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ROCHESTER UNIV N Y SCHOOL OF MEDICINE AND DENTISTRY
EFFECTS OF MICROWAVE EXPOSURE ON FUNCTIONAL MATURATION OF THE R--ETC(U)
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REPORT DOCUMENTATION PAGE

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1. REPORT NUMBER		2. GOVT ACCESSION NO.		3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) EFFECTS OF MICROWAVE EXPOSURE ON FUNCTIONAL MATURATION OF THE RAT,				5. TYPE OF REPORT & PERIOD COVERED Final rpt. 1 Mar 75-31 Aug 77 3/1/75 - 8/31/77	
7. AUTHOR(s) Sol M./Michaelson, Ronnie/Guillet Franklin W./Heggeness				8. CONTRACT OR GRANT NUMBER(s) 15) N00014-75-C-0845	
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Rochester Rochester, New York 14642				10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS N 66013	
11. CONTROLLING OFFICE NAME AND ADDRESS Office of Naval Research Resident Representative, Room 303, Federal Bldg. 100 State Street, Rochester, New York 14614				12. REPORT DATE 30 Nov 77	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)				13. NUMBER OF PAGES 25 (12 44 p.)	
				15. SECURITY CLASS. (of this report) NA	
				15a. DECLASSIFICATION/DOWNGRADING SCHEDULE NA	

AD NO. DDC FILE COPY

16. DISTRIBUTION STATEMENT (of this Report)
**ONR Biological Sciences Division
ONR Physiology Program**

DISTRIBUTION STATEMENT A
Approved for public release
Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)
NA

18. SUPPLEMENTARY NOTES
**To be published in Developmental Toxicology of Energy Related Pollutants
DOE Symposium Series, Oak Ridge, Tennessee**

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Microwaves	Development
Rat	Endocrines
Physiology	Oxygen Consumption

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JAN 18 1978

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

To investigate whether exposure of the developing rats to microwaves may influence functional maturation, female Long-Evans rats were exposed for one hour to 2450 MHz continuous wave (CW), 10 mW/cm² at nine or sixteen days of gestation or at 40 mW/cm² on the ninth, thirteenth, sixteenth or twentieth days of gestation. The dams were permitted to give birth and the offspring

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were studied for functional development through the 21-day nursing period.

In another study, neonatal rats were exposed to 40 mW/cm², 2450 MHz, CW, microwaves for ^{5 min} five minutes each day from day ¹ one to day ⁶ six of life. On postnatal day 7, the rats were either sacrificed, exposed to 2450 MHz, CW for a ^{7th} seventh time, or injected with ACTH.

Among animals exposed in-utero, there was no difference in litter sizes between exposed or control animals. Metabolic response to cold exposure was greater among offspring of exposed rats at the age of ⁰⁻² 0 to 2 days than among the sham exposed animals. The offspring from the 40 mW/cm² exposed rats showed a significantly higher corticosterone level than sham-exposed controls during the first 24 hours of life. Brain ^{and adrenal} weight in offspring from dams exposed to 40 mW/cm² at 13 or 20 days of gestation was not different from sham-exposed ^{or control} counterparts. Adrenal weight in pups from exposed dams did not differ from offspring of control dams.

→ A statistically significant increase in adrenal wet weight was noted in animals exposed to microwaves on the first ⁶ six postnatal days. Adrenal wet weight and adrenal-to-body weight ratios in ⁷ seven day old rats were significantly higher in microwave exposed animals in comparison to controls. Following either microwave exposure or ACTH injection on day 7, plasma corticosterone levels remained low (< 3 µg%) in both exposed and control animals but was significantly increased in microwave exposed (2.08 µg% ± 0.85 SD) over control (0.72 ± 0.60 SD) animals.

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THE INFLUENCE OF MICROWAVE EXPOSURE
ON FUNCTIONAL MATURATION OF THE RAT

by

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ABSTRACT

The young, developing organism may be more sensitive to pathophysiologic perturbations than the adult animal. Thus, the possible influence of microwaves on growth and development requires comprehensive, critical study and analysis.

It is recognized that an individual may be more responsive to adverse factors during the early period in its life than at later stages. If an insult is capable of inducing subtle pathophysiologic alteration, it may be more apparent in this early period than at a later time. On the other hand, rather than leaving an imprint for later pathophysiology, an early stimulus may enhance developmental processes or physiologic regulatory mechanisms.

To investigate whether exposure of the developing rat to microwaves may influence functional maturation, female Long-Evans rats were exposed for one hour to 2450 MHz continuous wave (CW), 10 mW/cm^2 at nine and sixteen days of gestation or at 40 mW/cm^2 on the ninth, thirteenth, sixteenth or twentieth days of gestation. The dams were permitted to give birth and the offspring were studied for functional development through the 21 day nursing period.

In another study, neonatal rats were exposed to 40 mW/cm^2 , 2450 MHz, CW, microwaves for five minutes each day from day one to day six of life. On postnatal day 7, the rats were either sacrificed, exposed to 2450 MHz, CW for a seventh time, or injected with ACTH. Twenty minutes after the final exposure

or injection, the pups were sacrificed.

Among animals exposed in-utero, metabolic response to cold exposure was greater among offspring of exposed rats at the age of 0 to 2 days than among the sham exposed animals. The offspring from the 40 mW/cm² exposed rats showed a significantly higher corticosterone level than sham-exposed controls during the first 24 hours of life. Thyroxine level tended to be decreased among one week old rats from dams exposed to 10 mW/cm² but increased during the second week of life. Among pups from 40 mW/cm² exposed dams, there was a statistically significant increase in thyroxin level at 14 and 16 days of age. Brain weight in offspring from dams exposed to 40 mW/cm² at 13 or 20 days of gestation was not different from sham exposed counterparts. Adrenal weight in pups from exposed dams did not differ from offspring of control dams.

The neonatally exposed rats demonstrated a slightly greater (but not statistically significant) growth rate during the first 6 days of life. A statistically significant increase in adrenal wet weight was noted in animals exposed to microwaves on the first six postnatal days. Adrenal wet weight and adrenal-to-body weight ratios in seven day old rats were significantly higher in microwave exposed animals in comparison to controls. No difference was found in plasma corticosterone concentrations between the rats previously exposed to microwaves and control animals that were not exposed. Basal plasma corticosterone concentrations were less than 2 µg% in both groups. Following either microwave exposure or ACTH injection on day 7, plasma corticosterone levels remained low (< 3 µg%) in both exposed and control animals but was significantly increased in microwave exposed (2.08 µg% ± 0.85 SD) over control (0.72 ± 0.60 SD) animals.

Whether exposure to microwaves during the gestational or early neonatal period perturbs or actually modifies ontogeny of neuroendocrine responsiveness

which involves neural as well as hormonal facets and is intimately involved in conjunction with blood circulation in temperature regulation, is an interesting concept which would be of significance in obtaining knowledge of maturational biology. These studies suggest such may be the case.

INTRODUCTION

Considerable evidence is available to indicate that an individual is usually more vulnerable to certain adverse factors during the period of development than at later times in life (Brent and Harris, 1976). Thus, if an environmental insult is capable of inducing subtle physiologic alterations in a mammal, it may be more obvious early in life rather than later. As an example, brain development and the maturation of body temperature control, which involve many neural and hormonal interactions may be especially susceptible to microwave exposure in the infant as compared to the adult.

Some animal species (i.e., rats) are behaviorally and neurologically quite undeveloped at birth and, to a large extent, the final development of the central nervous system occurs during the postnatal period (Fowler and Kellogg, 1975). The newborn rat has imprecise thermal regulation. As hair and fat accumulate and control over the cutaneous circulation is acquired, body temperature becomes increasingly independent of the environmental temperature (Adolph, 1957). At 15 days of age the animal can maintain constant body temperature over a fairly wide range of ambient temperatures (Lytle and Keil, 1974), although even at 21 days the rat does not possess fully developed capacities for maintaining a stable core temperature (Conklin and Heggeness, 1971).

The environmental hazards confronting the developing embryo and fetus have been the subject of numerous studies (Brent and Harris, 1976). Even before the

search for teratogens began in earnest in mid 1960's following the Thalidomide tragedy, well over sixty teratogenic agents were known to induce malformations in experimental animals (Wilson, 1959). The clinical significance of many of these, however, has not been clarified.

Infections such as rubella, influenza and smallpox occurring during early pregnancy are known to result in abortions, fetal resorptions or malformations (Gruenwald, 1947; Wilson, 1959). In general, it appears that fever in early stages of pregnancy in man or other mammals, is capable of producing these changes. There are numerous reports of fetal abnormalities following the induction of systemic hyperthermia of 2.5° to 4.5° C above the normal temperature for a particular species during specific critical developmental stages of the fetus. Fetal resorption, growth retardation, microphthalmia and other malformations affecting the central nervous system, musculo-skeletal system, and other organs, have been observed in many mammalian species e.g. the guinea-pig (Edwards, 1967, 1969a, 1969b, 1971a, 1971b; Edwards, Penny, and Zevnik, 1971) and the rat (Garrison, 1940; Hsu, 1948; Edwards, 1968). In rats, temperature increase of 4° to 4.5° C for 40 to 60 minutes produced increased fetal resorptions, retardation of growth, microphthalmia, anencephaly and defects of tails, limbs, toes, palate and body depending upon the gestational stage at which hyperthermia occurred (Edwards, 1968). These results indicate that the occurrence of fetal malformations in mammals from the fever accompanying viral or bacterial toxemia in early pregnancy are probably related not as much to the causative agent but to the hyperthermia occurring at a particular critical stage of organogenesis. The threshold appears to be an elevation of 2.5° to 4.5° C above the normal temperature of the species and sustained for an hour or more.

Maturation of temperature regulation is a complex phenomenon dependent on

many physiologic and biochemical changes occurring during early life. Central nervous system development may be critical in appearance of many maturation processes. A maturation process may be indirectly susceptible by virtue of its dependence on CNS controls. Experimental application of environmental stresses provides a tool for dissecting out the ontogeny of a particular regulation while also pinpointing perturbations which could suggest possible health hazards to man.

In recent years considerable interest has been expressed concerning the biologic effects and hazard potential of exposure to microwaves. There is no doubt that whole-body exposure of small animals to high power density ($> 100 \text{ mW/cm}^2$), for a sufficient period of time (i.e. 1 hour or more, which induces hyperthermia) can cause pathophysiologic consequences. Whether exposure to microwaves during the gestational or early neonatal period perturbs or actually modifies the ontogeny of temperature regulatory capacity is an important question that should be answered. The effects of microwaves on the development of the animal have not been adequately documented. Such studies would be of significance in obtaining knowledge of maturational biology. It is also possible that certain ages in neonatal life are "critical", and the presence of specific or general stressors, i.e. electromagnetic radiation, malnutrition, at specific moments may result in a permanent deficit or delay development.

"Shortwave" radiation (10 MHz) was administered periodically by Boak, Carpenter, and Warren (1932) to rabbits from the 29th day of life through several matings and pregnancies. The total exposure time ranged between 30 and 75 hours during which animals' temperatures were raised to 41-42° C. There was no interference with mating, fertilization or development of the young in utero. Litter sizes were not significantly different from the control animals.

Radiofrequency or microwave energy has been utilized in the past to increase the temperature of various parts of the body including the pelvic organs for therapeutic purposes. In the latter case, heating has been used in the treatment of gonorrhea, pelvic inflammatory disease, endometriosis, carcinoma of the uterus, and pelvic peritonitis (Gellhorn, 1928; Schumacher, 1936). Temperature elevations of 1 to 2 degrees centigrade are readily attained and can be maintained for two to three hours. Gellhorn (1928) raised pelvic temperature in women to 115° F. Although he was concerned about the harmful effects of the intensity of radiation he did not allude to specific complications, however, there was no indication of any patient follow-up.

Rubin and Erdman (1959) reported four case histories of women treated with microwave diathermy (2450 MHz, 100-watt output) for chronic pelvic inflammatory disease who were, or became, pregnant during the course of the therapy. Three women delivered normal infants, and the fourth, who received 8 treatments during the first 59 days of pregnancy, aborted on day 67. This woman delivered a normal baby following a subsequent pregnancy during which she again received microwave therapy. The authors concluded that microwaves did not interfere with ovulation, contraception, and pregnancy. Daels (1973, 1976) used microwaves to ease parturition in women. No evidence of injury has been manifested in a 1 year follow-up of the children.

Although experimental data do indicate that the increased temperature can result in fetal abnormalities, reports of clinical use of radiofrequency/microwave energy in pregnant women have shown no such derangement.

EXPERIMENTAL PROCEDURE

Prenatal Exposure

To investigate the relationship of microwave exposure and prenatal

thermogenesis on animal development, gravid Long-Evans hooded rats (Blue Spruce Farms, Altamont, N.Y.) were exposed for one hour to 2450 MHz, CW, 10 mW/cm^2 at 9 and 16 days of gestation or at 40 mW/cm^2 on the 9th, 13th, 16th, or 20th days of gestation. Because interest was primarily on functional maturation rather than teratogenesis, power densities were selected that would cause minimal temperature rise and hence avoid the thermally-induced teratogenic effects. In addition, a range of gestational periods was chosen to encompass critical times in the physiologic development of the individual.

A Raytheon PGM-100 generator, providing filtered 2450 MHz (CW) power, was used. Adjustment of the power directed to the chamber was controlled with a Waveline Type 20421 variable coupler. Forward power, sampled with a crossguide coupler and detected with a Hewlett Packard 8481A power sensor, was monitored continuously with a Hewlett Packard Model 435A power meter, during exposure. The microwave exposure facility is diagrammatically illustrated in Figure 1.

The rats were exposed in the far field zone (160 cm) of a Narda Model 644 S-band standard gain horn antenna. Incident power density measurements were made in the absence of the rats and exposure cages with a Narda Model 8315 radiation monitor, which was calibrated in this facility against an NBS, Model XD-1 probe. The average power density for each cage area is within 1 db from the average.

The exposure chamber (1.25 x 1.25 m) was designed to provide an anechoic microwave environment for the animals. It was limited in this regard by its size and by the fact that it is open at the top. The sides and floor of the chamber were lined with EHP-5 absorber (Rantec Division, Emerson Electric Co.) and Eccosorb HPY-12 absorber (Emerson and Cuming, Inc.) respectively. The 5 cm thick styrofoam "floor" provided support for the exposure cages and insulated the animal environment from the heat generated in the floor absorber during

exposure. The rats were observed during exposure by means of a mirror above the chamber. The back of the mirror was covered with Eccosorb SF microwave absorber (Emerson and Cuming, Inc.). No differences in the chamber field pattern were observed when the field was mapped with or without the mirror in place.

The exposure cages were constructed of 5 cm FR100 styrofoam lined with 0.013 cm methyl acetate. The cage floor was a polystyrene grid material and the lid was made of styrofoam and fiberglass screening. The cages were glued together with epoxy. The inside cage area was 20 x 20 x 30 cm. Animals were exposed individually.

Physical and functional development of the offspring were assessed. Metabolic response (oxygen consumption) to cold was measured by the volume displacement method of Watts and Gourley (1953). Plasma levels of thyroxine by radioimmunoassay (Pentex Immuno-T4 kit) and corticosterone by competitive protein binding (Murphy, 1967), were determined. Sacrifice was by decapitation.

Trunk blood was collected in tubes containing Na-EDTA. The blood was spun for 20 minutes at 2500 rpm, plasma removed and frozen at -20° C until assay. The sample for corticosteroid assay was extracted using ethanol; the source of CBG (corticosteroid binding globulin) was dog plasma; and dextran-coated charcoal was used to separate bound from free hormone. The adrenals and brain were removed, weighed, and preserved in buffered formalin for future study.

Neonatal Exposure

Pregnant Long-Evans hooded rats (Blue Spruce Farms, Altamont, N.Y.), obtained on the 15th day of gestation, were housed individually in metal maternity cages. They were maintained at $24 \pm 1^{\circ}$ C, light cycle 0600 - 1800 h, and allowed food and water ad libitum. On the first day after birth (day 1),

the litters were culled to 9 pups each. Thereafter, the pups were left undisturbed, except as noted below.

Beginning on day 1 until day 6, each pup was individually exposed to 2450 MHz, CW, 40 mW/cm^2 for 5 minutes each day. The pups were placed in styrofoam exposure cages (5 x 10 x 10 cm) in the far-field of a standard gain S-band horn antenna. A Raytheon, DMC-5 generator was used to produce the microwaves. The field was mapped with a Narda 8321 isotropic probe. The incident power was monitored with an H-P #430C meter. The ambient temperature within the exposure chamber was controlled at $34 \pm 0.5^\circ \text{ C}$ (Figure 2). Rats were weighed daily preceding exposure and the rectal temperatures were taken every other day immediately following exposure. Control rats were treated similarly except that they were placed in exposure cages in an incubator ($34 \pm 1^\circ \text{ C}$) instead of in the exposure chamber for 5 minutes each day for the first six days. The selection of 34° C was predicated by the "nest" temperature.

On day 7, to test adrenal responsiveness, one third of the pups were given ACTH (10 mU/100 g) i.p. and one third (control and experimental) exposed to 2450 MHz, CW, 40 mW/cm^2 for 5 minutes. These pups were sacrificed by decapitation 20 minutes following injection or exposure. The remaining pups were sacrificed immediately after removal from the nest.

RESULTS

Prenatal Exposure

The exposure did not adversely affect the pregnant rat. Body temperature increase at 10 mW/cm^2 was minimal for gestation day 9 rats (Table 1); however, in rats at 16 days of gestation, temperature increased $.5^\circ \text{ C}$. In all groups exposed to 40 mW/cm^2 , temperature increased up to 3° C ; parturition was normal with no change in gestation duration.

Litter sizes of 10 - 16 pups were not different from sham-exposed animals (Table 2). There were no apparent developmental or growth rate differences in pups from irradiated or non-exposed dams from birth to weaning on the 21st day (Figure 3).

Oxygen consumptions were tested at neutral temperature (temperature zone of minimal metabolism) and during cold exposure (5° C below neutral temperature) (Table 3). As expected, O₂ consumption in the cold was increased over that observed at neutral temperatures. At ages 0 - 2 days, metabolic response to cold exposure was greater among offspring of exposed rats than the sham exposed (control) animals.

Unstressed plasma corticosterone levels (Table 4) were somewhat variable. There appeared to be significant increases among the neonates of rats exposed on gestation day 16 to 40 mW/cm². Any biological significance of these changes is not clear at present. In reviewing data of this nature, one has to keep in mind that corticosterone as well as other hormones shows a fluctuating pattern during the early postnatal period. For the first few hours after birth, normal corticosterone levels are 10 - 20 micrograms % . After four hours, the level falls, reaching its nadir between the second and fifth days and remaining low until 12 - 14 days of age (Guillet, 1977). When certain days were combined, as noted in the lower portion of Table 4, it became evident that the offspring from the 40 mW/cm² exposed dams showed a significantly higher corticosterone level than sham-exposed controls. Because of the difficulty in determining exact time of birth, it is difficult to attach biological significance to this change particularly as litters are frequently born during the night. By separating the animals up to 2 days of age, it became apparent that the high levels are present during the first few hours of life. Although the higher corticosterone level is statistically significant from later levels,

one cannot be sure of the biological significance until more precise age-by-hour and corticosterone level correlations are obtained. It is to be noted, however, that throughout the first two weeks of life, there is a significantly higher corticosterone level in the offspring from the 40 mW/cm^2 exposed dams than those of sham exposed controls.

Thyroxine level tends to be decreased among the one-week-old rats from dams exposed to 10 mW/cm^2 but increased during the second week of life. Among pups from 40 mW/cm^2 exposed dams there is a statistically significant increase in thyroxine level at 14 - 16 days of age (Table 5).

Brain weight in offspring from dams exposed to 40 mW/cm^2 at 13 or 20 days of gestation are not different from sham-exposed counterparts (Table 6). Adrenal weight (Figure 4) in pups from exposed dams did not differ from offspring of control dams.

Neonatal Exposure

Average body weights for all exposed ($n = 18$) and control ($n = 18$) rats are shown in Figure 5. There was no significant difference in growth rate between exposed and control rats, but the exposed rats demonstrated a slightly greater growth rate during the first 6 days of life. Although maintained at comparable ambient temperature, the microwave exposed rats had a $1.5^\circ - 2.5^\circ \text{ C}$ higher colonic temperature than non-exposed rats.

Plasma corticosterone concentrations in control and exposed pups that were sacrificed immediately, injected with ACTH (10 mU/100 g) or exposed to 2450 MHz, CW, 40 mW/cm^2 incident energy for 5 minutes, are shown in Table 7. It is apparent that in the seven-day-old rat the basal corticosterone level is not altered by previous exposure to microwaves. The ratlets exposed to 40 mW/cm^2

microwaves show an adrenal responsiveness not quantitatively different from that produced by ACTH administration.

As indicated in Figure 6, adrenal wet weight and adrenal-to-body weight ratios in seven-day-old rats are significantly higher in microwave exposed animals in comparison to controls.

DISCUSSION

Rugh, Ginns, Ho, and Leach (1974, 1975) found that microwave exposure was teratogenic in mouse fetuses exposed at day 8 of gestation to absorbed 2450 MHz (CW) microwaves in the range of 3-8 cal/g (equivalent to 123 mW/cm^2 incident power). Gross anomalies such as hemorrhages, resorptions, exencephaly, stunting, and fetal death were observed.

In contrast to the studies of Rugh and associates, Chernovetz, Justesen, King, and Wagner (1975) exposed mice at 11 - 14 days of gestation to 2450 MHz (CW) at an absorbed dose of 40 mW/g (estimated incident energy 160 mW/cm^2 for 10 minutes) which induced a temperature increase of 2° C and resulted in 10% lethality in the dams. There was no evidence of teratogenesis nor effect on survival or learning ability of the offspring.

In another study, neonatal mice exposed to 10.5 MHz, 19.27 MHz, or 26.6 MHz pulsed in an H field of 55 amps/m and an E field of 8000 V/m did not show any evidence of alteration in growth and development (Stavinoha, Modak, Medina, and Gass, 1975). The test animals were exposed for 40 minutes a day for five consecutive days.

Budd, Laskey, and Howes (1970) exposed pregnant Sprague-Dawley rats for 8.5 minutes to whole-body 2450 MHz, 100 mW/cm^2 , CW, microwaves at 15 days gestation. Under these conditions the rectal temperature of the rats increased 4.2° C above that of the controls. Hematological parameters were measured in

the animals at 4 hours, 24 hours, and 5 days post-irradiation (shortly before the fetuses were removed). Body and spleen weights, and hematological changes were measured in the 20 day fetuses. No significant differences were found between the control and microwave exposed pregnant rats in body weight, total leukocyte count, erythrocyte count, hematocrit, or hemoglobin value. Microwave irradiated fetuses had significantly lower spleen weights, total leukocyte counts, and slightly lower hemoglobin values than controls. No differences in incidence of fetal resorption, body weight, or ^{59}Fe uptake in blood were observed between microwave irradiated fetuses and their controls.

Dietzel and Kern (1970, 1975) used 27.12 MHz radiation to produce hyperthermia (42.7°C) in pregnant rats. They found that a variety of teratological effects could be induced, specific abnormalities being related to the developmental stage of the fetus. Hyperthermia up to 42.7°C , following 10-minute exposure resulted in increased resorptions of both irradiated pre-implanted and post-implanted embryos. Elevation of the uterine temperature to 42°C for 10 minutes on the first and second day of pregnancy resulted in 65% of the zygotes failing to come to term. A similar exposure on the 7th - 8th day resulted in 28% resorptions.

Analysis of the literature indicates that temperature rise in the fetus irrespective of the manner in which it is produced, can result in alterations in animal development. During the first three weeks of life, there is a constantly-evolving pattern of maturation in the rat. After the first 24 - 48 hours, new-born animals respond minimally to stress as evidenced by lack of corticosterone increase. This capacity increases during ontogeny and is virtually complete by three weeks of age.

In the present study, newborn rats subjected to daily 5-minute exposures to 40 mW/cm^2 , 2450 MHz, CW, microwaves at 34°C ambient temperature for six

days did not result in apparent deleterious effects in spite of a 1.5° - 2.5° C body temperature rise. Growth rate was comparable for exposed and control rats maintained at similar (34° C) ambient temperature. It is presumed that if such exposure to microwaves were deleterious, weight gain, which is a very sensitive indicator of general development, would be depressed. It should be pointed out, however, that lack of evidence of influence on growth rate during this early period, does not preclude future alteration of growth rate.

Based on water calorimetry studies in this laboratory (Lotz and Michaelson, 1977), 40 mW/cm² incident power intensity with resultant 2 - 3° C rise in temperature is equivalent to 9 - 10 W/kg absorbed energy in these animals.

Of considerable interest is the finding that six daily exposures of newborn rats to 40 mW/cm² incident energy did not change basal corticosterone levels. There was no change in adrenal responsiveness to ACTH injection and the adrenal response to microwave exposure was comparable to ACTH injection. In this context, we had previously noted (Michaelson, Thomson, and Howland, 1961) that the hematologic response of adult dogs, exposed to 100 - 165 mW/cm², 2880 MHz pulsed microwaves for 1 - 3 hours, "resembles that reported to occur after slow continuous ACTH injection and may be indicative of hypothalamic or adrenal stimulation (stress effect)".

As compared with control rats, the rats exposed daily to microwaves did show a slightly greater adrenal responsiveness to ACTH or microwave-induced hyperthermia and a significantly increased ($p < .05$) adrenal wet weight. The magnitude of the increased adrenal weight is similar to that seen following injected Acthar (ACTH in depot form) 5 mU/g on days 1 - 6 (unpublished results). This increased adrenal weight may be functionally significant. It will be necessary to perform histologic examination of these glands to determine whether the larger weight is due to increased numbers of functional adrenal

cortical cells or connective tissue, fat, etc. Although such increased adrenal weight and responsiveness as a result of microwave exposure may be indicative of microwave, or thermally induced enhancement of developmental processes, additional study is indicated to establish this intriguing possibility.

On a functional basis, the increased consumption in the cold, and higher basal corticosterone levels in rats exposed in-utero suggests that microwave exposure may alter some maturational processes such as a change in set-point of the hypothalamic-pituitary-adrenal reactivity or possibly acceleration of ontogeny. Such speculation has to be approached with caution. One would like to consider acceleration of maturation as a beneficial process in the developing organism. It is essential nevertheless to make certain that such acceleration of maturation processes may not result in some physiologic deficit or impairment of other apparently unrelated maturational processes during later periods in the life of the animal.

It is of interest that in studies in which hyperthermia (microwave-induced or otherwise) is used as an adjunct in the treatment of cancer, tumor susceptibility to X-irradiation or chemotherapeutic agents, is related to temperature increase. Dickson and Ellis (1976) have shown that following heating of sarcoma implants in the rat to 40° C for 1 hour, there was a 50% increase in O₂ uptake and enhancement of tumor growth. On the other hand, 41 - 42° appears to suppress growth of this tumor.

Additional studies of this nature would be of considerable interest because we have here the possibility of a tool for dissecting out the ontogeny of a particular physiologic regulatory system. Such responses should also be correlated with temperature increases in the developing fetus to ascertain the level of hyperthermia that could be acceptable to the developing animal without compromising future growth and development.

This paper is based on work performed under Contract No. N0014-75-C-0845 with the Office of Naval Research, Department of the Navy and under contract with the U. S. Energy Research and Development Administration at the University of Rochester Biomedical and Environmental Research Project and has been assigned Report No. UR-3490-1157.

TABLE 1

Colonic Temperature in Pregnant Rats Exposed to 2450 MHz (CW) Microwaves

Exposure (1 hour) / Day of Gestation	9	13	16	20
Sham	37.5 ± 0.07 (13)*	37.3 ± 0.17 (9)	37.6 ± 0.09 (9)	37.2 ± 0.14 (8)
10 mW/cm ²	37.7 ± 0.08 (12)	-----	38.1 ± 0.10 (8) t = 3.72 p < .01	-----
40 mW/cm ²	38.7 ± 0.12 (16) t = 8.64 p < .001	38.5 ± 0.09 (20) t = 6.24 p < .001	39.5 ± 0.06 (20) t = 17.57 p < .001	38.6 ± 0.07 (16) t = 8.94 p < .001

* °C mean ± SEM (n)

TABLE 2

Litter Size of Pregnant Rats Exposed to 2450 MHz (CW) Microwaves

Exposure (1 hour) \ Day of Gestation	9	13	16	20
Sham	11.1 ± 0.68 (14)*	10.6 ± 2.7 (8)	12.9 ± 2.21 (8)	10.0 ± 1.6 (6)
10 mW/cm ²	11.7 ± 0.62 (9)	-----	10.4 ± 0.75 (8)	-----
40 mW/cm ²	10.4 ± 1.03 (14)	11.8 ± 1.9 (16)	11.4 ± 0.79 (22)	12.7 ± 2.2 (14)

* number of pups mean ± SEM (n)

TABLE 3

Oxygen Consumption in Rats Exposed In-Utero to Microwaves
(2450 MHz, CW, 40 mW/cm², 1 hour)

Age (days)	Day of Gestation	9	13	16	20
0-2	sham	39.0/52.7*	45.9/74.1	41.9/67.3	55.0/65.1
	Rx	49.1/63.3	65.1/124.7	49.1/79.9	63.3/89.8
6-8	sham	37.1/72.3	40.5/68.2	32.4/42.9	44.9/67.2
	Rx	30.6/63.4	39.9/78.1	42.8/82.3	47.9/69.1
14-16	sham	37.1/62.3	33.9/45.7	40.8/64.3	33.4/59.5
	Rx	30.4/46.5	34.9/63.1	33.9/74.3	35.9/52.5
18-21	sham	40.4/54.5	33.2/53.7	29.6/57.2	33.1/61.0
	Rx	33.5/44.8	37.1/56.3	50.0/53.4	40.9/52.7

* (ml O₂/min/kg) neutral temperature/cold temperature

Note: Cold temperature 5° C less than neutral temperature

TABLE 4

Corticosterone Levels in Rats Exposed In-Utero to 2450 CW Microwaves
(gestation day 16)

Age (days)	Sham Exposed	10 mW/cm ² *	40 mW/cm ² *
0	12.6 ± 2.50 (4) **	-----	20.4 ± 1.18 (12) t = 2.82 P < .01
1	8.2 ± 1.58 (22)	-----	4.3 ± 2.29 (6)
2	1.8 ± 0.15 (3)	-----	-----
0-2	8.2 ± 1.33 (29)	6.6 ± 1.1 (11) t = 2.41 P < .05	15.0 ± 2.10 (18) t = 2.76 P < .01
6-8	2.6 ± 0.57 (7)	1.8 ± 0.33 (6)	5.7 ± 0.41 (6) t = 4.42 P < .01
9-12	1.1 ± 0.49 (5)	0.84 ± 0.18 (8)	2.6 ± 0.31 (9) t = 2.59 P < .05
14-16	-----	-----	1.9 ± 0.32 (8)
17-21	-----	-----	4.4 ± 1.19 (11)

* 1 hour exposure

** µg% - mean ± SEM (n)

TABLE 5

Thyroxine Level in Rats Exposed In-Utero
to 2450 MHz (CW) Microwaves
(gestation day 16)

Age (days)	Sham Exposed	10 mW/cm ² *	40 mW/cm ² *
3-5	1.2 ± 0.23 (10)	0.8 ± 0.24 (15)	
6-8	2.2 ± 0.45 (7)	0.9 ± 0.24 (7) t = 2.55 P < .05	2.1 ± 0.39 (8)
9-12	2.0 ± 0.20 (12)	3.4 ± 0.33 (40) t = 3.63 P < .001	1.6 ± 0.17 (11)
14-16	2.9 ± 0.39 (7)	4.6 ± 0.34 (11) t = 3.29 P < .01	4.7 ± 0.24 (14) t = 3.93 P < .001
17-21	-----	-----	3.0 ± 0.21 (10)

* 1 hour exposure

** µg% mean ± SEM (n)

TABLE 6

Brain Weight of Rats Exposed In-Utero
to 2450 MHz (CW) Microwaves

Age (days) \ Gestation day	Sham-exposed		40 mW/cm ² - 1 hour	
	13	20	13	20
4-5	—	4.6 ± 0.37*	4.8 ± 0.49	—
7-10	—	4.5 ± 0.18	5.3 ± 0.33	5.0 ± 0.21
11-12	—	4.8 ± 0.15	4.7 ± 0.33	5.0 ± 0.30
14-19	4.5 ± 0.09	4.4 ± 0.26	4.9 ± 0.23	4.4 ± 0.19
20-21	3.6 ± 0.32	3.9 ± 0.21	4.2 ± 0.15	—

* g/100g body weight, mean ± SEM, n = 5

TABLE 7

Plasma Corticosterone Concentration¹ in Neonatal Rats

Treatments		Treatment on Day 7		
Days		Control	ACTH (10 mU/100 g)	2450 MHz (40 mW/cm ²) 5 min
1-6				
Incubated Pups		0.73 ± 0.45 (3)	1.63 ± 0.45 (3)	0.72 ± 0.60 (3) ^a
Radiated Pups		0.67 ± 0.24 (5) ^b	2.29 ± 0.60 (6) ^c	2.08 ± 0.85 (6) ^d
Control Pups ²		0.58 ± 0.20 (3) ^e	1.32 ± 0.28 (3) ^f	1.82 ± 0.43 (3) ^g

¹ µg/100 ml, mean ± S.D. (n)

² Pups remained with dams until 7th day

a-d p < .05; b-c p < .001; b-d p < .01; e-f p < .05; e-g p < .05

FIGURE 1

Controlled temperature/microwave unit for whole-body exposure of adult rats.

FIGURE 1

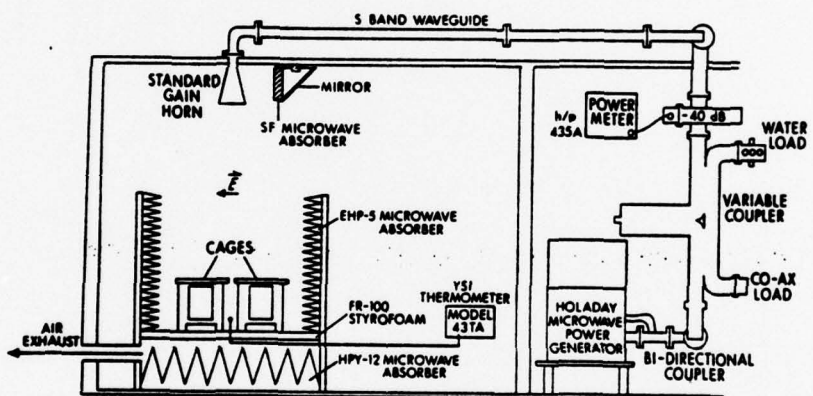


FIGURE 2

Controlled temperature/microwave exposure unit for neonatal rats.

FIGURE 2

CONTROLLED TEMPERATURE / MICROWAVE EXPOSURE UNIT

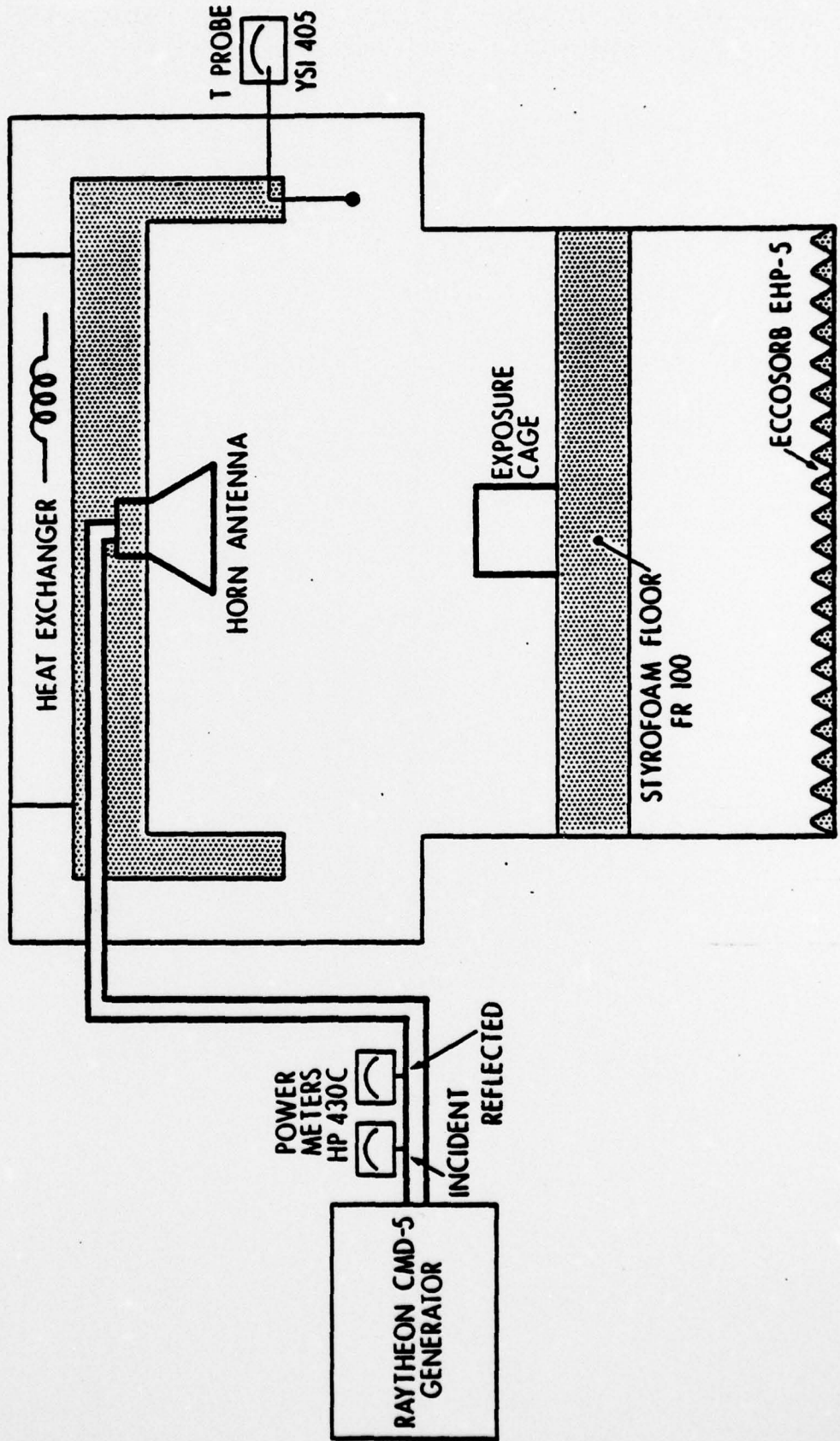


FIGURE 3

Weight (mean \pm 2 S.E.M.) of rats exposed in utero to 40 mW/cm^2 for one hour.

FIGURE 3

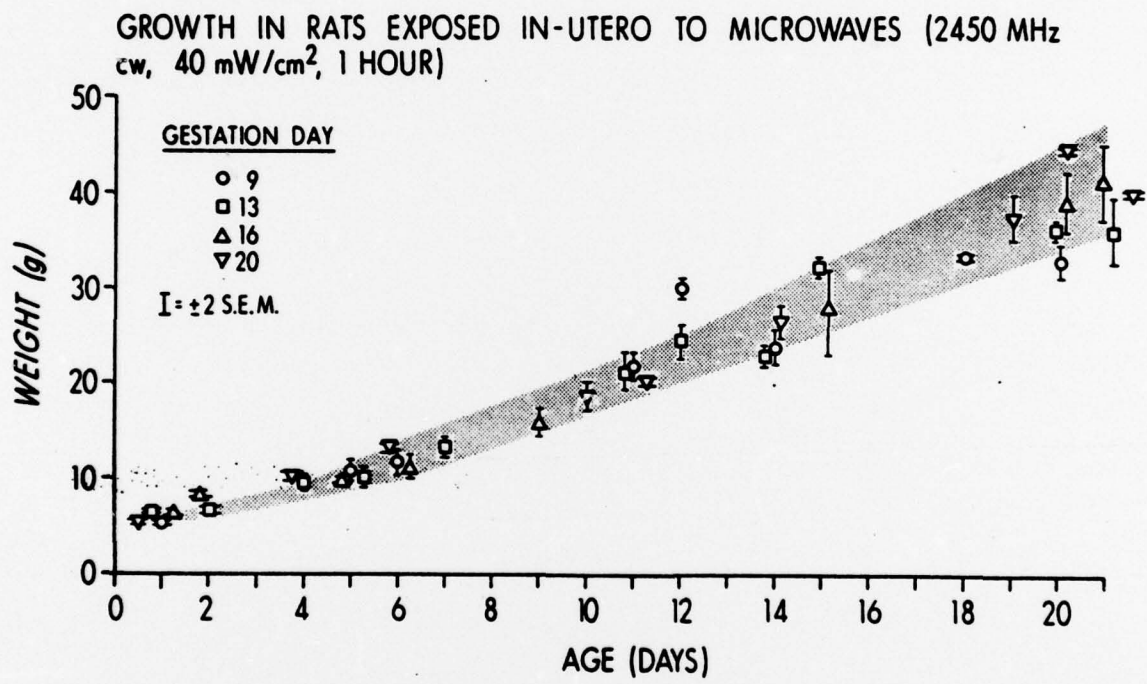


FIGURE 4

Weight (mean \pm S.E.M.) of adrenal glands of rats exposed in utero to 10 or 40 mW/cm² for one hour.

FIGURE 4

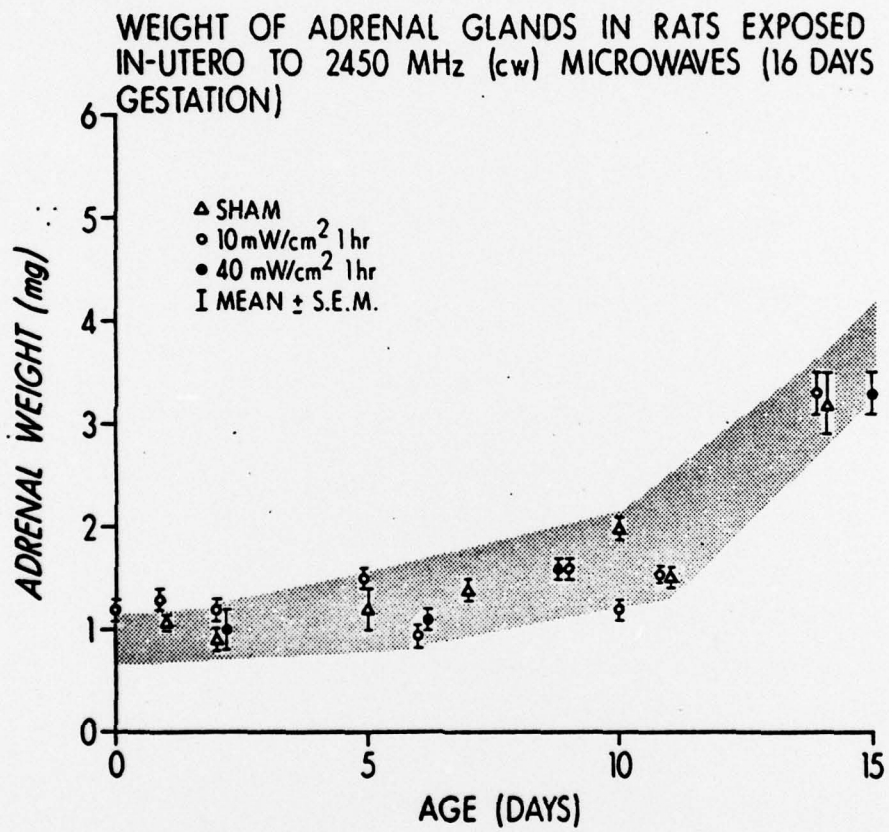


FIGURE 5

Weight in grams of neonatal rats from first through seventh day of age. Pups were exposed six or seven times for five minutes to 2450 MHz, CW, microwave radiation at a power density of 40 mW/cm^2 . Control pups were sham-radiated. All pups were maintained at the "maternal temperature" of 34°C during radiation or sham radiation.

FIGURE 5

GROWTH IN RATS EXPOSED TO MICROWAVES

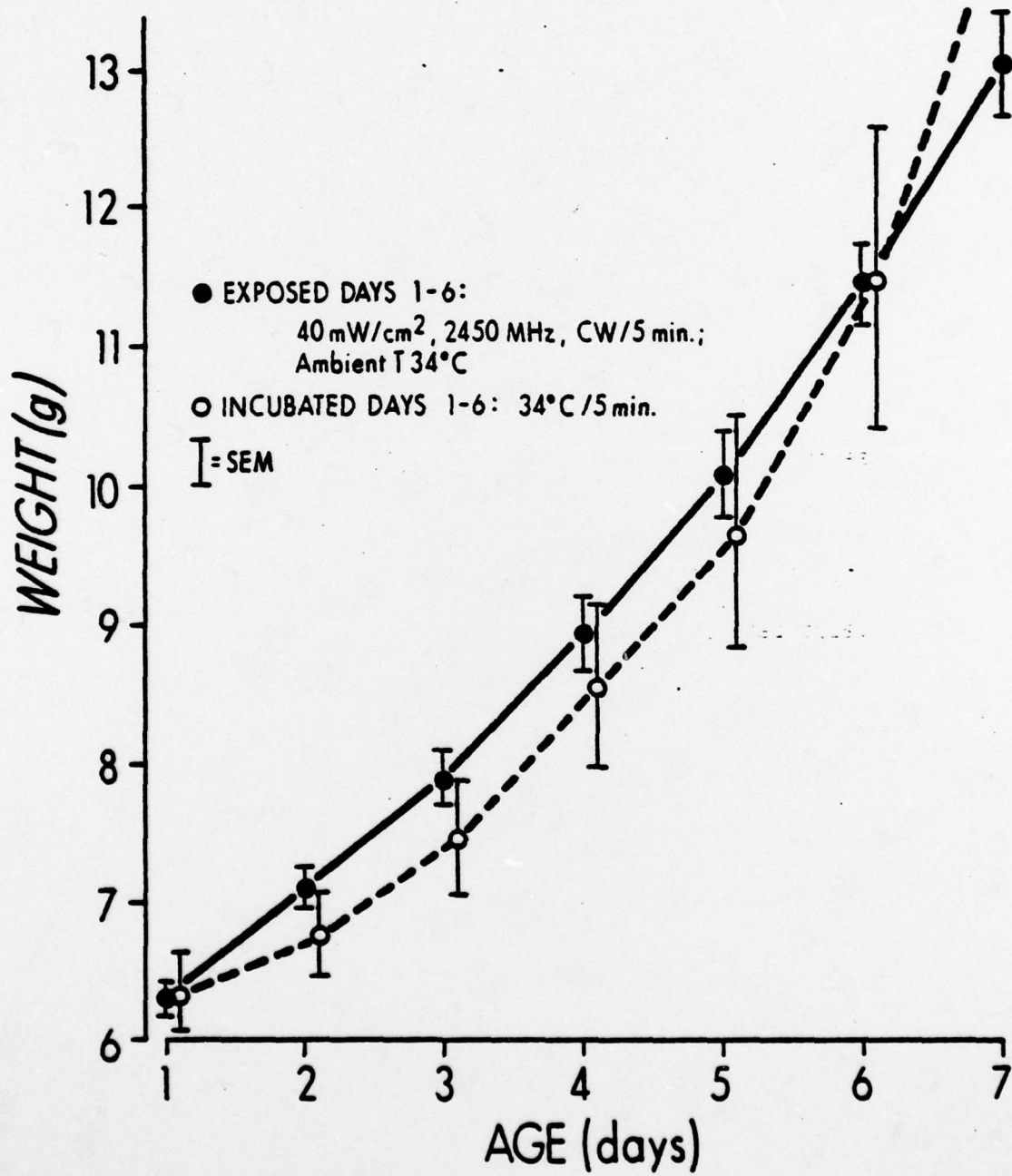
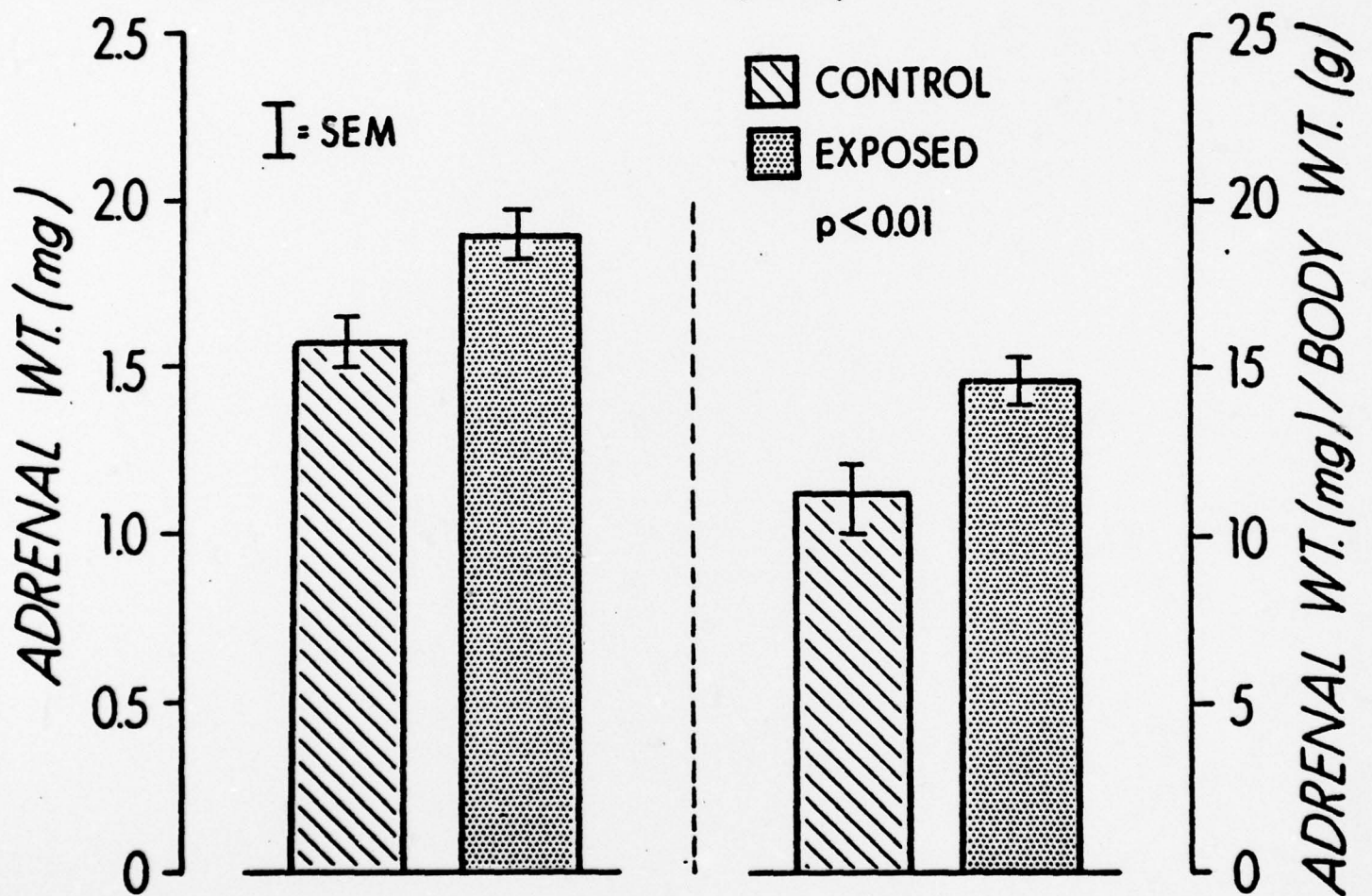


FIGURE 6

Mean adrenal weight (left) and ratio of adrenal-to-body weight (right) of sham-radiated controls and of pups that had been radiated six or seven times by microwaves. The adrenal glands were harvested on post-natal day 7.

FIGURE 6

ADRENAL WEIGHT IN RATS
EXPOSED TO 2450 MHz (CW)

REFERENCES

- Adolph, E.F., 1957, Ontogeny of physiological regulations in the rat, Quarterly Rev. Biol., 32: 89-137.
- Boak, R.A., C.M. Carpenter, and S.L. Warren, 1932, Studies on the physiological effects of fever temperatures. II. The effect of repeated short wave (30 meter) fevers on growth and fertility of rabbits, J. Exp. Med., 56: 725-739.
- Brent, R.L., and M.I. Harris (eds.), 1976, Prevention of Embryonic, Fetal, and Perinatal Disease, 411 pp., DHEW Publication No. (NIH) 76-853, Bethesda.
- Budd, R.A., J. Laskey, and M. Howes, 1970, Hematological response of fetal rats following 2450 MHz microwave irradiation, Radiation Bio-Effects Summary Report, pp. 33-35, USDHEW, PHS, BRH Publication No. BRH/DBE 70-7, D.M. Hodge (ed.), Bethesda.
- Chernovetz, M.E., D.R. Justesen, N.W. King, and J.E. Wagner, 1975, Teratology and reversal learning after fetal irradiation of mice by 2450 MHz microwave energy, J. of Microwave Power, 10: 391-409.
- Conklin, P., and F.W. Heggeness, 1971, Maturation of temperature homeostasis, Amer. J. of Physiol., 220: 333-336.
- Daels, J., 1973, Microwave heating of the uterine wall during parturition, Obstet. Gynecol., 42: 76-79.
- , 1976, Microwave heating of the uterine wall during parturition, J. Microwave Power, 11: 166-168.
- Dickson, J.A., and H.A. Ellis, 1976, The influence of tumor volume and the degree of heating on the response of the solid Yoshida sarcoma to hyperthermia (40-42°), Cancer Res., 36: 1188-1195.
- Dietzel, F., 1975, Effects of electromagnetic radiation on implantation and intrauterine development of the rat, Ann. N.Y. Acad. Sci., 247: 367-376.

- , and W. Kern, 1970, Abortion following ultra-shortwave hyperthermia animal experiments, Arch. Gynaekol., 209: 237-255.
- Edwards, M.J., 1967, Congenital defects in guinea pigs following induced hyperthermia during gestation, Arch. Path. 84: 42-48.
- , 1968, Congenital malformations in the rat following induced hyperthermia during gestation, Teratology, 1: 173-178.
- , 1969a, Congenital defects in guinea pigs: fetal resorptions, abortions, and malformations following induced hyperthermia during early gestation, Teratology, 2: 313-328.
- , 1969b, Congenital defects in guinea pigs: prenatal retardation of brain growth of guinea pigs following hyperthermia during gestation, Teratology, 2: 329-336.
- , 1971a, The experimental production of clubfoot in guinea-pigs by maternal hyperthermia during gestation, J. Path., 103: 49-53.
- , 1971b, The experimental production of arthrogryposis multiplex congenita in guinea-pigs by maternal hyperthermia during gestation, J. Path., 104: 221-229.
- , R.H.C. Penny, and I. Zevnik, 1971, A brain cell deficit in newborn guinea-pigs following prenatal hyperthermia, Brain Res., 28: 341-345.
- Fowler, S.J., and C. Kellogg, 1975, Ontogeny of thermoregulatory mechanisms in the rat, J. Comp. Physiol. Psychol., 89: 738-746.
- Garrison, L., 1940, The effect of fever on the development of the rat incisor, J. Dent. Res., 19: 215-225.
- Gellhorn, G., 1928, Diathermy in gynecology, J. Amer. Med. Assoc., 90: 1005-1008.
- Gruenwald, P., 1947, Mechanisms of abnormal development. I. Causes of abnormal development in the embryo, Arch. Path., 44: 398-436.

- Guillet, R., 1977, The Development of the Adrenal Axis in the Neonatal Rat, Ph.D. Thesis, University of Rochester, Rochester, New York.
- Hsu, Chih-Yun, 1948, Influence of temperature on development of rat embryos, Anat. Rec., 100: 79-90.
- Lotz, W.G., and S.M. Michaelson, 1977, Stimulation of the adrenal axis in the microwave exposed rat, Am. J. Appl. Physiol. (in press).
- Lytle, L.D., and F.C. Keil, 1974, Brain and peripheral monoamines: possible role in ontogenesis of normal and drug-induced responses in the immature mammal, K. Fuxe, L. Olson, and Y. Zotterman (eds.), Dynamics of Degeneration and Growth in Neurons, Pergamon Press, New York.
- Michaelson, S.M., R.A.E. Thomson, and J.W. Howland, 1961, Physiologic aspects of microwave irradiation of mammals, Am. J. Physiol., 201: 351-356.
- Murphy, B.E.P., 1967, Some studies of protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay, J. Clin. Endocrinol. Metab., 27: 973-990.
- Rubin, A., and W.J. Erdman, II, 1959, Microwave exposure of the human female pelvis during early pregnancy and prior to conception, Am. J. Phys. Med., 38: 219-220.
- Rugh, R., E.I. Gims, H.S. Ho, and W.M. Leach, 1974, Are microwaves teratogenic? Biologic Effects and Health Hazards of Microwave Radiation, Proceedings of an International Symposium, Warsaw, Poland, October 15-18, 1973, P. Czerski, K. Ostrowski, M.D. Shore, C. Silverman, M.J. Suess, and B. Waldeskog (eds.), pp. 98-107, Polish Medical Publishers, Warsaw.
- , 1975, Responses of the mouse to microwave radiation during estrous cycle and pregnancy, Radiation Res., 62: 225-241.

Schumacher, P.H., 1936, Kurzwellentherapie in der Gynäkologie, Zentralbl. Gynak., 60: 1923-1924.

Stavinoha, W.B., A. Modak, M.A. Medina, and A.E. Gass, 1975, Growth and Development of Neonatal Mice Exposed to High-Frequency Electromagnetic Fields, SAM-TR-75-51, USAF School of Aerospace Medicine, Aerospace Medical Division, (AFSC) Brooks Air Force Base, Texas.

Watts, D.T., and D.R.H. Gourley, 1953, A simple apparatus for determining basal metabolism of small animals in student laboratories, Soc. Exper. Biol. Med. Proc., 84: 585-586.

Wilson, J.G., 1959, Experimental studies on congenital malformations, J. Chron. Dis., 10: 111-130.