exposure sample. This region returns to normal by 8 days post exposure.
No. R 34, 25 mW/cm^2 3 hours 1/24/73 (pulsed wave)	The immediate pre-exposure (1/24/73) shows one large post transferrin 3 peak as compared to two smaller post transferrin 2 and 3 peaks in the baseline sample from 1/2/73. This may be a trip effect. No noticeable change in 1 day post exposure sample. At 3 day post exposure there are very small post transferrin 2 and 3 peaks and at 7 days post the post transferrin 2 peak has shifted to the right into the β peak and the post transferrin 3 fraction is greatly diminished in size and is spread out. No significant alterations were noted in the α_1 region baseline.
No. R 33, 2 hours 25 mW/cm^2 on 1/23/73, (pulsed wave)	The post transferrin 2 fraction is small in the baseline sample 12/27/72 and the post transferrin 3 peak appears to be merged with the post transferrin 4 region. In the immediate pre-exposure sample on 1/23/73, the post transferrin 3 peak is large, there is no discernable post transferrin 2 peak and the peak is significantly smaller than the baseline sample. (this again may be a trip effect). The immediate post exposure sample

Table II (continued)

Animal Number and Treatment	Serum Protein Alterations
No. R 33 (continued)	is similar to the pre exposure except that the post transferrin 3 peak has decreased the post transferrin 2 peak is very small. At 1 day post exposure post transferrin 2 has increased slightly; post transferrin 3 had decreased and the post transferrin 4 fraction, component 2, has increased in amplitude. At 4 days post exposure post transferrin 2 has increased and is equal to post transferrin 3, but both are small relative to post transferrin 4 which has increased significantly in size. At 7 days post exposure post transferrin 3 has increased somewhat and component 2 of the post transferrin 4 region has further increased. An increase in the baseline in the α_1 region is noted at 1 and 4 days post exposure with a return to normal by day 7 post exposure.
No. R.32, 25 mW/cm^2 for 3 hour on 1/23/73, (pulsed wave)	Some changes in the relative amplitudes of the two components of the post transferrin 4 fraction are seen in the baseline sample of 12/27/72 and the immediate pre exposure sample of 1/23/73. The post transferrin 2 component is absent in the baseline sample but present in the pre exposure sample. Immediately after irradiation the post transferrin 2 peak disappears. At 1 day post exposure the post transferrin 3 peak is absent but post transferrin 2 reappears; at 4 days post exposure component 2 of the post transferrin 4 peak has increased in size; a large post transferrin 3 peak is seen but post transferrin 2 is not seen. At 7 days post exposure a small post transferrin 2 and a diminished post transferrin 3 and a somewhat broader but normal amplitude double peaked post transferrin 4 region is noted. An increase in the amplitude of the entire α_1 fraction relative to the post transferrin region appears at day 1 post exposure but returns to normal by day 7.
R 31, 25 mW/cm^2 2 hours on 1/22/73, (pulsed wave)	Baseline sample (12/7/72) and immediate pre exposure sample (1/22/73) are similar except that the post transferrin 4 region migrated as two distinct peaks in the pre exposure sample as contrasted to one two component peak in the baseline sample. The post transferrin 2 peak is not seen in the post exposure sample, the other peaks remained about the same. At 1 day post exposure the post transferrin 2 and 3 peaks are larger relative to post transferrin 4. At 3 days post exposure post transferrin 2 is absent and post