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AD # A004-024

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TITLE

→ EFFECTS OF MICROWAVE IRRADIATION ON EMBRYONIC BRAIN TISSUE ←

TYPE OF REPORT (TECHNICAL, FINAL, ETC.)

FINAL

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DATE

November 20, 1974

U. S. ARMY RESEARCH OFFICE

CONTRACT / GRANT NUMBER

DAHC04 74 C 0004

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This report describes the work done and the results obtained in the initial phase of a proposed program of research, the objective of which was to develop an animal model for the investigation of the effect of non-ionizing radiation on mammals. The specific aim of this phase of the proposed program was to replicate the observations of Dr. Joseph C. Sharp and his colleagues (1, 2, 3) on the effect of microwave irradiation on the development of the rat fetus, with particular reference to the growth of the cerebral cortex. These workers found that, in contrast with the destructive effects of ionizing radiation, radiation with low doses of microwaves (2450 MHz) on the 13th day of gestation stimulated the growth process. The average weight of the fetuses was larger and, in particular, the size of the cerebral cortex was greater than in rats similarly handled, but not irradiated.

One of the referees who read the original proposal pointed out that albino rats not infrequently showed congenital abnormalities of the visual system, including abnormalities of the occipital cortex. He recommended that rats of the Long-Evans, "hooded" strain be used. The initial experiments were therefore performed on rats of this strain.

Microwave irradiation was carried out either in one of the anechoic chambers of the Department of Microwave Research, Division of Neuropsychiatry, Walter Reed Army Institute of Research, or in the oven used for the original experiments by Dr. Sharp and his colleagues referred to above. The rats were restrained in rectangular lucite boxes, 6" x 3" x 3" with holes in the sides for ventilation. In the anechoic chamber three rats were irradiated

at a time. One was placed at the center of the microwave beam and the others on each side, one foot apart measured to the center of the restraining box. The longitudinal axis of the holder was perpendicular to the E vector of the beam. The chamber was maintained constant at a temperature between 70 and 78° F during radiation. Control rats were similarly restrained and placed in the chamber at the same time, but shielded from all radiation. In the oven rats were irradiated individually and the modal stirrer was in operation. The oven was calibrated measuring the rate of rise of temperature of 100 c.c. water exposed in a rectangular lucite container. The following equation was used:

$$w/cm^2 = \frac{\text{Vol. X } \Delta C^{\circ} \text{ X } 4.18}{\text{Time (in secs.) X Area (in cms}^2\text{)}}$$

The intensity of irradiation was varied in different cases from 5 to 30 mw/cm² and the duration from 20 minutes to 16 hours. All exposures to radiation excepting in the final experiment were made between 0700 and 1100 hours. In the final experiment exposure was from 1700 to 1600 hours or overnight

In most instances the rats were sacrificed on the 19th day of gestation. The fetuses were rapidly removed, weighed and their brains dissected out and fixed in Bouin's solution. The brains were examined grossly after fixing and were then imbedded in paraffin. Serial sections at 10 M were cut and two out of every eight were mounted and stained with cresyl violet for microscopic examination.

Of the first nine Long-Evans hooded rats, four were not pregnant, two were exposed to radiation in the chamber at 1700 MHz constant wave and 30 mw/cm² for 45 minutes, starting at 1423 hours. Three rats were kept as controls.

Two of the 14 rats in the second run were not pregnant and one, which was apparently in extremis after two hours' exposure in the anechoic chamber, at 1700 MHz and 20 mw/cm² was sacrificed. Two other rats received the same two-hour radiation, were stressed by it, but survived. One was sacrificed at 19 days, the other brought to term. Two rats were kept as controls, one of which was brought to term. The others, 7 rats, were radiated in the anechoic chamber at 1700 MHz and 20 mw/cm² for 20 minutes for three or four exposures at one or two per day. Two of these were brought to term. Five were sacrificed at 19 days and the fetal brains sectioned.

The four rats brought to term delivered without incident. At 12 days of age the control litter weighed slightly more on average than the radiated litters of the same size. At 24 days one of the radiated litters averaged 10 gms more per rat, the two others were less. No behavioral differences between the radiated and the control young were noted on gross observation. Two brains from each litter were prepared for histological examination. No anatomical differences between the radiated and control brains were found.

The brains of the fetal rats terminated on the 19th day of gestation were examined under a dissecting microscope. No abnormalities were found. Three pairs of brains, one radiated and one control, from fetuses of equivalent weight were compared, blind, by two observers. One observer picked the radiated brain as the larger in all three, the other observer had two right and one wrong--essentially chance. On microscopic examination no abnormalities or differences between the radiated and control brains were found. No appreciable difference in size could be determined.

The complete absence of any of the effects of radiation previously found raised the question as to whether a genetic factor might be responsible. Albino rats (Fischer strain) with presumed dated pregnancies were therefore obtained from a local source. Only 15 of 60 rats proved to be pregnant. Five of these were controls, four were exposed in the oven, and six in the anechoic chamber. The power in the chamber was pulsed, 50% duty cycle, at 12, 120 or 1000 Hz. Exposure was at 2450 MHz and 20 mw/cm² for 20 minutes.

No significant differences in the weights of the control and radiated fetuses were found at autopsy on the 19th day of gestation. However, cortical abnormalities were found in four of the control and in two of the exposed rats. In two of the controls the occipital pole of the cerebral cortex unilaterally showed a fold into the ventricle instead of being normally distended. In some sections this fold appeared as a small intraventricular gyrus. The total size of the cerebral cortex of the hemisphere appeared normal. Two of the control and two of the exposed rats showed small depressions of the external surface of the hemisphere unilaterally with some thinning of the underlying cortex. The depressions occurred, one just dorsal to the rhinal fissure and the other lateral to the longitudinal sulcus, in the same hemisphere. No associated changes in the adventitious tissues were noted at autopsy.

Following these findings albino rats of the Holtzman strain were used for the further experiments. 25 rats were studied. Seven were controls, nine were exposed in the oven at 20 mw/cm² and 2450 MHz and nine in the chamber at 20 mw/cm² and 1700 MHz constant wave. All exposures except one were for 20 minutes and on 1, 2, or 3 days starting on the 13th day of gestation. One rat was exposed in the oven for 40 minutes--the fetuses

were found to be resorbed. All exposures were carried out between 0800 and 1030 hours. At autopsy no significant differences between the weights of the fetuses of the control and exposed rats were found. Microscopic examination of serial sections of sample brains showed no differences between the different groups.

20 albino rats of the Holtzman strain were studied with Dr. Don R. Justesen at his laboratory in the Veterans Administration Hospital, Kansas City, Missouri. Ten of these were kept as controls, two rats were exposed on one day and five on four successive days in the oven, with the modal stirrer on, at 30 mw/gm and 2450 MHz for 20 minutes. Exposure was between 1300 and 1500 hours. No significant differences between control and exposed fetuses were found. Microscopic study showed no abnormalities.

17 albino (Holtzman) rats were divided into a control (7 rats) and two experimental groups. Six rats were exposed in the chamber at the Walter Reed laboratories to microwave radiation at 10 to 15 mw/cm² and 1700 MHz for one hour a day at 1700 hours on the 5th to the 8th and the 12th to the 16th (inclusive) days of gestation. (Exposure was for two hours on the 6th day of gestation. Five rats were exposed overnight, 14 to 16 hours, at 5 to 7.5 mw/cm² and 1700 MHz, on the same days. All rats were sacrificed on the 20th day of gestation.

The average weight of the fetuses of both exposure groups was significantly greater than that of the control group, $P < 0.005$. The average fetal weight of the one-hour exposure group was 4.03 \pm 0.28 gms, of the overnight group, 4.23 \pm 0.48 gms, and of the control group 3.73 \pm 0.28 gms. That is, the control group weighed approximately 10% less. Histological study was confirmatory, the

brains of the control group being approximately 10% smaller than those of the exposed fetuses.

The last experiment has been discussed on the telephone with Dr. Sharp and Dr. D. R. Justesen. In the original studies the rats were exposed to radiation in the oven at 2450 MHz, with the modal stirrer operating, in the late afternoon. In the present series all except the last group were exposed earlier in the diurnal cycle. The question naturally arises as to whether there may be a critical period in the diurnal cycle (circadian rhythm) during which radiation is effective in increasing the rate of growth, the same radiation being ineffective at other periods. This question would seem to be important to investigate. Should such a critical period be found its relation to the rhythmic variations in rate of cell mitotic division as found by Sharp and Paperiello (4) in the intestinal epithelium of the rat should be also investigated.

No publications have resulted from this work.

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