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EFFECTS OF MICROWAVE IRRADIATION ON EMBRYONIC BRAIN TISSUE.(U)

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FINAL REPORT

EFFECTS OF MICROWAVE IRRADIATION ON EMBRYONIC BRAIN TISSUE

LEVEL II

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David McK. Rioch, M.D., and George H. Koustenis, M. A.

March 1979

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This report describes a research program that investigated the biological and behavioral effects of low-level microwave irradiation on embryonic brain tissue in the rat. Pregnant Sprague-Dawley albino and Long Evans hooded rats were irradiated during nine consecutive overnight sessions between the 6th and 16th days of gestation. Each session was from 14 to 16 hours long and the subjects were exposed in a field intensity of 5 th 7.5 mw/cm^2 at 1700 MHz with the E vector perpendicular to the horizontal body plane. Some litters were sacrificed late in gestation and the fetuses were autopsied to determine if

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gross anatomical differences existed between exposed and control groups. The remaining litters were brought to term and observed to detect any differences in the biological and/or behavioral development of the two cohorts. These groups were subsequently sacrificed at about 8 months of age and were compared through the use of detailed autopsies and neurohistological techniques. The results of the anatomical and behavioral testing revealed no significant or consistent difference between the irradiated and control groups.

FOREWORD

In conducting the research described in this report, the investigators 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.

EFFECTS OF MICROWAVE IRRADIATION ON EMBRYONIC BRAIN TISSUE

INTRODUCTION

The data to be presented in this report consist of anatomical studies of fetuses and of mature rats which had been exposed to microwave radiation earlier in fetal life. The data are thus chiefly significant in relation to the preceding experimental programs. The two sets of experiments on which the work reported here was based will therefore be very briefly reviewed in the following paragraphs headed "Background." More detailed data on specific experiments will be included with the anatomical descriptions of certain groups of brains in the main body of this report under "Anatomical Data." In the last section of this report, "Summary and Conclusions," some of the questions raised by the findings will be briefly discussed.

BACKGROUND

The series of experiments described in this report were originally undertaken in order to replicate and extend the preliminary studies described by Dr. Joseph C. Sharp and others at the DOD Electromagnetic Research Workshop, Washington, D.C., 1971. These studies indicated that low dosage microwave irradiation of pregnant rats resulted in the increased growth of the fetuses, including increased growth of their brains. In Appendix 1*, a number of attempts to replicate the earlier experiments are briefly described. None of the experiments replicating the original procedures resulted in significant differences between the irradiated and the control fetuses in the growth of the whole body or of the brains. In one experiment, however, the rats were irradiated overnight (14 to 16 hours) on 9 nights between the 5th and the 16th days of gestation in a field intensity of 5 to 7.5 mw/cm^2, cw, the E vector perpendicular, at 1700 MHz (p.5, App.1). The irradiated fetuses weighed approximately 10% more than the control fetuses, and their brains were proportionately larger.

In order to further test the effects of prolonged microwave irradiation early in fetal life, a contract was obtained with the Office of Naval Research, and a series of experiments were conducted as described in Appendix 2**, (2450 MHz, 5 mw/cm^2, cw, E vector perpendicular). Two batches of four datemated female rats each (albino, Sprague-Dawley [S-D]) and two batches of ten rats each (hooded, Long-Evans [L-E]) were irradiated on 9 nights overnight between the 6th and the 16th days of gestation. Control rats were similarly treated, except that they were sham-exposed in the chamber shielded from radiation. Six of the irradiated and 6 of the control S-D rats were pregnant.

* Appendix 1 consists of the Final Report, "Effects of Microwave Irradiation on Embryonic Brain Tissue," by David McK. Rioch, M.D., November 20, 1974, U.S. Army Research Office - Durham, Contract No. DAHC04-C-0004.

**Appendix 2 consists of the Final Report, "Effects of Microwave Irradiation on Embryonic Brain Tissue," by David McK. Rioch, M.D., and George H. Koustenis, M.A., November 8, 1977, Office of Naval Research, Contract No. N00014-75-C-0969.

Ten (all of the second batch) of the L-E rats were found to have purulent endometritis on autopsy and were discarded. Four irradiated and four controls of the other 10 L-E rats were pregnant. Autopsies were performed on the 19th L (S-D) and 20th (~~E~~-E) days of gestation. In all three batches, the irradiated fetuses were heavier than the controls.

As shown in Table 2 (p. iii of Appendix 2), the difference in weights of the irradiated and control fetuses was significant in all cases when the degrees of freedom were determined by the number of fetuses. However, congenital factors, the size of the litters, the handling of the pregnant rats (stress in shipping, etc.), and so on, are so important for determining fetal weight that it seemed preferable to use the numbers of litters for determining the degrees of freedom rather than the numbers of fetuses. When this was done, the differences between the irradiated and control fetuses of the first group of Sprague-Dawley rats and of all three groups of rats taken together were no longer statistically significant.

Twenty hooded Long-Evans rats--nine of which were irradiated overnight on 11 nights as described earlier, but at 7. mw/cm^2--were brought to term. All the rats were born between noon on the 21st and noon on the 22nd days of gestation. The litters were weighed and no correlation of the litter weight and the temporal order of birth was found. The average weight of the irradiated fetuses was less than that of the control fetuses, but the difference was not statistically significant. These data would support the conclusions from the earlier experiments, namely, that no consistent effects on the growth of fetuses or of embryonic brain tissue results from low dosage microwave irradiation--electrical field intensity 5 to 7.5 mw/cm^2, E vector perpendicular to body axis, 1700 MHz for 9 to 11 exposures overnight (12 to 16 hours each) between the 6th and 17th days of gestation.

The rate of growth, the sequence in development of behavior patterns, righting reflexes, etc., and the time of maturation of behavior of the control rats and of the rats irradiated during fetal life are described in Appendix 2. The rate of growth was the same in the two groups and although some behavioral patterns were first seen in the irradiated pups no difference in the behavior or in the rate of maturation of the two groups could be used consistently for differentiating them. The most pronounced difference appeared first on the 4th day of life when the irradiated pups were more active and agile and showed better motor coordination than the control pups. In a locomotion test made on days 7 and 8, the irradiated pups traveled at least 30% further than the control pups from the starting point (the center of a 30 cm x 30 cm board) in 60 seconds. On the 10th day, however, the control pups traveled further, and thereafter the two groups were equal. Figure 5 in Appendix 2 shows a curious break in both the curves produced by plotting the distances traveled in 60 seconds against the age of the pups in days. The short duration of the effect and small degree of difference in the muscular coordination necessary to account for the improved performance would require replication to demonstrate its relation to irradiation. It would also be necessary to control the temporal relation of testing to feeding, sleeping, mother's activity, etc.

To briefly summarize the observations of behavior and of the maturation of behavior, one may say that microwave irradiation as delivered in the present experiments resulted in no functional deficiencies. Several observations recorded in Appendices 1 and 2 suggest microwave irradiation may increase growth and the rate of the development of behavior early in life. A number of factors (genetic, stress on the mothers, diurnal rhythm and so on) would have to be controlled more rigorously than was feasible in the experiments reported to validate these suggestive data.

The observations of the behavior of the experimental animals were discontinued at 8 months of age. There were 20 litters, 9 of which had been irradiated during fetal life, 9 had been manipulated in the same manner as the irradiated rats--that is, they had been restrained in holders and placed in the radiation chamber at the same times as the irradiated rats--but had been shielded from irradiation. Two litters had been kept in their home cages throughout gestation and until weaning.

ANATOMICAL DATA

At between 8 and 9 months of age 2 rats from each of the 9 irradiated and the 11 control litters were anesthesized with veterinary nembutal intraperitoneally. When unresponsive to peripheral stimulation, the chest of each rat was opened and the heart exposed. The right ventricle was widely incised and the left ventricle promptly infused through a 20 guage needle. After flushing with normal saline, the body was perfused with 10% neutral formaldehyde. The brain was then removed and stored in the same fixative. The viscera were then removed and individually examined for gross abnormalities. None were found.

Half of the brains were processed by George H. Koustenis, M.A., in the anatomy laboratory of the Walter Reed Army Institute of Research under the supervision of Mrs. Michie Vane, head technician. The other brains were sent to Dr. William R. Mehler in the Life Sciences Division of the Ames Research Center, NASA. Dr. Mehler kindly had them processed there, briefly reviewed them microscopically, and returned the sections for further study here.

After fixation was complete, the brains were dehydrated and imbedded in paraffin. Serial sections were cut at 15 micra and approximately every 20th section stained with cresyl violet and mounted.

On microscopic examination, no abnormalities other than artifacts resulting from preparation of the sections were found. The cytoarchitectonics of the cortex appeared normal throughout. The striatum and globus pallidus were well defined. The thalamic and hypothalamic nuclei showed normal organization and structure. The colliculi were well developed and the reticular formation of the mid- and hind-brains showed the typical cell types and distribution. The cranial nerve nuclei were distinct, the cerebellum was well developed, and the large fiber systems (pyramids, medial lemnisci, brachia conjunctiva, brachia pontis, restiform bodies, and so on) could be readily delineated.

The different cell types, such as the motor cells of the motor nuclei (III, IV, V, VI, VII, X, XII), the sensory cells of the trigeminal, vestibular, N. of the Tr. solitarius, the specialized cells (Locus coeruleus, Purkinje, magnocellularis reticularis) and so on showed their usual characteristics. The ependymal lining of the ventricles was intact and the Choroid plexus in the lateral, third and fourth ventricles appeared well developed.

The microscopic structure showed none of the abnormalities which had previously been found in the fetal brains. There were no old scars and no gliosis. Attempts to differentiate anatomically between irradiated and control brains failed.

SUMMARY AND CONCLUSIONS

It was noted earlier that microwave irradiation of rats during fetal life results in no abnormalities or deficiencies of behavior at the low dosages used here. The microscopic examination of sections 0.3 to 0.5 mms apart of these brains showed no anatomical abnormalities.

In Appendix 2, p. 29, the irradiated and control fetuses whose brains were microscopically examined are summarized in a table. In the early experiments (January to July, 1974), 1 to 3 exposures to radiation at 10 to 30 mw/cm^2 for 20 to 60 minutes were used. The proportion of fetuses with lesions was less in the irradiated than in the control groups, suggesting some beneficial effect of microwave irradiation. The effect was reversed in the later experiments in which the intensity of the electric field was reduced, but the duration of irradiation was increased (to more than 12 hours), more exposures were given (9), and were started at an earlier stage of gestation (the 6th or 7th day). The table did not include the rats reported on here, though they were mentioned in the discussion. When these rats are added to the long-irradiated, low-dosage group, the picture changes considerably. In the control group there are now 35 fetuses and rats with no lesions, 72.9%; and 13 with one or another type of lesion, 27.1%. In the irradiated group there are 23 with no lesions, 52.3%; and 21 with lesions, 47.7%.

It should also be noted that a very high rate of lesions, all of the same type (here designated as Type 1) were found in the L-E fetuses autopsied January 6, 1976, Appendix 2, p. 28. This finding may be correlated with the stress the rats underwent during shipment. A strike which grounded the planes of one airline, the seasonal rush in late December, and a blizzard resulted in approximately 50 hours travel time. The group of rats shipped together with this group all proved to have a purulent endometritis at autopsy (Appendix 2, p. 5, and Figure 2). In contrast, the 20 rats brought to term whose young were found to have no central nervous lesions, as described above, were shipped on 20 March, arriving at the laboratory approximately 6 hours after leaving the farm.

Besides the stress on the mother during gestation, genetic factors need to be considered in any attempt to determine the effects of microwave irradiation at low dosage. Ideally, to conduct such research, animal raising and holding facilities should be available adjoining the radiation facilities and should be sufficiently large to permit genetic studies and to supply as many as 20 date-mated, sperm positive rats at least once a month.

APPENDIX I

APPENDIX I

TITLE

EFFECTS OF MICROWAVE IRRADIATION ON EMBRYONIC BRAIN TISSUE

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AUTHOR (S)

DAVID McK. RIOCH, M.D.

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THE FINDINGS IN THIS REPORT ARE NOT TO BE CONSTRUED AS AN OFFICIAL DEPARTMENT OF THE ARMY POSITION, UNLESS SO DESIGNATED BY OTHER AUTHORIZED DOCUMENTS.

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 radition. 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:

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

The intensity of irradiation was varied in different cases from 5 to 30 mw/cm^2 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/cm^2 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/cm^2 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/cm^2 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/cm^2 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/cm^2 and 2450 MHz and nine in the chamber at 20 mw/cm^2 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/cm^2 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/cm^2 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, $P \leq 0.005$. The average fetal weight of the one-hour exposure group was 4.03 ± 0.28 gms, of the overnight group, 4.23 ± 0.48 gms, and of the control group 3.73 ± 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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APPENDIX II

APPENDIX II

INSTITUTE FOR BEHAVIORAL RESEARCH, INC.

EFFECTS OF MICROWAVE IRRADIATION ON EMBRYONIC BRAIN TISSUE

FINAL REPORT

David McK. Rioch, M.D. and George H. Koustenis, M.A.

November 8, 1977

OFFICE OF NAVAL RESEARCH

Contract No. N00014-75-C-0969

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.

References:

AR 70-18. Laboratory Animals, Procurement, Transportation, Use, Care, and Publicity.

AR 70-31. Standards for Technical Reporting.

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EFFECTS OF MICROWAVE IRRADIATION ON EMBRYONIC BRAIN TISSUE

Introduction

The work to be reported here was undertaken in an attempt to replicate an experiment in a previous study by Sharp, Berk and Rioch (1) described in the Final Report on Contract #DAHC04-74-C-0004 with the U.S. Army Research Office-Durham. A copy of the report is attached as Appendix 1. In one experiment in that study it was found that long term irradiation of pregnant rats at a low dosage [2450 MHz; 5 to 7.5 mw/cm^2; cw; for over 12 hours (overnight) on 9 nights between the 6th and 16th days of gestation] resulted in approximately 10% increased growth of the fetuses as compared with similarly handled, but not irradiated, control animals. This observation seemed worth further investigation.

It also seemed desirable to investigate another aspect of microwave irradiation, namely, the possibility that the effects of the irradiation may vary with the stage of the circadian rhythm in which the irradiation is given. In the earlier work of Sharp, Brizzee and Justesen referred to in the attached report (Appendix 1) the irradiation was routinely carried out in the late afternoon, i.e., at about 1600 or later (Sharp, personal communication). In the experiments conducted by Sharp, Berk and Rioch (described in the report in Appendix 1) irradiation was carried out between 0700 and 1300. It was therefore proposed to irradiate some pregnant rats between 0800 and 1200 and others at different times between 1600 and 2000. The weights of the fetuses could then be compared with each other and with the weights of non-irradiated, control animals to determine the effects, if any, of the circadian rhythm.

The third objective of the studies reported here was to determine whether prolonged irradiation at low dosage during fetal life would measurably affect the behavior - including the maturation of behavior - of the young rats directly after birth or later in their lives. This problem was considered independently of the problem of the effects of irradiation on fetal growth rate.

The work done on the three problems noted in the preceding paragraphs will be described in Sections I, II and III of this report. In Section IV Mr. George Koustenis will describe experiments he conducted on irradiated and control rats between 80 and 150 days of age.

The brains of one or two fetuses from most of the litters were serially sectioned and stained for histological examination. The brains of two of the rats from each litter in the study on behavior were removed and similarly prepared. The types of abnormality found in this material will be briefly described and the distribution of the lesions in the brains of irradiated and control animals will be summarized in Section V of this report.

The dated pregnancy Sprague-Dawley (albino) rats were obtained from the Holtzman Co. of Madison, Wisconsin, and the Long-Evans (hooded) rats, used in 1976, from the Blue Spruce Farms, Altamont, New York. The animals were kept in the rat colony at the Behavioral Biology Center of the Institute of Behavioral Research. For irradiation they were transported in carrying cages by automobile approximately 300 yards to the laboratories of the Department of Microwave Research of the Walter Reed Army Institute of Research.

All irradiation was conducted in one of the anechoic chambers. Mr. Peter Brown was in charge of the equipment for generating and transmitting the microwave irradiation. After a warm-up period of some 45 minutes this equipment performed in a very stable manner and seldom needed adjustment. In all our experiments the E vector of the microwave beam was perpendicular. As the position of the rat in the holder was usually horizontal and at right angles to the direction of the beam, the E vector was at right angles to the long axis of the rat's body.

In the earlier studies the rats were restrained in plexiglass boxes, 3 x 3 x 6 inches, with holes in the sides to provide ventilation. In the studies reported here larger holders were made, measuring 5 x 5 x 7 inches (inside dimensions). These holders consisted of round extruded acrylic rods, 5/16" diameter, forming the top, bottom and sides and set in 1/4" plexiglass plates forming the ends. The top acrylic rods moved freely in the holes in the end plates. They thus provided a sliding door and were fastened by rubber bands wound around the ends. A further small modification before the last experiment was a hinged door for the top made of acrylic rods fastened into two short cross pieces. Longer rods extending through the cross pieces and the end plates of the holder provided hinges and fasteners. These holders gave the animal room to turn around easily; there was ample ventilation; and urine and feces fell out into a tray.

In the initial experiments of this series (April and May, 1975) four animals were irradiated at a time. The holders were placed in the corners of a 3 x 3 ft. set of shelves, so there was a distance of 2 ft. from center to center of the holders. At this distance there was no significant distortion of the field around one animal due to the presence of another. If the distance between the cages was reduced to 1 foot, there was pronounced disturbance of the electric field.

Since irradiating only four rats in long term experiments was uneconomical, Oliva and Capravas (1977) of the Armed Forces Radiobiology Institute developed a system for placing 10 holders at different distances from the transmitting horn and at different distances above, below and lateral to the center of the beam, so that the electrical density at the different positions would be approximately equal. This system with minor modifications was adopted in the experiments conducted during the later experiments of the present series.

An attempt was made to improve the rat holders by making them out of styrofoam -300, painted with a saturated solution of quinine sulfate to keep the rats from gnawing their way out. This was a failure, as Long-Evans hooded rats were not deterred by the quinine and gnawed their way out in a few minutes. The plexiglas-acrylic rod holders, therefore, continued to be used.

Another modification of the irradiation procedure was introduced with simultaneous irradiation of 10 rats. If all the irradiated and control rats were pregnant this would require autopsying 20 rats or more in a day on terminating an experiment. In order to avoid an excessive number of autopsies on one day the rats were obtained in 2 cohorts, mated 24 hours apart. On the first day of irradiation only the first cohort was used and the second cohort was started the next day. Irradiation was also discontinued and the autopsies were performed 24 hours apart on the two cohorts. This schedule reduced the delay between first and last autopsies sufficiently to keep the extra growth time of the later-terminated fetuses from becoming significant.

I

Three Experiments on Repeated Irradiation for Over 12 Hours (Overnight)

Twenty-two female Sprague-Dawley (albino) rats mated on the night of 1 - 2 April 1975 were obtained from the Holtzman Company, Madison, Wisconsin. They were shipped by air on 3 April and received the morning of 4 April. As impregnation probably occurred in the early morning of 2 April, that day was counted as day 0 for calculating the duration of gestation. The rats were weighed the day of arrival and rank-ordered by weight. They were divided into four groups of four rats each (such that the average weight of the rats in each group was approximately equal) and two additional groups of three rats each, including five rats 20 - 30 gms lighter than the rest. The rats were kept in individual cages with food and water ad lib in the colony at a temperature between 74° and 78° F.

One group of four rats was designated for 12 hour (overnight) irradiation and another group of four for controls. (The other groups were used in experiments to determine the effect of irradiation at different stages of the circadian cycle, to be described in Section II). Irradiation was conducted as described earlier, starting at 1930 on 8 April until 0800 9 April. Irradiation was at 2450 MHz and 5 mw/cm^2, cw. The control rats were similarly handled in all respects and were placed on the floor of the chamber in the far corner some 20 feet beyond the experimental rats. They were shielded from irradiation by blocks of microwave absorbent material. The temperature of the chamber was maintained between 70° and 72° F. In the morning the rats were removed from the holders and returned to their home cages, with food and water ad lib. On successive nights the rats were rotated through the different positions at the corners of the styrofoam shelves so that they would all get the same radiation in case there were irregularities in the field density.

One of us, George Koustenis, slept in the laboratory during the overnight irradiation in April and May, 1975. An alarm bell was connected so that it would be sounded in case of any appreciable changes in the intensity or frequency of the irradiation.

The rats were irradiated on 9 occasions on the nights of 8, 9, 10, 11, 14, 15, 16, 17, and 18 April. They were then kept in their home cages until 22 April, the 19th day of gestation. They were given 1 cc veterinary nembutal i.p. and, when anesthetized, the fetuses were removed, blotted on absorbent paper, and weighed. The time from the first to the last autopsy was approximately 90 minutes.

The results are presented in Table 1 (S - D I Rad and Con). Three control rats had 20 fetuses with an average weight of 4.38gms. Three irradiated rats had 24 fetuses with an average weight of 4.7 gms. The irradiated fetuses, thus, averaged 0.32 gms. heavier, or 6.3%. In calculating the statistical significance of this difference by t test, the question arises as to whether the degrees of freedom should be based on the number of fetuses or on the number of litters. Using the first criterion - number of fetuses - the difference in weight of the control and irradiated rats is significant by t test at the .05 level, but it is not significant if based on the number of litters (Table 2). It may be noted that the smaller control litters favored larger fetuses, so the difference between the control and irradiated fetuses is probably less than equal sized litters would have given. (See Figure 1).

The second group of rats used in this experiment were also Sprague-Dawley albinos from the Holtzman Company. They were mated the night of 15 to 16 April, shipped on 17 April and received on 18 April, 1975. There were 20 rats. They were divided into 5 groups of four rats each on the basis of weight so that the average weight of the rats in each group would not differ significantly. As in the first experiment of 8 to 22 April, a control and a 12° (overnight) irradiation group were selected. The procedure was the same as on the former occasion in all respects. Irradiation was conducted on 22, 23, 24, 25, 28, 29, 30 April and 1 and 2 May. The autopsies were performed on 6 May. Three control rats had 11, 11 and 2 fetuses respectively. Three irradiated rats had 8, 9 and 4 fetuses. The average weight of the control fetuses was 3.67 gms and of the irradiated was 3.98 gms (Table 1). The difference, 0.31 gms or 8.5%, is significant by t test at the .01 level if the degrees of freedom are based on the number of fetuses, but is not significant if the number of litters is used (Table 2 and Figure 1).

The third 12-hour (overnight) experiment was carried out in December 1975 - January 1976. The use of albino rats raised some questions as they are known to have rather frequent congenital abnormalities of the visual system. It was therefore decided to use Long-Evans hooded rats. Twenty dated pregnancy, sperm positive rats were obtained from the Blue Spruce Farms, Altamount, New York. Ten of these were mated 16-17 December and the other 10 on 17-18 December 1975. Both groups were shipped on 21 December in two cartons fastened together, the second group below the first. Due to an airline strike, the Christmas rush, and a blizzard, the rats spent more than 50 hours in transit and were probably exposed to considerable cold. They appeared active and healthy on arrival, however. In order to let them rest, drink and feed before irradiation, the experiment was started with the first group on 24 December and the second group on 25 December 1975, i.e., each on the 7th day of gestation instead of the 6th. In both groups five rats were irradiated and five used as controls. Thus from 25 December to 6 January 10 rats were irradiated simultaneously at 2450 MHz, and 5.0 mw/cm^2, cw. On the 2nd day one of the irradiated rats escaped in the preparation room and was loose for 2 days before being caught. One of the control rats of the second group was transferred to the irradiation contingent and the escaped rat kept in her home cage - as a "home-cage control" with food and water ad lib after she was caught. It may be noted here that at autopsy this rat's fetuses were slightly heavier on the average than any other irradiated or control fetuses. The question thus arose as to whether the restraint overnight in the anechoic chamber without food or water represented sufficient stress to reduce fetal weight. If so, one might have to assume that the irradiation counteracted this stress, since the irradiated fetuses were heavier than the "chamber" controls.

Irradiation was given on 11 nights, 24, 25, 26, 28, 29, 30, and 31 December 1975 and 2, 3, 4, 5, and 6 January 1976. On the night of 1 - 2 January there was equipment failure and the rates were irradiated for only 1 1/2 hours. The temperature of the chamber was maintained between 70° and 72° F throughout.

In the group of rats mated 16 December and autopsied on 6 January there were four pregnant irradiated rats and four pregnant control rats. The number of fetuses per litter and their average weights is shown in Table 1 (L.E. Rad and Con). In the four irradiated litters there were 42 fetuses with an average weight of 4.33 gms. In the four control litters there were 53 fetuses with an average weight of 4.07 gms. The difference was 0.26 gms, or 5%. When the degrees of freedom were based on the 93 fetuses this was significant at the

.0005 level. With 8 litters used instead, giving 6 degrees of freedom, significance was at the .01 level (Table 2 and Figure 1).

In Figure 1 is plotted the relationship of the number of fetuses per litter to the average weight of the fetuses per litter for the two groups of Sprague-Dawley rats and the first group of Long-Evans rats. The statistical significance of these three groups separately and when combined is shown in Table 2, both by t test and by analysis of variance. Also, the calculations were carried out first on the basis of the number of fetuses and then on the basis of the numbers of litters. The latter seems to be the preferable basis for this set of experiments although the small number of litters results in reducing the degree of significance.

The second group of rats, mated on 17 December 1975, came to autopsy on 7 January 1976. It was then found that the pregnant rats all had a purulent endometritis, which showed as a greyish yellow exudate forming a ring around the placenta as well as over the implanted surface. The irradiated rats showed much less exudate and had more fetuses, all living. Only three of the five control rats had fetuses, including several dead and resorbed (Figure 2). The purulent endometritis was diagnosed when sections were taken of an affected placenta. No bacteria were recognizable in the sections. The finding was not anticipated and no cultures were taken. The endometritis was probably related to the stress during travel, although no symptoms of illness were recognized during the period of irradiation, 23 December to 7 January. The pronounced difference between the irradiated and control animals suggests that the irradiation had some salutary effect. None of the rats of this group could be used for estimating the effects of microwave irradiation on fetal growth.

If one combines the three groups of rats which were irradiated for 12 or more hours 9 to 11 times between the 6th and 16th days of gestation and the respective control groups, there are totals of 20 litters and 182 fetuses. The difference between the average weights of the irradiated and of the control fetuses is significant at the .0005 level by t test if the degrees of freedom are based on the 182 fetuses. However, if the 20 litters are used as the basis for the degrees of freedom, the difference is not significant (Table 2).

It would appear that there are a number of factors, including the weight of the mother, the number of fetuses per litter, and inherited characteristics which are as potent as or more potent then microwave irradiation for determining fetal growth rate. A parametric study to measure the effect of microwave irradiation would require, at a minimum, a large enough rat colony to be able to supply dated pregnancy females of known characteristics as needed for the experiments. This would avoid shipment and the attendant stress which probably contributed much of the variability encountered in the measures reported here.

In the experiment on comparison of the behavior of rats irradiated during intra-uterine life with that of non-irradiated rats (see Section III, p. 13), the opportunity occurred to weigh each litter on completion of parturition. This was done and all but 3 or 4 litters were weighed immediately after the birth of the last fetus. All litters were weighed within one hour of the completion of parturition. Taking the average weight of the newborn pups of nine irradiated litters compared to that of seven chamber-control litters, it was found that the control pups weighed more, but that the difference-- 0.2 gms or 3.6%--was not significant by t test using the number of litters to determine the degrees of freedom. Two rats were maintained as home-cage controls in this experiment. They were kept in their home cages with food and water always available. Both were pregnant. The average weight of the young of one of them was among the heaviest, and of the other amongst the lightest found in this experiment. The difference in the average weights of the fetuses could not be explained either by differences in the weights of the dams or by the numbers of fetuses in the two litters, which differed only slightly.

The results of 12 hour (overnight) irradiation are summarized in Table 1. Although there was a tendency for the irradiated fetuses to weigh more than the controls, this was not significant by t test when the degrees of freedom were based on the number of litters involved (rather than on the number of fetuses). It appears clear that the irradiation used--2450 mHz, 5 mw/cm^2, cw, for 12 or more hours on 9 to 11 nights between the 6th and 16th days of gestation--resulted in no overt abnormality. Indeed, the microwave irradiation may well have been salutary in the case of the group of rats with purulent endometritis.

II

The Effect of Irradiation for Shorter Periods at Different Times in the Diurnal Cycle

During the same periods that the overnight irradiation of the Sprague-Dawley rats was carried out, other dated pregnancy Sprague-Dawley (Holtzman) rats were divided into groups for 1 hour irradiation in the morning and in the late afternoon as well as a nonirradiated control group. Fourteen rats mated on the night of 1-2 April were divided into two groups of four each and two of three each. One group of four was irradiated at 2450 MHz, and 10 mw/cm^2, cw, from 0900 to 1000 AM. A group of three rats was handled similarly, but shielded from irradiation, also from 0900 to 1000. A group of four was similarly irradiated from 1640 to 1740 and a group of three from 1800 to 1900. Unfortunately only two rats in the morning irradiation group were pregnant, one with five and one with only two fetuses. Only two of the three control rats were pregnant. These were the critical groups. In the other two groups (irradiated 1630 to 1730 and 1800 to 1900) one rat only had two and another only three fetuses. The third in this group was not pregnant. No conclusions could be drawn from these data.

A similar experiment was carried out in conjunction with the second 12-hour irradiation run, i.e., using rats mated 16-17 April. Twelve Sprague-Dawley rats were obtained and divided into three groups of four each. One group was irradiated 0900 to 1000, another from 1630-1730 and the third was confined in the holders in the irradiation chamber, but shielded from irradiation, from 0900 to 1000. All were terminated on 6 May 1975. Unfortunately, three of the four control rats were not pregnant, one of the morning irradiated group was not pregnant, another had only one fetus and one of the afternoon irradiated rats was not pregnant. The groups thus were too small to permit any valid comparisons.

A third experiment on short-term irradiation of dated pregnancy rats morning and afternoon was carried out from 14 and 15 January to 4 and 5 February 1976. Twenty-one Long-Evans dated pregnancy rats (mated on 1-14-76) were obtained on 1-19-76 and divided into five sub-groups as follows:

five rats in each of two irradiation groups--0900 to 1200 and 1630 to 1930 (at the same time as the first contingent) on 12 occasions from 22 January to 3 February; two rats in the a.m. and four rats in the p.m. chamber-control groups; and three rats as home cage controls.

The results of irradiation were as follows:

9 rats irradiated 0900-1200 average fetal weight	3.31 gms.
10 rats, control (4 chamber; 6 home cage)	3.21 gms.
8 rats irradiated 1630-1930	3.25 gms.
10 rats, control (4 chamber; 6 home cage)	3.24 gms.

None of the differences between the fetal weights was significant when the degrees of freedom were based on the number of litters. The greater average weight of the fetuses irradiated in the forenoon was directly counter to the hypothesis on which the experiment was based. Further, the results of irradiation for three hours repeated 12 times from the 6th to the 17th days of gestation showed no significant effect on the growth rate of the fetuses.

III

The Effects of Microwave Irradiation During Fetal Life on Behavior After Birth and Later in Life

The third area investigated was, in many respects, independent of the effects of irradiation on anatomical growth rate. Instead it proposed to investigate the question as to whether microwave irradiation during fetal life would affect the form of behavior or the maturation of behavior of the irradiated fetuses after birth and later in life. In order to investigate this area, 22 dated pregnancy Long-Evans (Blue Spruce Farms) rats were obtained. They were mated on the night of 16-17 March and shipped and arrived 20 March. The rats were weighed, identified by punching holes in their ears and housed in individual cages with food and water ad lib. Two rats were kept as home cage-controls. Twenty were divided randomly into two groups, the irradiated (R) and controls (C). Both groups were treated in an identical manner excepting for the irradiation. Both groups were transferred from their home cages to carrying cages and taken by automobile to the Department of Microwave Research of the Walter Reed Army Institute of Research. They were then transferred to the holders, 10 for exposure to microwave radiation and 10 shielded in the far corner of the chamber for chamber controls. The two home cage control rats were left undisturbed in their cages with food and water ad lib.

Irradiation was at 2450 MHz and 7.5 mw/cm^2,cw. It was given for over 12 hours (12.5 to 14 hours) on each of the nights of 23, 24, 25, 26, 28, 29, 30, and 31 March and 1, 2, and 3 April - a total of 11 nights from the 6th to the 17th days of gestation. The temperature of the radiation chamber was maintained at 72° F. throughout. The 10 experimental rats were irradiated simultaneously as previously described and were rotated through the 10 positions in the field during the course of irradiation by being advanced one position each night.

From the 18th day of gestation the rats were housed in polystyreme breeding cages with food and water ad lib and shredded news-print for building nests. One of us (George Koustenis) observed the rats hourly from the 19th day of gestation in order to compare parturition in the irradiated and control rats. However, no behavioral differences between the R and C rats were observed during the period of irradiation, the pre-delivery period, or the period of delivery and cleaning the newborn pups. A tendency of the R mothers to be away from the pups and nests to a somewhat greater extent than the C mothers during the first half of the period of nursing and the greater tendency of the C mothers to retrieve young during the middle of the period of nursing will be mentioned again later.

One each of the R and C rats proved not to be pregnant. Twenty litters were delivered between noon on the 21st and noon on the 22nd days of gestation. It may be of passing interest that whereas the C litters were spaced an hour or two apart throughout the period, 7 of the 9 R litters were delivered between approximately 0300 and 0530 on the 22nd day. In most of the animals parturition was completed in 40 to 60 minutes (9 to 15 pups). Two C females, however, which started delivery at 2200 and 2330 hours on the 21st day, gave birth to 4 pups each, cleaned them and started nursing. The observer at the time (DMR) concluded that delivery was complete, weighed the litters and continued other observations. In the morning these two rats were found to have 10 and 11 pups respectively. (They are omitted from Table 1) In the last R and the last three C animals to deliver, however, parturition continued from 2 to 3 hours and 2 pups in one C litter and 1 pup in another were dead.

As soon as a litter was delivered and cleaned by the mother, she was removed from the nest and placed in a holding cage. The observer then picked the pups up by hand and weighed the whole litter, returning it promptly to the nest. The mother was then returned to the nest. The whole operation required between 1 and 2 minutes. Apart from some brief sniffing the pups and the nest area, the mothers showed no disturbance. Handling the pups with the fingers rather than using forceps saved considerable time and was less likely to result in trauma. The pups were similarly lifted and manipulated by hand in testing the righting reflexes, locomotion, response to a tilting platform or rotating platform, and so forth. The decision to handle the pups by hand was based on the reputation of Blue Spruce Long-Evans rats for gentleness and calmness on being handled. Preliminary tests of the procedure were carried out in an earlier study of Long-Evans female rats and their litters.

One R litter had only 5 pups, but the others had from 9 to 15 pups each. On day 3 all litters above 10 were reduced to a maximum of 10 pups and on day 4 they were further reduced to 7. Since the majority of the litters were delivered between 2200 and 0600 on the night of 7 to 8 April the age of the pups was recorded assuming 8 April as the date of birth.

Observations were made on the mothers and pups several times a day and late evening. Attention was paid to the progressive maturation of behaviors (cf. Bolles and Woods, 1964), such as locomotion, and also to the time of appearance of new patterns, such as the startle response to a hand clap or click, the mature righting response during free fall, and so forth, (Hard, et al, 1975). It was not possible to make quantitative determinations of much of the behavior observed. In most of the slowly developing patterns - e.g. locomotion - there was great variability in performance by one animal from time to time, even from day to day. A few patterns of behavior could be more precisely timed or measured. However, the mother's schedule of feeding and the consequent schedule of the pups' sleep could determine whether one litter or another showed a particular reaction earliest. It must also be noted that on any one day tests could only be run on a fraction of the litters on account of time limitations.

In the following paragraphs the appearance, development, and maturation of a number of behavior patterns will be described briefly. The main attention in the course of this work was to determine whether reliable differences between the R and C rats occurred, rather than to describe the ontogenesis of behavior.

Each litter was weighed as a whole at the time of birth and every three days until weaning. The average weight of the pups was determined and is shown for the first 17 days in Figure 3. The same proportionate differences found at birth were maintained throughout this time. The young rats were less frequently weighed after weaning, as shown in Figure 4. No difference in average weight or in rate of growth between the males and females was found during the first five weeks after birth. Thereafter the males gained weight faster than the females as shown in Figure 4. During the first 17 days - Figure 3, the difference in weight of the irradiated, the chamber control and the home cage control litters remained the same, indicating no difference between them in the rate of growth.

8 April. On the day of birth, locomotion was largely by squirming and wriggling, the legs fully extended in abduction laterally. The head weaved from side to side, but the snout was not raised from the floor. The legs were swung forward and back, with some slight downward pushing on the backward stroke helping to move the pup forward. No flexor movements of the legs occurred in locomotion. The forelegs at times alternated, but at times seemed to move independently. No coordination between the hind legs of the two sides or between the fore- and hind legs was seen. When placed on their backs, pups usually responded promptly, but might lie for up to one or two minutes without moving. In righting, the hind legs were adducted and fully extended caudalward. The head and shoulders were ventro-flexed and then turned to one side or the other quickly. This might carry the pup far enough over so that some further wriggling would bring it upright. The process required several seconds or might be interrupted by "rest" periods and not accomplished for a minute or more.

Any contact with the mother or other pups resulted in greatly increased activity. Each pup tried to burrow under the next, but crawled over if burrowing failed. In the nest they tended to lie parallel and there was continuous movement except during "sleep". The uppermost pups would burrow down between pups below them, the snout being continually waved from side to side, probing for a space to burrow into and then seemingly used as a wedge. No difference between the irradiated and control pups was apparent.

9 April, Day 2, the degree of activity of all pups increased and they raised their heads off the floor in swinging them from side to side. The snout seemed to be a major source of sensory input, especially in directing action on contact with siblings or the mother. When a pup was placed horizontally on a tilted board (approximately 25°) the head turned toward the upper side and all four legs were extended. The movement of the head was slight, only some 5° to 10° from the midline. Locomotion was inhibited. No differences between the R and the C pups were noted.

It may also be mentioned here that during the first week to ten days the R mothers were found off the nests and away from the pups more frequently than the C mothers when the observer came into the colony room at different times of the day. The growth curves of the pups demonstrated that the R pups did not suffer any food deprivation, but the relative restiveness of the mothers may have played a role in the slight precociousness which the R pups showed in the development or use of some behavior patterns as noted later in this report

10 April. By day 3 righting had become prompt and occasional flexion of the forelimbs was apparent. When placed on a tilted board the head was turned toward the upper side, the upper limbs began to be flexed, and the lower extended. When placed head downwards all four limbs showed increased extension. With the head to the upper side all activity was reduced. No difference was found between the irradiated and control pups.

On day 3 the litters were reduced to 10 pups, maximum, and on day 4 to 7.

11 April. On day 4 it was clear that flexor movements of the legs were being used by the pups in locomotion, in balancing and in righting when rolled over on to their backs. The legs were beginning to be adducted during locomotion, though they did not yet support noticeable weight. Locomotion was still chiefly by wriggling on the stomach, using the legs, extended laterally, to push forward. The righting response, when placed on the back, had become active, prompt and brisk, requiring only 1 or 2 seconds. This was facilitated by flexion of the under and extension of the over foreleg. The hind legs, however, still might merely be dragged or used fully extended without any clear coordination. If one hind leg remained under the pup when it turned over, or when it lost balance and fell, the pup usually pivoted around on the hip as a center until the hind quarters rolled upright. Locomotion then occurred with the use of all four legs extended laterally.

There was no qualitative difference between the R and C pups, though slightly greater activity of the controls was noted by Mr. Koustenis.

12 and 13 April. On days 5 and 6 the changes in behavior were continuous, but hard to define as there were no qualitative changes. Coordination of the trunk and leg movements gradually improved with increased use of flexor movements. The legs were steadily more and more adducted, so that they began to bear some weight. This was noted late on day 5 and more generally on day 6. With the weight bearing a coarse tremor of about 8 to 10 per second appeared. This was first noted in a few of the irradiated rats, but was general within 24 hours. The head was raised well up and continued to be waved from side to side during locomotion but was not necessarily coordinated with foreleg movements. The snout still appeared to be the important source of information for guiding forward movement.

The greater activity of the irradiated compared with the control pups suggested on day 4 was obvious and was noted by both observers on day 5. The irradiated pups also showed better coordination in locomotion than did the control pups. No new behaviors were noted other than the tremor associated with weight bearing on the legs.

14 and 15 April. On day 7 a more quantitative method for testing locomotion was devised by Mr. Koustenis. A flat board, approximately 30 x 30 x 0.5 cms., was covered with adhesive tape to improve traction. It was divided into

4 quadrants. A pup was placed at the center of the board and the distance traveled from the center in 60 seconds was measured. Four pups from each of half the litters were tested on alternate days, so all the litters were sampled. The gradually-improving ability to stand, walk and run was noted and the direction of movement recorded.

Most pups would pivot around on one hip, with that hind leg under them for up to some 30 seconds before getting "started", as it were. This activity was promptly initiated, but actual travel was always delayed. On the first trial and on the succeeding three days the irradiated pups traveled 30% or more distance than the control pups. The latter spent more time pivoting at the start and whenever they stumbled or lost balance and fell. (See Figure 5.)

16 April. On day 9 two observers examined in succession six litters--three irradiated and three control--"blind" (i.e., without knowing which was which). On the basis of the agility, coordination of movement, and overall activity of the pups they correctly differentiated five of the six groups. It was also noted that the behavior of the irradiated and control pups differed when they came to the edge of the test board. Fourteen out of 20 irradiated pups continued off the board over the surface of the table until they came to the edge of the table and one of the forelegs extended over the edge. They then stopped, pulled back, and turned. Only seven of the 20 control pups continued off the test board. (It may be noted here that the eyes did not open until day 13.)

17 April. On day 10 it was noted that both the irradiated and control pups were walking without the belly touching the floor. Walking was accompanied by the 8-10/sec. tremor, but its amplitude was reduced. However, walking was awkward, with frequent loss of balance and falls. Recovery from falling and resumption of walking were more rapid for the R than for the C pups. The R pups continued to out-distance the C pups in the one minute locomotion test situation. Measurements of the distance traveled in 60 secs by the R and C pups were continued through day 14.

When the average distance traveled by the R and C pups was plotted by days a curious phenomenon appeared, shown in Figure 5. There seemed to be two processes controlling locomotion with a shift from the earlier to the later occurring on the 10th day in the R pups and on the 11th day in the C pups. The more mature process gives a much more regular and smooth curve than the earlier process. It is of interest that Flexner (1955) described the growth of fibers from the forebrain from about the 7th to the 11th day of life which brought lower centers under forebrain control.

On day 10 rudimentary grooming behavior was seen for the first time. It consisted of bringing both forepaws up to the sides of the head and snout with some abortive movements along the snout, but no licking of the paws. It occurred when the pups were lying in the nest with others. This was seen in all of the four irradiated pups which were watched for several minutes each but in none of the control pups similarly watched. This behavior became general in both groups during the next two days.

18 April. On day 11 the control pups for the first time out-distanced the irradiated pups on the board for testing locomotion. Following this reversal of the previous findings, the two groups responded similarly and the distance traveled no longer differentiated between them.

It was noted that when the pups tested were returned to the breeding cages, the control mothers were much more active than the irradiated mothers in seizing the pups and carrying them promptly back to the nest area. On occasion they would grab the pup out of the experimenter's fingers as he was putting it in the cage. The irradiated mothers showed no such "interest". This maternal behavior lasted two days and then subsided.

On day 11 "sniffing" behavior was first noted in both R and C pups. The head would bob to one or the other side, the snout extended, or the head would be extended in the midline, up and back, with full extension of the neck. Movements of the muzzle then occurred - twitching of the nose and of the vibrissae. Both irradiated and control pups demonstrated this behavior.

The rudimentary self-grooming behavior seen in four R pups on day 10 was now also seen in C pups. It could not be used to differentiate the two groups in either frequency or form.

<u>19 April.</u> On day 12 a moderately loud, high-pitched click evoked a startle response in all pups of both groups. None showed this response on day 11. The external meatus of both ears was now found to be open for the first time.

Hard's test for righting in free fall was introduced (Hard et al., 1975). When the pups of both groups were gently held upside down until they stopped struggling and were then dropped a distance of 40 cms. they landed on their backs (on paper covering some loose shredded newsprint).

<u>20 April.</u> On day 13 one R pup's eyes were partially open. A few more opened on day 14.

<u>21 April.</u> On day 14, 2/12 irradiated pups and 4/12 control pups righted during a free fall of 30 cms., but not of less.

<u>22 April.</u> On day 15, 12/34 irradiated and 25/33 control pups had their eyes open. In free fall from 10 cms. at 1330, 7/10 irradiated and 6/10 control pups righted and landed on their feet. However, at 1600 16/18 irradiated and 12/18 control pups righted when dropped from 5 cms. above the landing level and landed on their feet. Muscular coordination steadily improved so that walking and trotting at times appeared adult. The tremor still persisted, but the amplitude was much reduced. No difference could be observed between the irradiated and control pups as to stance and locomotion at this time. Also both groups balanced equally poorly when placed on a narrow (1/2 inch) board. The hind legs fell off every few steps.

<u>23 April.</u> From day 16 on both the irradiated and control pups righted during free fall of 5 cms. on most occasions when dropped.

<u>24 and 25 April.</u> Play was first seen between two irradiated pups on day 17, but rapidly became general.

When observed in an open area surrounded by a sheet iron boundary--2 ft. x 4 ft.--the pups huddled in a near corner. One or two would then leave the huddle briefly and run along the margin in the angle formed by the boundary with the floor. At first they promptly returned, but gradually "explored" further and were followed by others. During the first two or three days when tested in this open area, the R pups appeared more active and explored further and oftener than the C pups. After a few trials no difference between the two groups was apparent. However, when the lighter of the home cage control litters was tested, the pups remained in the huddle they formed in a corner of the enclosed area and made no attempt to explore further during the 20 to 30 minutes under observation. When picked up and placed 2 to 3 feet away from the huddle they promptly ran back. Locomotion, however, was performed just as well as by the pups which promptly explored the environment. This home cage control litter had not been tested or handled routinely, as had the other litters. One gets the impression that although discrete behaviors, such as, walking, trotting, and so on, are not noticeably affected by "practice", the objectives to which they are directed are strongly determined by previous experience.

26 April. On day 19 R and C pups were observed to eat solid food -- rat pellets in the food hopper. They were also seen to engage in play fighting and wrestling and in mutual grooming as well as in self-grooming around the genitals. A rapid hopping locomotion as well as trotting was observed. The rapid coarse tremor had almost disappeared. Pups were also seen to sit on their haunches and bring the body upright without support from their forelegs. This stance was maintained for only three or four seconds. Sitting on their haunches bent over forward to groom had been present two or three days.

29 and 30 April. The pups were separated from their mothers and housed one or two to a cage, all males or all females, on day 22. Some rudimentary sex behavior--attempts at mounting--which changed promptly to play boxing and fighting were seen on day 23. During the succeeding week, when the pups of one litter were observed together, mounting behavior by males and females indiscriminately occurred not infrequently. None of these behaviors differentiated the R and C groups.

A number of exploratory experiments were conducted on the young rats, both irradiated and control, during the period from 40 to 80 days of age. The objective of these tests was to determine whether any qualitative or any pronounced quantitative differences in behavior between the two groups could be demonstrated. Any such difference would be useful in longitudinal studies to follow the course of the changes occurring in the irradiated and control rats with increasing age. No clear and obvious differences between the two groups were found. Consequently, more detailed studies of maze running and of taste preference threshold for Valenstein's saccharin-glucose solution were conducted by Mr. Koustenis during the 3rd, 4th and 5th months of the lives of the young rats.

The exploratory tests between the 40th and 80th days included the following: rate of learning and swimming a water maze built in the form of a Lashley III maze; behavior in an open field, 4 feet by 2 feet in size; threshold of head turning, in the counter rotational direction, when the rat was placed at the center of a rotating table at different rates of acceleration; response to rapid rotation for several seconds (20 to 30).

After the studies on maze running and taste preference threshold--i.e., at 6-1/2 to 8-1/2 months of age, 15 September to 15 November--a further attempt to repeat earlier tests was made. Taste preference threshold was tested on two of the irradiated rats and 2 control rats. No difference between them was found. Several attempts to study maze running, even after food deprivation had reduced the rats' weights to 80% of eating ad libitum, were unsuccessful. On reaching the "goal" box the rats would merely sniff the food there and walk away, sniffing here and there in a slow stereotyped fashion.

Neither the irradiated, nor the control rats would run the maze. They also would hang on to a narrow board, 4 feet x 4 inches x 1/4 inch, and remain motionless unless a strongly aversive noise--a loud clap--was made repeatedly close behind them. They also failed to rope-climb to escape swimming in a tank of water at approximately 22° C.

Most of the animals tested had lived during the summer in individual cages with food and water ad lib. Some, however, had been used in studies providing more activity, such as running in cages with running wheels. No explanation for the inactivity of the irradiated and control rats when tested in the fall was apparent. However, there was no difference between the two groups in this regard.

At approximately 65 days of age, four irradiated females and four control females from the young rats irradiated during fetal life were kept in cages with running wheels of the type designed by Dr. Curt P. Richter. This study of activity--i.e., running behavior--proved unsuccessful, as the rats failed to develop a steady pattern of running. There were several difficulties which are sufficient to explain the irregularity of the results and which were not corrected during the period of the trials. The project had to be terminated before the errors could be corrected. The errors included irregular noise and disturbance in the laboratory, as an isolated room had been temporarily requisitioned for some post-operative infant monkeys. Much of the disturbance was due to noises at night and other irregular times from adjoining units in the building housing our laboratories. Mechanical difficulties causing the running wheels to stick also were not corrected during this period. However, the irregularity of the rats' running behavior showed no differences between the irradiated and control groups.

From approximately the 80th to the 150th days of age, Mr. George Koustenis studied maze running and taste preference threshold on a number of irradiated and control rats. He has described these experiments in Section IV of this report, which follows.

IV

(George H. Koustenis, M.A.)

Several sets of experiments were conducted during June, July, and August, 1976. In each experiment, equal numbers of rats exposed to microwave radiation during fetal development and similarly handled chamber control rats were used. The experiments were designed to compare experimental and control groups across a number of behavioral and biological parameters. The purpose of this series was to determine if discriminable differences existed between irradiated and non-irradiated subjects, and to identify any such variances.

Taste Preference

Valenstein (1967) has reported that rats will consume massive amounts of a solution combining specific proportions of saccharin and glucose. The reinforcing properties of this solution are so powerful that a rat may consume a volume equal to or greater than its body weight within a 24-hour period. The present investigator employed this solution to determine the taste preference in both irradiated and control rats.

Baseline measures of daily water consumption were first obtained. These data did not indicate any differences between the two groups with regard to daily water intake. The amounts of fluids drunk were measured by the use of 100 ml calibrated drinking tubes. The animals were presented Valenstein's solution, which is 0.25% saccharin and 3.0% glucose by weight dissolved in tap water (S-G solution), in one calibrated tube and tap water in another. The animals replicated Valenstein's reports and consumed the entire 100 mls within several hours, ignoring the water.

When the consumption of a given concentration of the S-G solution reached a stable rate for three days, the solution strength was changed. Each change in concentration thereafter was a 50% dilution of the preceding steady state strength. Thus, from 0.25% saccharin-3.0% glucose, the next concentration was reduced to 0.125% saccharin-1.5% glucose. A total of 10 different concentrations were used during the course of the experiment. The most concentrated solution was 0.25% saccharin-3.0% glucose, and the most dilute was 0.0005% saccharin-.00625% glucose.

Initially, the 24-hour continuous presentation method as reported by Valenstein was used for testing the taste preference. However, Cagan and Maller (1974) reported that post-ingestional factors may play a role in taste preference experiments. They suggested that a brief, single stimulus presentation method should be used to determine what role, if any, those post-ingestional factors may play in such studies. In the present experiment, both methods were employed to study the responses of the irradiated and control rats. Testing was continued until the daily rates of water and S-G consumption were observed to be equal for a five-day period.

Figure 6 presents points representative of average consumption rates for experimental and control rats over the range of concentrations which were tested. The ratio of the mls of water consumed divided by the mls of the S-G solution consumed was calculated to illustrate the degree of preference within and between the groups. The control rats preferred the saccharin-glucose solutions to water down to the extremely small concentration of 0.001% saccharin and 0.0125% glucose. However, the irradiated rats preferred the S-G solution when the strength was reduced to a concentration of half that strength, namely, 0.0005% saccharin and 0.00625% glucose. At this same strength, the control rats were consuming equal amounts of water and the S-G solution. This finding was replicated several times during the course of the experiment with six different pairs of rats.

A method was designed to compare the food consumption rates of irradiated and control rats.* Six pairs of rats, matched for weight and sex, irradiated and control, were employed for these observations. A quantity of

* The original intention was to compare taste preference using food instead of water. Saccharin and glucose in the proportions found to be optimal by Valenstein (1967) were added to the food by the method described by Teitelbaum (1962). The experiment was not carried further than described here due to repeated difficulties with the air conditioning equipment in the laboratory.

standard lab chow was weighed to the nearest 0.1 gram and given to the rats for their daily feeding. The cages were examined after 24 hours and the remaining chow was removed and again weighed to the nearest 0.1 gram. Sheets of aluminum foil had been placed beneath the cages to catch excess food, which fell through the cage, as well as the feces and urine. The foil was also weighed before placement and again after 24 hours. From the data the consumed food could be determined with sufficient accuracy for the purposes of the experiment.

At this time the air conditioning system of the laboratory broke down and could not be repaired for four days. During this breakdown, the laboratory temperature reached 84° F. The observations on food consumption were continued throughout the period of hyperthermia. Prior to the failure of the system, both irradiated and control rats consumed approximately equal amounts of chow. During the hyperthermic conditions, food consumption decreased in both groups. However, the controls were observed to decrease consumption by 25% of baseline rates, while the irradiated rats decreased their consumption by 13-15%. When the laboratory temperature was returned to normal, both irradiated and control rats returned to similar rates of consumption.

This incidental observation was not further investigated. It is reported here for its own interest and also because a behavioral change in the same direction, correlated with an increase in ambient temperature, was later found in tests of maze running activity. Another breakdown of the air conditioning equipment, lasting 24 hours, occurred with ambient temperature in the laboratory reaching 84° F. During this period, as will be described more fully later (page 17) the irradiated rats again showed less change in behavior than the control rats.

Maze Learning

Another component of the irradiated-control test battery used was training a rat to traverse a modified* Lashley III maze (Lashley, 1963) in order to obtain food reinforcement and then to study maze behavior.

The 8 rats--4 irradiated and 4 control--used in the maze studies were 60 to 80 days old when first exposed to the maze under deprivation conditions. They were first food-deprived until they were reduced to 75% of their free feeding body weight. The rats were also deprived of all food for 20-22 hours preceding each session. Standard food pellets (Noyes, 0.045 gram) were placed in a feeding cup at the finish position in the maze. In shaping the rat to run the maze, it was first necessary to allow the animal to discover that food was available. The first series of shaping behaviors involved placing the rat and food at opposite ends of the same channel. When the observer released the animal, it would usually dash immediately to the food cup some 3.5 feet away. This procedure was continued for several days, until the rat reliably would run straight for the food cup when released. The next phase of shaping involved placing the rats just beyond the gate which opens into the final channel; thus, the rat must now pass through the gate, turn and then proceed down the final alley to obtain food. This phase continued until the rat would consistently pass through the gate and then race for the food cup.

* The maze was modified by adding a "start" and a "goal" channel, making 5 channels in all, instead of start and goal boxes and 3 channels. The rats were started from the end of Channel 1 and the food was placed at the end of Channel 5.

The weights of the rats were carefully monitored every day after each session to ensure that weight was maintained at a healthy level. Minimum food requirements were determined for each subject, and just enough food was delivered after the sessions to maintain weight at 75% of the free feeding weight.

The rat, having been shaped to turn through a gate and then run down the alley to obtain food, was now placed in the first channel. A total run would now require a series of alternating right-left turns, through five channels, each 3.5 feet in length. When first placed in the maze under the new contingencies, the rat would usually spend some time exploring the channel. Then he would proceed from one channel to the next, until finally reaching the fifth channel and would then race to the feeding cup. After several trials of exploring and committing errors in turning and by back-tracking through the maze, both irradiated and control rats began to traverse the maze with high levels of speed and accuracy.

Observations of the subjects throughout the maze trials included number of errors, type of error, time to traverse the maze, and clinical observations of behavior while in the maze. Both groups of subjects were equivalent in time, number of correct trials, and type of error; however, the control rats committed more errors throughout the experiment than did the irradiated. Figure 7 presents the sum of the errorless trials for the irradiated and control groups during the sessions on running the maze. There were 5 trials per session. The difference between the two groups is not significant, though after the 25th trial the irradiated rats performed better.

An incidental observation was made during a 24-hour period in which the ambient temperature of the laboratory reached 84° F. Prior to the temperature change, the irradiated and control rats were showing similar error rates. During the trials when the temperatures reached 84° F, the irradiated rats performed almost without error. However, the error rate of the control rats increased dramatically during the same time (the 31st to the 35th trial inclusive). Both groups returned to previous patterns of error frequency when the temperature returned to normal. (Figure 8).

In summary then, it appears that irradiated and control rats do not respond to severe heat in a similar manner with regard to motor behavior, consumption of solids and liquids, and trial performance. These observations remain clinical and should be replicated under more controlled conditions. They should also be continued over longer periods of time.

Sexual Behavior

Ten irradiated and 10 control rats were used to study sexual behavior. The 20 rats were divided into 10 pairs with one female and one male per cage, both irradiated or both controls. This was the first time since four weeks of age that the rats had been together in a cage. At this time, the rats were approximately five months old. Comparative data and material on the normal sexual behavior of the rat was obtained from Munn (1950) and Barnett (1963).

The males were placed in the breeding cages for 24 hours prior to introducing the females, to allow for adaptation and reduce the probability of the male attacking the female. The females were introduced in the late evening, as 2400 to 0400 is the time reported for best breeding results. Almost immediately, the two rats would begin sniffing and grooming each other, and within twenty minutes all of the pairs were observed to copulate. All aspects of behavior for both males and females were well within normal limits, and no differences between irradiated and control pairs were observed.

The pairs were left together for ten days, which should have allowed for at least two estrous cycles in the females. At this time, the males were returned to their individual cages and the females remained in the breeding units. The females were examined periodically for signs of pregnancy.

The results of the mating were identical between the two groups, as three out of five of both the irradiated and the control females became pregnant. They were allowed to come through to parturition and were closely observed throughout gestation. In all animals the duration of pregnancy and the accomplishment of delivery were within normal limits. Observations were continued on the pups through weaning. There were no indications that the litters were in any way different from each other, and maternal behavior was also quite stereotyped.

V

Post Mortem Observations

In this section brief descriptions are presented of the types and distributions of the lesions of the brains of all the fetuses and rats examined in the course of the experiments performed in 1974, 1975 and 1976. In the experiments conducted in 1974, brains from both the control and irradiated fetuses were randomly selected for histopathological examination. They were fixed in Bouin's solution, imbedded in paraffin and serially sectioned at 10 micra. Two of every eight sections were mounted on slides and stained with cresyl violet.

In the experiments terminated on April 22 and on May 6, 1975, the brains of two fetuses from each litter were fixed in Bouin's solution. These were processed, serially sectioned and stained in hematoxylin-eosin in the histopathological laboratories at the Ames Research Center through the kindness of Dr. Webb Haymaker, since facilities were not available at the laboratories of the Institute for Behavioral Research at the time. The rats in the experiment terminated on 6 January, 1976 provided 4 irradiated and 4 control litters. The brain of each of the two largest fetuses in each litter was removed and fixed in Bouin's solution. Through the kindness of Dr. James Petras, Chief of the neuroanatomical laboratories and of Mrs. Michie Vane, senior histological technician, these brains were serially sectioned at 15 micra and every tenth section mounted and stained with hematoxylin-eosin.

The rats born on 8 April, 1976, and studied behaviorally were sacrificed when 8 months of age. Of these rats a total of 42, divided equally between irradiated and control, and between males and females, were selected for autopsy. This procedure was two-fold in purpose: 1) to obtain information on the animals through gross postmortem examination, and 2) to remove the brains for histological sectioning and examination.

They were injected intraperitoneally with sodium pentobarbitol, and within minutes reached a state of deep anesthesia. Before the rats were cut, three criteria were satisfied: 1) no response to ear pinch, 2) absence of eye-blink when the cornea was touched, and 3) no response to pinching the sensitive tissue between the toes.

The thoracic cavity was opened, and the right atrium of the heart incised. Immediately, the rat was perfused through the left ventricle with saline followed by neutral formalin. The brain was removed and placed in neutral formalin. It was later dehydrated, embedded in paraffin and was subsequently sectioned. One brain from each litter was sectioned and stained with cresyl violet in the anatomical laboratory, NASA, Ames Research Center through the kindness of Dr. William Mehler. A second brain from each litter has been similarly sectioned and stained under contract No. DAMD 17-77-C-7028 with the Department of the Army Medical Research and Development Command. These preparations are still being studied under the said contract. However, cursory examination of all the sectioned brains and detailed study of a few showed no lesions other than artifacts produced during the histological preparation.

After the brain had been removed, a gross postmortem examination was performed on the remaining organs. These examinations did not reveal any gross pathology.

When the brains for histological study were obtained, the remaining rats in this experimental colony were killed by chloroforming.

In the neurohistological studies reported here attention was directed mainly to the forebrain as this was the region which showed the major changes in the original experiments on which the present series was based. The anterior half of the midbrain was also surveyed, but the serial sections were not continued more caudally. The lesions found were almost all in the neocortex, with a few in the paleocortex, and only in two brains were abnormalities of the midbrain seen. No differences between the control and irradiated rats were found in the basal ganglia, the thalamus, the hypothalamus or rostral tegmentum.

The cytoarchitecture showed little or no differences from one part of the neocortex to another. The most prominent feature was the densely packed, rather broad layer of neuroblasts lining the lateral ventricles, excepting over the surface of the septum which was covered by immature ependyma. This layer varied in thickness, being thinnest in the cortex of the medial wall of the frontal region. It was continuous with the cells making up the immature caudate nucleus. In virtually every section of irradiated and control rats several cells in the process of division could be seen in this layer, chiefly in those bordering the ventricle. This densely packed layer of deeply staining cells did not extend into the 3rd ventricle, aqueduct, etc., which were lined by a well-organized layer of large ependymal cells. The septal nuclei, excepting their rostral poles, were also covered by a well-differentiated ependyma. Thus, the deep, dense cellular layer seemed to be limited to the cerebral vesicles, including those of their derivatives which were still actively developing.

Another layer of cells, less dense than the deep layer noted above, extended under a rather broad molecular layer over the whole neocortex. It also varied in thickness, though not as much as the inner dense layer. The molecular layer was covered by the pia arachnoid, which at places appeared thickened. The molecular layer showed no intrinsic structure and small, scattered nuclei. The cells in the dense layer below the molecular layer were not as tightly packed as those of the inner layer, but in both layers most of the cells were bipolar, oriented with their long axes perpendicular to the inner and outer surfaces of the hemispheres.

Between the two dense layers extended a broad, pale zone with scattered cells and fibers. It was divided into a narrower outer and a broader inner zone by a narrow horizontal layer of cells with a somewhat reticular appearance.

This general six-layered structure of alternating layers, chiefly of cells or chiefly of fibers, was maintained in most of the neocortical abnormalities found. The thin, reticular layer in the mid-region of the cortex was most likely to be disturbed or absent.

The commonest lesion, designated here as Type IA, was found in both the irradiated and control fetuses. It consisted of a U-shaped depression of the cortical surface, a narrow V-shaped cleft, or a longer, almost linear interruption of the normal cytoarchitecture. The molecular layer was continuous into these lesions, sometimes folded with a few cells of the pia extending into the fold. The external dense layer of cells of the cortex was thinner as it extended around the base of the lesion. The cell-poor zone of the cortex was encroached on, but the deep layer of closely packed neuroblasts was usually not affected.

Some of these lesions turned on entering the upper cortical layers and extended through the outer dense cellular layer more or less horizontally, with or without superficial branching extensions toward the surface and other branches downward into the cell-poor zone. Above these horizontal lesions the cortex showed a more dense band of cells adjoining the molecular layer and less densely packed cells below, adjoining the lesion. Below the lesion, however, the cortical layer again showed densely packed cells in contact with the lesion and less dense cells below, as the cell-poor zone was approached. These structural features demonstrated that the long, cleft-like lesions as well as the shorter depressions were not recent artifacts, but that the tissue of which they were composed was sufficient to control the growth of the adjoining neurons.

Irregular "clefts" or V-shaped lesions were occasionally seen extending from the ventricular surface into the deep layer of densely packed cells. These irregularities of the walls of the ventricles extended between the cells of the densely packed layer, but resulted in no modification of the form or arrangement of the cells. There is no appearance of a lining or infolded layer or of any syncytial or other tissue in these lesions such as is seen in the Type IA lessions penetrating the external layer of densely packed cells.

In some brains, lesions which appeared similar to the Type IA lesions in structure extended through the whole wall of the hemisphere and were often associated with fracture of the wall and free communication between the ventricle and the subarachnoid space. These lesions, with complete fracture of the wall of the hemisphere, were designated Type IB lesions.

In a few brains there appeared to be a pseudo-gyrus formation with two Type IA lesions extending into the cortex on each side of the "gyrus." More caudalward the lesions might join ventrally and the "gyrus" would then end, its caudal pole unconnected with the adjoining cortex. Most lesions with formation of pseudo-gyri showed more extensive disorganization of the cortex and were designated Type IC.

In some brains the cortex of the hemisphere was so distorted by large lesions and intervening masses of typically layered cortical tissue that the structure could not be analyzed from the available histological sections. In these distorted hemispheres there may have been more cerebral tissue than in a normal hemisphere. On general observation, however, there appeared to be a loss rather than a gain in mass of tissue.

In contrast to the lesions noted in the preceding paragraphs there were two other types of lesion in which there seemed to be an abnormal increase in tissue. In three brains--in 2 control animals and in 1 irradiated--the medial wall of the occipital cortex was invaginated into the ventricle and partially filled it. These were designated as Type II lesions. In one of the brains the invagination extended to the occipital pole. The position of the hippocampus was altered caudally so that it also protruded into the occipito-temporal horn of the ventricle.

Another abnormality--designated a Type III lesion--involved the olfactory stalk and, in two brains, the olfactory tubercle and neighboring paleocortical areas. The smallest expression of this abnormality consisted in some thickening, particularly caudalward, of the junction of the olfactory stalk with the ventral surface of the frontal pole. This increase in size of the stalk was somewhat more than the normal variation of the junction and was found in three brains in one batch of rats. A considerably larger abnormality also occurred in which the junction of the stalk with the frontal lobe and ventricle in one hemisphere was in the form of an inverted T, with one of the branches of the olfactory stalk extending caudalward and ending in a blind pouch. This lesion was found in three Sprague-Dawley albino rats which had been irradiated for 10 overnight (12 hour) periods. In one of these rats the lesion was combined with unorganized growth of the surrounding paleocortex which extended back to the level of the anterior commissure. This irregular, overgrown tissue included some choroidal tissue in an irregular depression on the ventrolateral surface of the brain.

The different kinds of lesions and the proportion of abnormal brains were not evenly distributed in the different batches of rats studied. In the following paragraphs, therefore, the different batches of rats, irradiated and control, will be described separately according to the date of autopsy.

<u>1/16/74</u>

Long Evans — Controls 4 (3 pregnant) Irradiated 5 (2 pregnant)

1700 MHz, 30 mw/cm^2, 45 mms. Exposure: once on the 13th day of gestation.

CONTROLS: #6-3 No abnormality found.

#3-2 U-shaped Type IA lesion and clefts in wall of ventricle. Break in ependyma of aqueduct with cleft extending in lower layer of right colliculus.

IRRADIATED: ##1-4, 2-1 and 2-4 No abnormalities found.

#1-2 U-shaped Type IA lesion with infolding of molecular layer and of pia arachnoid.

<u>2/26/74</u>

Long Evans — Controls 1 (1 pregnant) Irradiated 9 (7 pregnant)

One rat had had 20 mw/cm^2 for 2 hours and appeared to be <u>in extremis</u>. It was anesthetized and killed. In addition, 4 rats, (1 control and 3 irradiated) were brought to term and delivered. No behavioral abnormalities of the young were noted. The brains of 1 pup from each litter were examined and no abnormalities were found.

1700 MHz, 20 mw/cm^2, cw, for 20 minutes on 13th day of gestation.

CONTROLS: #31-1 No abnormalities found.

IRRADIATED: ##24-1, 24-2, 25-1, 25-2, 26-2, 27-2 and 27-8 showed some artifacts but no definite intrinsic abnormalities.

#27-3 Medial wall of right hemisphere was thin with irregular ventricular clefts. More caudally this was continuous with a break through the wall, i.e., A Type IB lesion.

#29-2 Type IA lesions with a pseudogyrus formation between them in the lower left frontal lobe.

<u>4/8/74</u>

Fischer strain — Fifty were mated on 3/20/74 in our laboratories.

CONTROLS: 6 (None pregnant)

IRRADIATED: 44 (3 pregnant)

##39-1 and 39-2 2450 MHz, 15 mw/cm^2, pulsed at 12 pps in oven. No abnormalities seen.

##62-1 and 62-2 2450 MHz mw/cm^2, 20 mins. in oven; pulsed at 1000 pps. Some artifactual changes. No intrinsic abnormalities seen.

#54-1 2450 MHz, 15 mw/cm^2, 20 mms in oven. Small Type IA lesion dorsum left cortex.

4/22/74

Sprague-Dawley (Holtzman) Controls 9 (4 pregnant) Irradiated 21 (8 not pregnant)

CONTROLS: (Chamber)

##71-2 and 71-4 No abnormalities.

##73-3 and 73-5 Ventricles irregular. Lesions of Type IA and Type IB.

(Oven)

#86-2 Right olfactory stalk bent across midline and the tissue extends caudal to the ventricular junction, i.e., a small Type III lesion.

#86-3 Invagination of medial wall of occipital cortex into occipital horn of ventricle, filling horn of ventricle and resulting in a cleft across the occipital pole. Type II lesion.

#86-5 Teratomatous growth under right brainstem attached through a break in the tissue to the ependyma at the infundibular leve', also attached to the external surface of the hypothalamus. Extends forward under the optic chiasm. Possibly includes some anterior tissue. Type III lesion.

#87-2 Olfactory stalk tissue on left extends somewhat caudal to junction with ventricle. Type III lesion.

#87-3 Left medial hemisphere wall is distorted frontally and broken through--Type IB lesion--more caudally. The hypothalamus is distorted at the infundibulum. There appears to be an artifactual break, but the right hypothalamus is enlarged and lacks the usual ventricular markings.

#87-4 Olfactory stalk extends a short distance caudal to the ventricular junction.

IRRADIATED: In anechoic chamber at 2450 MHz; 20 mw/cm^2; pulsed at 120Hz 50% duty cycle for 20 mins.

#88-2 No abnormalities seen.

#89-4 No abnormalities seen.

#74-4 Small irregular elevation of inner dense layer of cells in right ventricle, but none of the more frequent types of lesions seen.

#75-6 Type IB lesion frontal medial wall of left hemisphere with Type IA lesions continuous in front and behind.

#79-6 Small Type IA lesion left frontal pole.

#81-5 Two pseudo-sulci with a pseudo-gyrus between (Type IA lesions) in frontal lobe. Invagination of medial wall of occipital lobe into occipital horn of ventricle on left, Type III lesion.

#91-5 Cleft between the superior collicali from surface into aqueduct. This is probably an artifact.

In calibrated oven with mode stirrer operating.
2450 MHz, 20 mw/cm^2; 20 minutes.

##88-2 and 89-4 No abnormalities except artifact at level of superior collicali in #89-4.

#91-5 Thin medial wall of left frontal lobe with Type IA lesions before and behind Type IB break into ventricle. Invagination of right medial wall of occipital lobe into ventricle, Type III lesion. Abnormality of development between the superior colliculi, extending into the aqueduct.

<u>5/28/74</u>

Sprague-Dawley (Holtzman) Controls 7 (all pregnant) Irradiated (5 not pregnant)

CONTROLS: ##98-4 and 102-4 No abnormalities seen.

IRRADIATED: In oven, 2450 MHz; 20 mw/cm^2; cw; 20 minutes on 1, 2, or 3 days.

##107-2 (x3), 108-2 (x3)*, 122-2 (x1), 125-2 (x1) No abnormalities seen.

#103-1 (x3) Type 1A lesion, small, on right.

Chamber, 1700 MHz; 20 mw/cm^2; cw; 20 minutes on 1, 2 or 3 days.

##113-4 (x3), 114-4 (x2), 116-2 (x2), 118-2 (x1) and 124-2 (x1) No abnormalities seen.

<u>7/15/74</u>

Sprague-Dawley (Holtzman)

Controls 10 (all pregnant) Irradiated 10 (3 not pregnant)

This experiment was carried out in the Experimental Neuropsychological Laboratory, VA Hospital, Kansas City, Missouri, under Dr. Dan R. Justesen. The calibrated oven in use there was employed for irradiating the rats. Two rats were exposed on the 13th day of gestation, five received 1 exposure on each of 4 days, the 13th to 16th of gestation. The irradiation was at 30 mw/gms, 2450 MHz for 20 minutes with the modal stirrer on. Irradiation was conducted between 1300 and 1500.

CONTROLS:

##1-9, 8-6, 9-7, 11-4, 14-4, 17-4, 19-3 and 20-5 No abnormalities seen.

#12-10 had a small, broad Type IA lesion in the right lateral cortex.

IRRADIATED:

##2-1, 2-3, 3-4, 3-9, 7-5, 7-8, 13-3, 13-4 and 16-1 (irradiated on 4 days) and 18-5 (irradiated on the 13th day of gestation only) No abnormalities seen.

#16-4 (irradiated on 4 days) showed a small narrow Type IA lesion in the left frontal pole as well as some irregularities of the ventricular walls.

<u>8/27/74</u>

Sprague-Dawley (Holtzman)

Controls 7 (All pregnant) Irradiated 12 (1 not pregnant)

Rats divided into 3 groups:

Group I, 6 rats, irradiated for 1 hour at 1700 MHz; cw, 10 mw/cm^2 on 5th and 6th days gestation and the same, but 15 mw/cm^2 on the 7th, 8th and 12th to 16th days of gestation.

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* (x1), (x3) indicate exposures at 1 per day for 1, 2 or 3 days, respectively).

Group II, 6 rats, irradiated for 14 to 15-1/2 hours at 1700 MHz, cw, and 5 mw/cm^2 on 5th and on the 12th to 16th days of gestation, and at 7.5 mw/cm^2 on the 6th, 7th and 8th days of gestation. However, on the 15th and 16th days the air conditioning failed to function and the power dropped for an unknown time during the overnight irradiation.

Group III, control groups for Groups I and II. Left in their home cages. Group I controls had food and water available, 2 rats. Group II controls were deprived of food and water during the night, 5 rats.

CONTROLS:

Group III: (Controls for Group I)
Not examined.

Group III: (Controls for Group II)
##16-4, 18-1 and 18-2 No abnormalities seen.

##15-1 and 15-2, several small and one deeper Type IA Lesions.

IRRADIATED:

Group I:
#9-1 No abnormalities seen.

#8-4 Irregularities of ventricles.

#7-1 Small Type IA lesion and irregularities of ventricles.

Group II:
#12-1 No abnormalities seen.

#2-1 Long Type IA lesion extending horizontally through right lateral cortex.

#11-1 Narrow, deep Type IA lesion, right lateral cortex, continuous caudally with Type IB lesion more caudally. More caudally still, the break through the hemisphere wall appears due in part to artifactual injury.

#3 Small broad Type IA lesion, left frontal lobe. The junctions of the olfactory stalk with the lateral ventricles appear more oblique than usual.

<u>4/22/75 and 5/6/75</u>

Sprague-Dawley (Holtzman)

Two batches of 8 each, mated 4/1-2/75 and 4/15-16/75, respectively, were obtained. Four rats were irradiated overnight for 10 nights between 8 and 19 April and 4 of the second batch were irradiated similarly between 22 April and 3 May. Irradiation was at 2450 MHz, 5 mw/cm^2, cw, for 12 or 15 hours. Control rats were similarly restrained and handled, but shielded from radiation. The brains of 2 fetuses from each litter were serially sectioned in Dr. Webb Haymaker's laboratory for histological examination and were stained with hematoxylin-eosin. One rat of each control and each irradiated group was not pregnant.

CONTROLS: (4/22/75) ##B11-1, B11-3, B15-3 and B15-4; and (5/6/75) ##G4-1, G13-3, G14-1 and G14-2 showed no abnormalities.

#B7-3 Cortex of both hemispheres markedly distorted, the right more than the left, Type IC lesion. A ventral occipital portion of the right cortex is attached to the dorso-medial surface of the left superior colliculus.

#B7-4 Large Type IA lesion of right frontal pole becomes continuous cardalward with break into the lateral ventricle (Type IB lesion). Caudally the hemisphere wall is whole, but the cortex is in 3 segments (Type IC lesion). On the left there is what appears to be a teratomatous growth extending under the brain from the olfactory stalk to the level of the optic chiasm, Type III lesion.

#G4-1 Large Types IA and IB lesions of left cortex with considerable distortion.

#G4-2 Type IB lesion and considerable distortion of neocortex of left hemisphere. Also the right olfactory stalk continues caudal to the attachment to the frontal lobe and ends in a blind pouch, Type III lesion.

#G13-2 Small Type IA lesion of left dorsal cortex.

IRRADIATED: #B4-2 Type III lesion.

#B4-3 Type IA lesion, small, at least of frontal lobe.

#B10-3 Teratomatous growth of neuroblastic, choroidal and possibly glandular tissue under surface of the brain from preoptic area candalward. Break of ependymal lining of aqueduct and growth of neuronal tissue down into the aqueduct from the superior colliculi.

#B10-4 Large teratomatous mass including neuronal and choroidal tissue starts around attachment of R't. olfactory stalk to frontal lobe, Type III lesion, and extends back to interpeduncular nuclei. Left hypothalamus enlarged and abnormal. Right hemisphere distorted throughout, Type IC and Type III lesions, and a part is ventro-laterally attached to the caudo-lateral hypathalamus. Both occipital poles of the hemisphere are attached to the dorsal surfaces of the superior colliculi. Tissue from the midline structures between the superior colliculi invades the aqueduct as in B10-1.

##B18-1 and B18-4 No abnormalities seen.

#G12-1 No abnormalities seen.

#G1-1 Very small Type IA lesions in frontal and occipital cortices.

#G1-3 Moderately large Type IA lesions in both hemispheres.

#G2-2 The optic chiasm is deformed by an abnormal growth from the ventral surface of the right hypothalamus. Also a small Type IA lesion in the medial right cortex.

#G12-4 Very small type IA lesion laterally on right.

1/6/76

Long Evans Controls 4 Irradiated 4

Ten were mated 12/16/75, received 12/23/75. Five were irradiated 2450 MHz, 5mw/cm^2, cw for 13.5 hours on 11 nights from 12/24/75 to 1/5/76. Four others were similarly handled, but shielded from radiation. One was irradiated on 12/24/75 only, escaped and was loose in laboratory without food and water for 36 hours before being caught. It was then kept in its home cage with food and water ad lib until sacrificed. One of the control rats was not pregnant. Consequently there were 4 control and 4 irradiated animals in this group.

CONTROLS: ##2-1, 10-2 and 10-3 No abnormalities seen.

#2-4 Several small and some large Type IA lesions with limited Type IC lesions.

4-1 and 4-2 Several small to moderate Type IA lesions of frontal poles.

#7-1 Several small to medium Type IA leasions and a small Type IC lesion.

#7-4 Several small Type IA lesions.

#10-1 Very small Type IA lesion in cortex adjoining Corpus callosum.

IRRADIATED All 8 fetuses showed small to medium sized Type IA lesions.

#5-1 Also showed a break in the ependyma of the aqueduct with growth of neuronal tissue extending through it.

##5-4, 8-1 and 8-2 showed Type IB and small Type IC lesions.

The series of experiments carried out may be divided into two parts. From January to July, 1974, 6 batches of rats were irradiated, mostly on 1 occasion on the 13th day of gestation. In some experiments the rats were irradiated 2, 3 or 4 times on the 13th and 14th days of gestation. Irradiation was at 1700 or 2450 MHz, at 10 to 30 - mostly at 20 - cw/cm^2, in a chamber or, less often, in a modified oven. Most of the radiation was continuous wave, but in some experiments it was pulsed at 12, 120 or 1200 p.p.s. The duration of irradiation was 20 minutes to 1 hour per session.

In the experiments terminated in August, 1974, April and May, 1975 and January, 1976, the rats were irradiated at 2450 MHz, 5 mw/cm^2, for 12 to 15 hours (overnight) on 9 to 11 occasions between the 6th and the 17th days of gestation and were autopsied on the 19th or 20th days of gestation.

TABLE 3

	Control Rats			Irradiated Rats		
	No lesions found	Lesions	Total	No lesions Found	Lesions	Total
I Experiments Jan-Jul '74	15 brains	9	24	33 brains	8	41
	62.5%	37.5%	100%	80.05%	19.5%	100%
II Experiments Aug '74 to Jan '76	13	13	26	5	21	26
	50%	50%	100%	19.2%	80.8%	100%

The table presents a summary of all the brains studied histologically. Under the heading "Lesions" the number of all the brains showing any abnormality other than what was most probably an artifact due to the removal or preparation of the specimen. Thus, very small lesions of Type IA have been included. I have not been able to identify the nature of these lesions in the present material, but as there is a continuous series from small Type IA lesions to marked abnormalities of a teratological nature in Types IB and IC it has seemed preferable to include all such lesions as abnormalities of growth. They are definitely not artifactual, as shown by the cellular organization of the lesion.

The table shows that the control fetuses of Group I (January to July, 1974) showed fewer lesions than those of Group II (August, 1974 to January, 1976). However, the difference - 37.5% of Group I to 50% Group II - is much less than the marked difference found in the irradiated fetuses, namely, 19.5% in Group I to 80.8% in Group II. As the change in this direction occurred in all four experiments, though to different degrees, it may be taken as a reliable observation. In this respect it is very different from the small, variable and suggestive differences between the control and irradiated groups with respect to fetal weight and behavior. Coming to any conclusions about the effects on living organisms of low doses of microwave irradiation is unsafe, however. Thus, a preliminary survey of the serial sections of the brains of the rats whose behavior was studied showed no obvious abnormalities. These rats had been irradiated during fetal life at 2450 MHz; 7.5 mw/cm^2; cw, for over 12 hours on 11 occasions between the 6th and 17th days of gestation. The anatomical examinations of these brains is continuing in more detail, but no lesions comparable to those found in the fetal brains are present.

The distribution of the brain lesions (more lesions and particular types of lesions being found in different batches and in different litters of rats) suggests that genetic factors, increased stress in handling or shipping and possibly other factors are more important than the dosage of microwave irradiation used in the reported experiments for producing brain abnormalities.

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LEGENDS FOR TABLES

Table 1

Upper Division. Summary of numbers and average weights of fetuses in the litters of irradiated (Rad) and control (Con) rats in 3 experiments on the effects on fetal growth of 12° (overnight) irradiation repeated 9 to 10 times between the 6th and the 16th days of gestation.

Lower Division. Summary of data on the litters immediately (or less than 1 hour) post partum in the experiment described in Section III, page 13.

Table 2

The significance of the difference in weight of the irradiated and control fetuses in 3 experiments on the effects of repeated exposures of 12° duration each on 9 occasions between the 6th and 16th days of gestation. The significance is calculated by t test in the left column and by analysis of variance in the right column. The degrees of freedom are derived from the number of fetuses in the lines labelled FETUS and from the number of litters in the lines labelled LITTER.

Table 3

Comparison of numbers and proportions of brains with and without lesions in irradiated and control fetuses in the groups irradiated 1 to 4 times starting on the 13th day of gestation and in those irradiated for over 12 hours on 9 or 10 occasions between the 6th and 16th days of gestation.

TABLE 1

Strain of rats and treatment	Number of fetuses per litter and average weight in grams ( ).	Range of weight of fetuses	Total Number of fetuses and average weight in grams ( ).	Difference in av. wt. of rad. & con. fetuses
S-D I Rad	4(4.07) 9(4.66) 11(4.95)	3.6 - 5.3	24(4.7)	
				+0.32 gms 6.3%
S-D I Con	6(4.95) 8(3.97) 6(4.28)	3.4 - 5.3	20(4.38)	
S-D II Rad	8(3.90) 9(4.01) 4(4.08)	3.3 - 4.4	21(3.97)	
				+0.31 gms. 8.4%
S-D II Con	11(3.56) 11(3.78) 2(3.59)	3.1 - 4.1	24(3.66)	
L.E. Rad	8(4.45) 9(4.31) 12(4.27) 13(4.31)	3.32 - 4.88	42(4.33)	
				+0.26 gms. 5%
L.E. Con	11(4.19) 14(4.17) 13(3.99) 15(3.97)	2.89 - 4.80	53(4.07)	

	No. of young per litter and av. wt. of young in (gms).*	Total number and av. wt. of young	Diff. in wt. in gms. and %. ** Significance
Rad	9(5.1); 11(5.7); 12(5.4) 15(5.4); 11(5.6); 13(5.6) 15(5.5); 5(5.9); 11(6.0)	103(5.6)	(0.2grms.)
Con	10(5.3); 12(6.1); 12(5.5) 9(6.0); 9(5.7); 13(5.8) 15(5.9)	80(5.8)	(3.6%) n.s.

* 2 rats omitted from control tabulation. See text p.

** Parenthesis ( ) used to indicate that the average irrediated pup was lighter than the average control pup.

TABLE 2

SPRAGUE-DAWLEY I
12 HOUR EXPOSED-CONTROLS

FETUS:	t,	2.52	(43)	p = .01	F,	3.96	(1,43)	appro. .05
LITTER:	t,	.39	(4)	p = NS	F,	.09	(1,4)	NS

SPRAGUE-DAWLEY II
12 HOUR EXPOSED-CONTROLS

FETUS:	t,	3.67	(43)	p = .0005	F,	15.42	(1,43)	p = .001
LITTER:	t,	4.16	(4)	p = .01	F,	18.00	(1,4)	p = .025

LONG-EVANS I
12 HOUR EXPOSED-CONTROLS

FETUS:	t,	3.95,	(93)	p = .0005	F,	15.77	(1,93)	p = .001
LITTER:	t,	3.58,	(6)	p = .01	F,	12.00	(1,6)	p = .025

SPRAGUE-DAWLEY I + II + LONG-EVANS I
12 HOUR EXPOSED-CONTROLS

FETUS:	t,	4.98,	(183)	p = .0005	F,	24.47	(1,183)	p = .0005
LITTER:	t,	1.55,	(18)	NS	F,	2.34	(1,18)	NS

TABLE 3

	No. of Brains, Control Rats				No. of Brains, Irradiated Rats			
I Experiments Jan.-July '74	Lesions:	None 15 62.5%	Present 9 37.5%	Total 24 100%	Lesions:	None 33 80.5%	Present 8 19.5%	Total 41 100%
II Experiments Aug. '74 - Jan. '76		13 50%	13 50%	26 100%		5 19.2%	21 80.8%	26 100%

LEGENDS FOR FIGURES

Figure 1

Summary of experiments on Groups 1 and 2 of Sprague-Dawley (Holtzman) rats and on Group 1 of Long-Evans (Blue Spruce Farms) rats. Distribution of litters when the number of rats per litter (abscissae) is plotted against the average weight of the rats per litter (ordinates). Open symbols show irradiated litters, solid symbols show control litters. Triangles and circles represent the first and second groups of Sprague-Dawley rats respectively and the squares represent the Long-Evans rats.

Figure 2

Summary of the data on Group 2 of the Long-Evans (Blue Spruce Farms) rats as in Figure 1. The open circles represent the number of fetuses per litter of the irradiated rats. The solid circles represent the data on the control rats. The absence of overlap between the two groups is dramatic, even in view of the small number of subjects.

Figure 3

Average growth curves for the first 17 days of (top curve) the heavier of the two home-cage control litters, of (middle curve) the chamber control litters and of (lower curve) the irradiated litters. The differences in weights maintained the same proportions throughout demonstrating similar growth rates. The litters were weighed every 3 days. Abricissae: age in days. Ordinates: average weight of the fetuses.

Figure 4

Growth rates of irradiated and chamber control young rats. The pups were weighed as litters for 4 weeks and individually thereafter. Between the 5th and 7th weeks the males began growing faster than the females. There were negligible differences between growth of the irradiated and the control pups. Open circles represent irradiated and solid circles control animals. Male and female are designated by m and f. Abricissae: age in weeks. Ordinates: average weight in grams.

Figure 5

Test of locomotion as the average number of centimeters travelled in 60 seconds - of the irradiated and control pups as a function of age in days. The greater distances travelled and somewhat greater variability of the irradiated pups until the 10th day of age and the smoother curves from the 11th day on may be noted. Each point represents the average distance travelled by an irradiated pup (open circles) or a chamber control pup (solid circles) as determined from the data from the performance of 4 pups from each of half or all the litters of both groups measured daily. When only half the litters were tested the alternate half was tested on the next such occasion. Ordinates: Average distance in centimeters travelled by a pup in 60 seconds. Abricissae: Age of pups in days.

Figure 6

Ratio of the amount (in cc.) of Valenstein's saccharin-glucose solution to the amount of plain tap water (in cc.) drunk by irradiated and control rats at different concentrations of saccharin. Each point is the average value of 6 irradiated and 6 control rats after a steady ratio of consumption had been maintained for 5 days. Ordinates: The ratio of tap water to S-G solution in cubic centimeters imbibed. Thus, "0" indicates 100% S-G solution and "1" indicates equal amount of S-G and tap water. Abricissae: concentrations by weight of saccharin in the S-G solution. Open circles represent behavior of control and solid circles the behavior of irradiated rats. It may be noted that the taste preference threshold is lower for the irradiated rats.

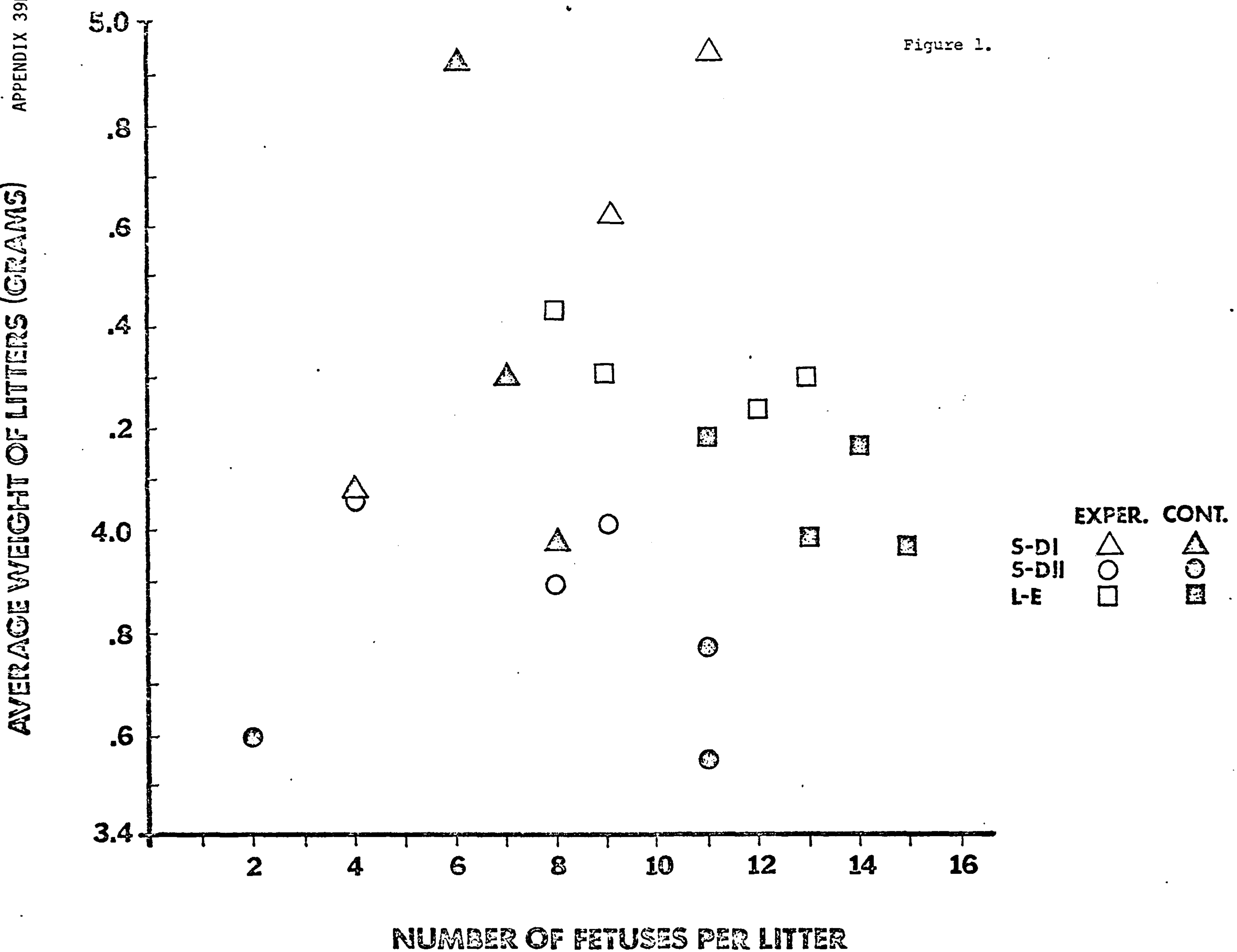
Figure 7

Cummulative rate of errors in maze running. After rats had learned a modified Lashley maze III the control rats continued to make errors at a slightly higher rate than the irradiated rats. The cummulative curves of errors committed demonstrate this. A pronounced difference occurs from trial 31 to 34 when the ambient temperature reached 85° F due to mechanical failure of the air conditioning equipment. The errors committed by the control rats increased sharply, whereas the irradiated rats committed practically no errors. Ordinates: Number of errors committed - cummulative curve. Abricissae: Number of trials. Upper curve: performance of control rats. Lower curve: performance of irradiated rats.

Figure 8

Number of errors committed by control rats (open circles, dashed lines) and by irradiated rats (solid circles, continuous lines) when tested on 5 days during the middle one of which the ambient temperature rose to 84° F due to equipment failure.

Figure 1.



AVERAGE WEIGHT OF LITTERS (GRAMS)
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.8
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.2
4.0
.8
.6
3.4
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12
14
16
NUMBER OF FETUSES PER LITTER
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CONT.
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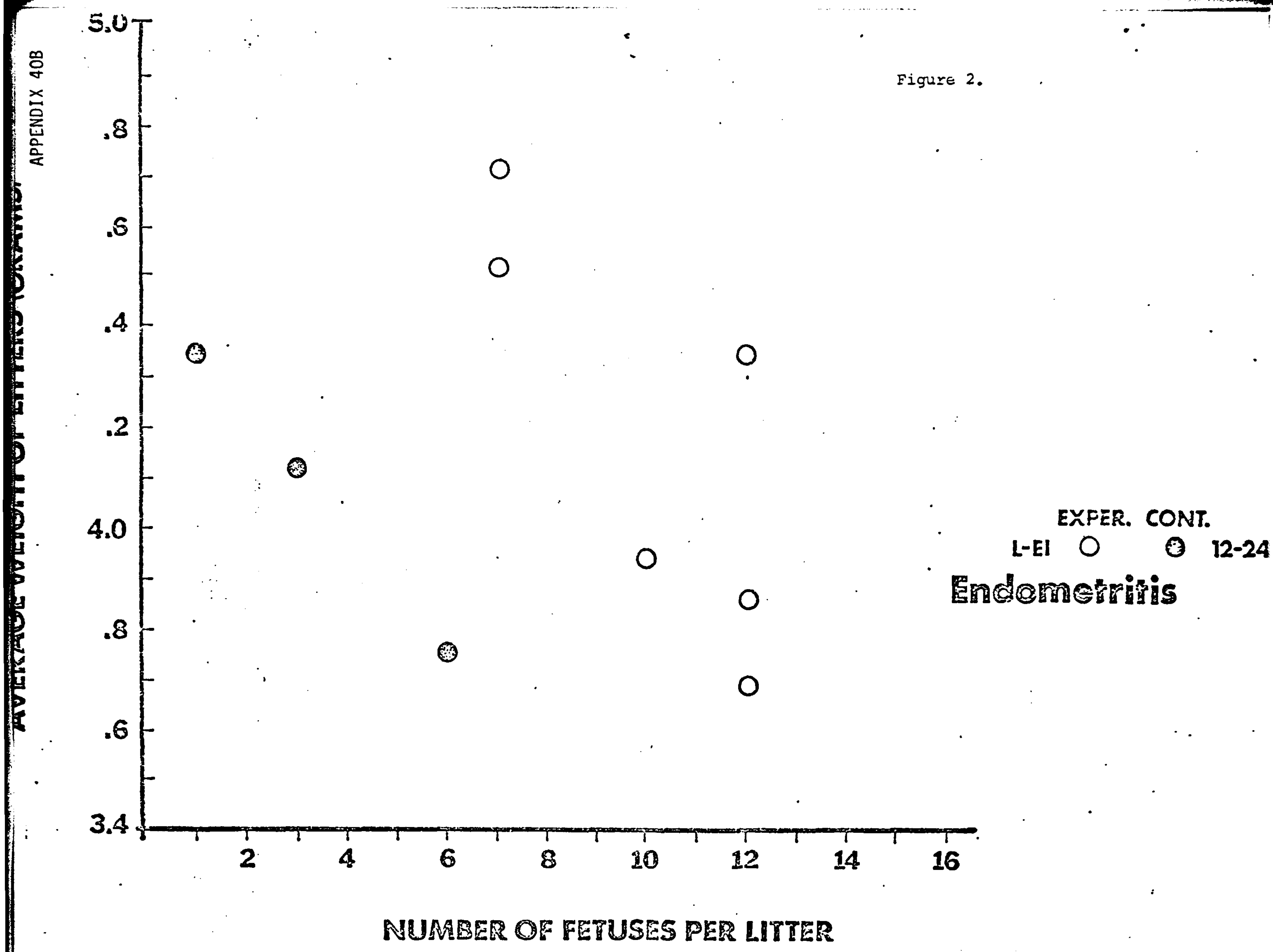
S-DII
L-E

Figure 2.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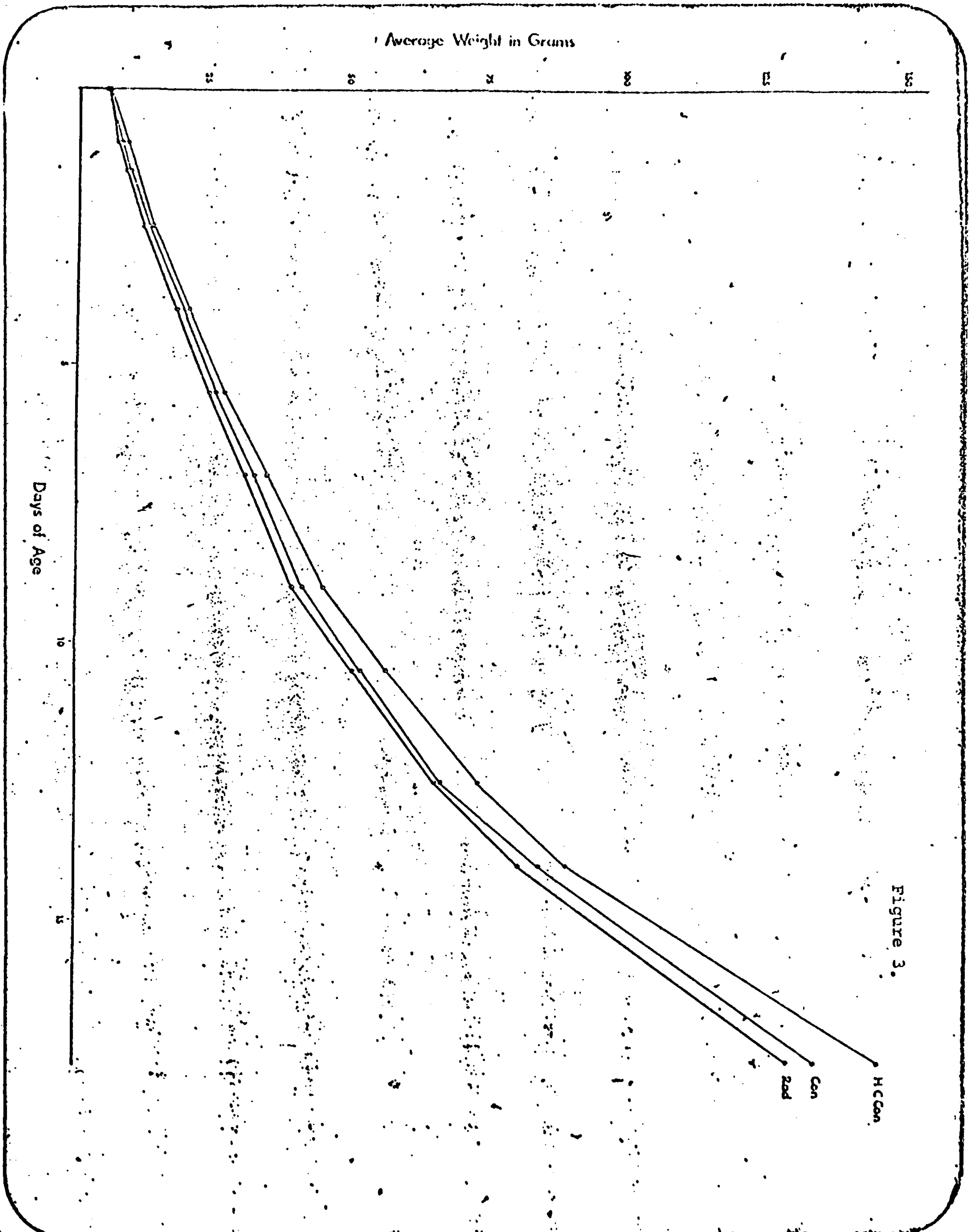
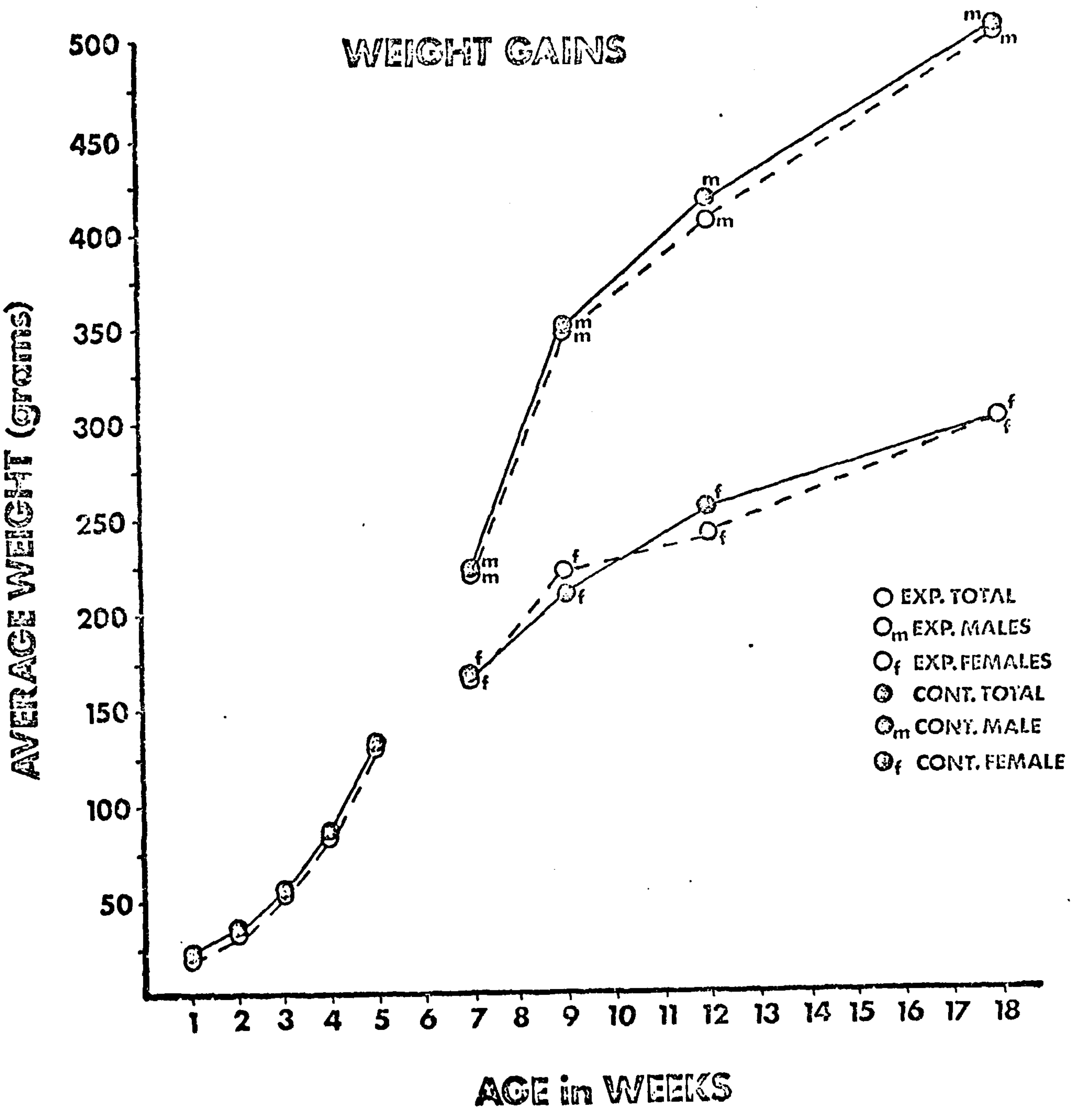
16
NUMBER OF FETUSES PER LITTER
EXPER. CONT.
L-EI
12-24
Endometritis



WEIGHT CURVES OF THE RATS IRRADIATED DURING FETAL LIFE


WEIGHT GAINS
AVERAGE WEIGHT (grams)
AGE in WEEKS
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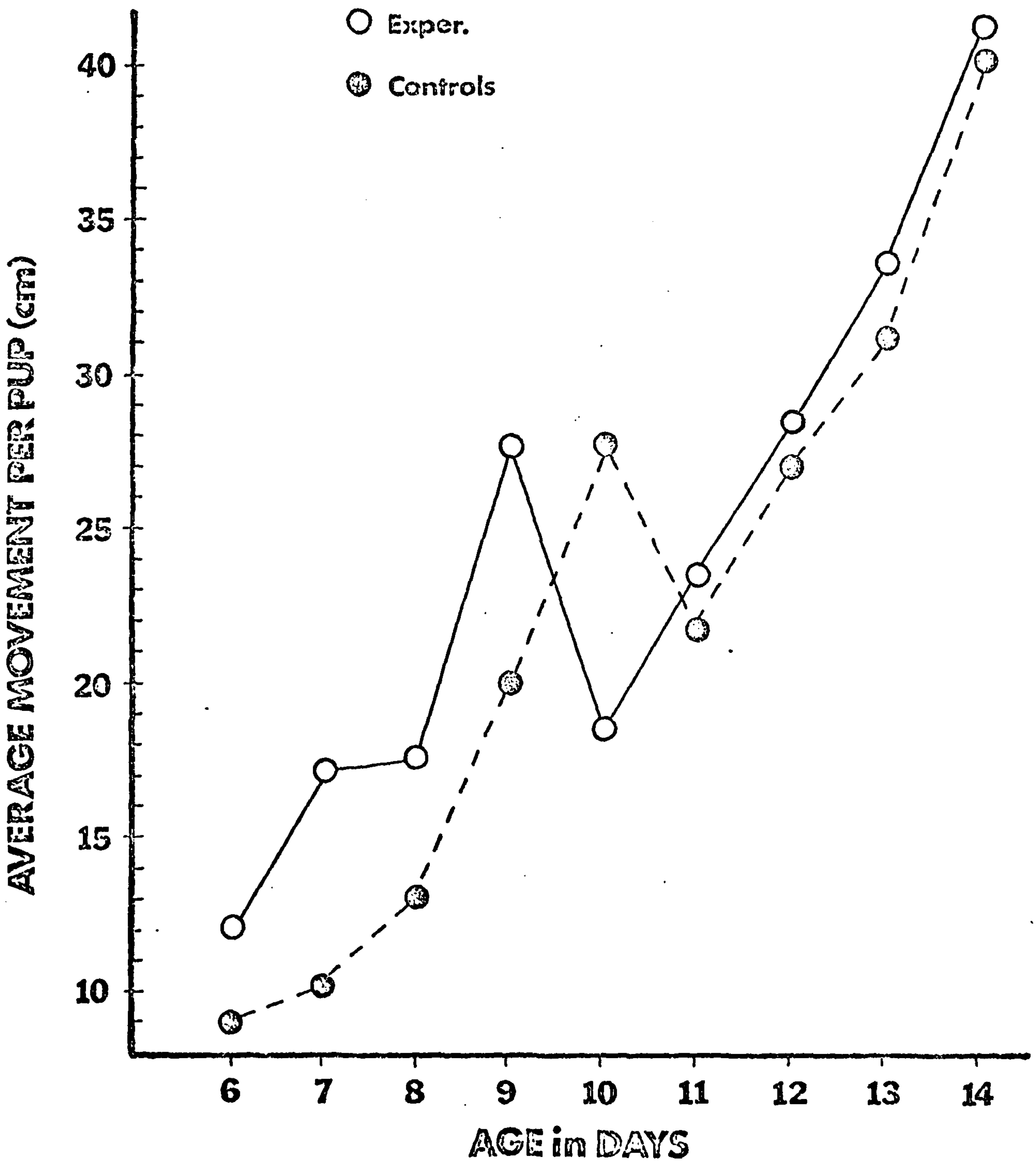
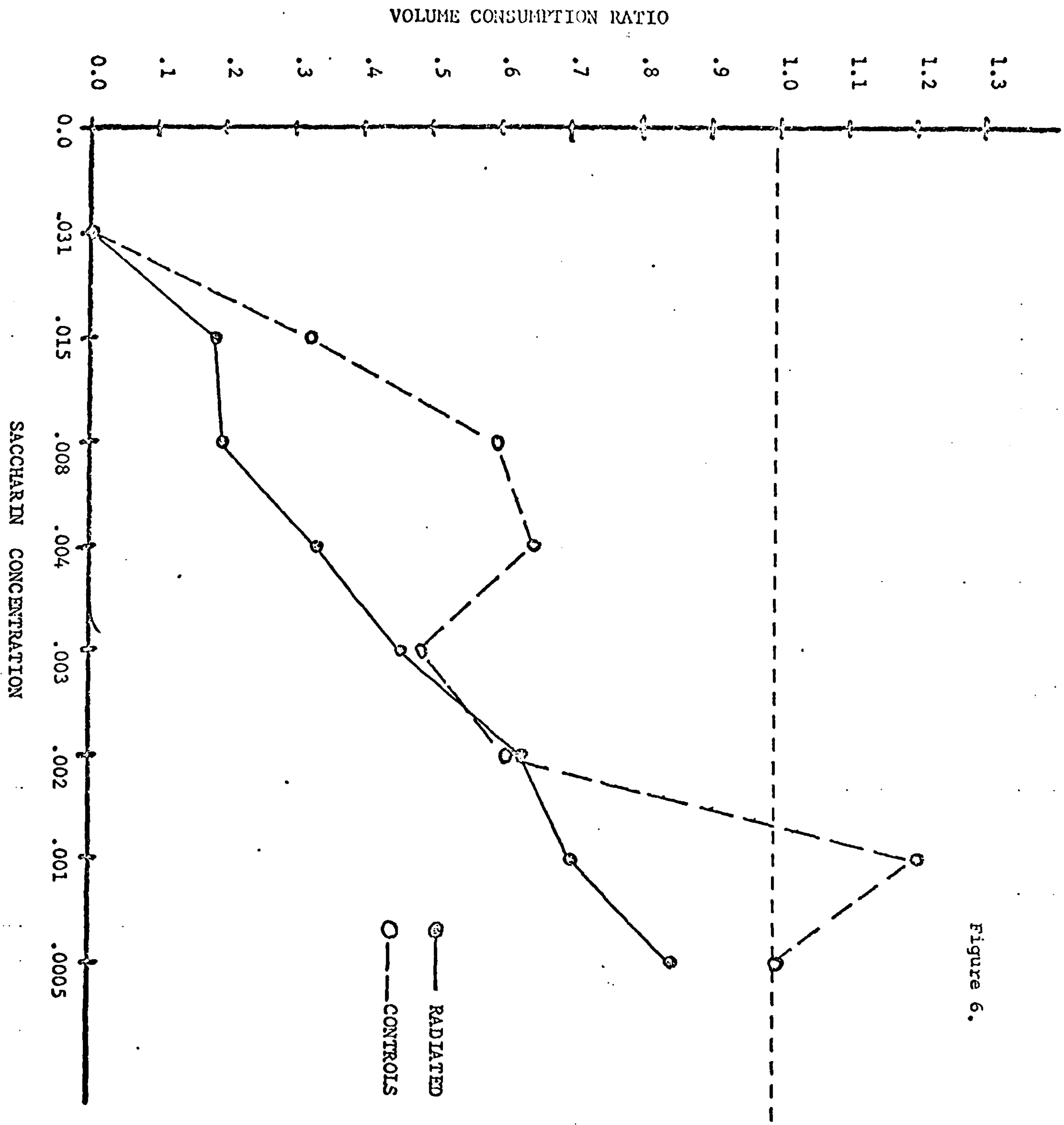
450
400
350
300
250
200
150
100
50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
EXP. TOTAL
EXP. MALES
EXP. FEMALES
CONT. TOTAL
CONT. MALE
CONT. FEMALE

Figure 4.



EARLY MOTOR BEHAVIOR

Figure 5.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1.2
1.3
SACCHARIN CONCENTRATION
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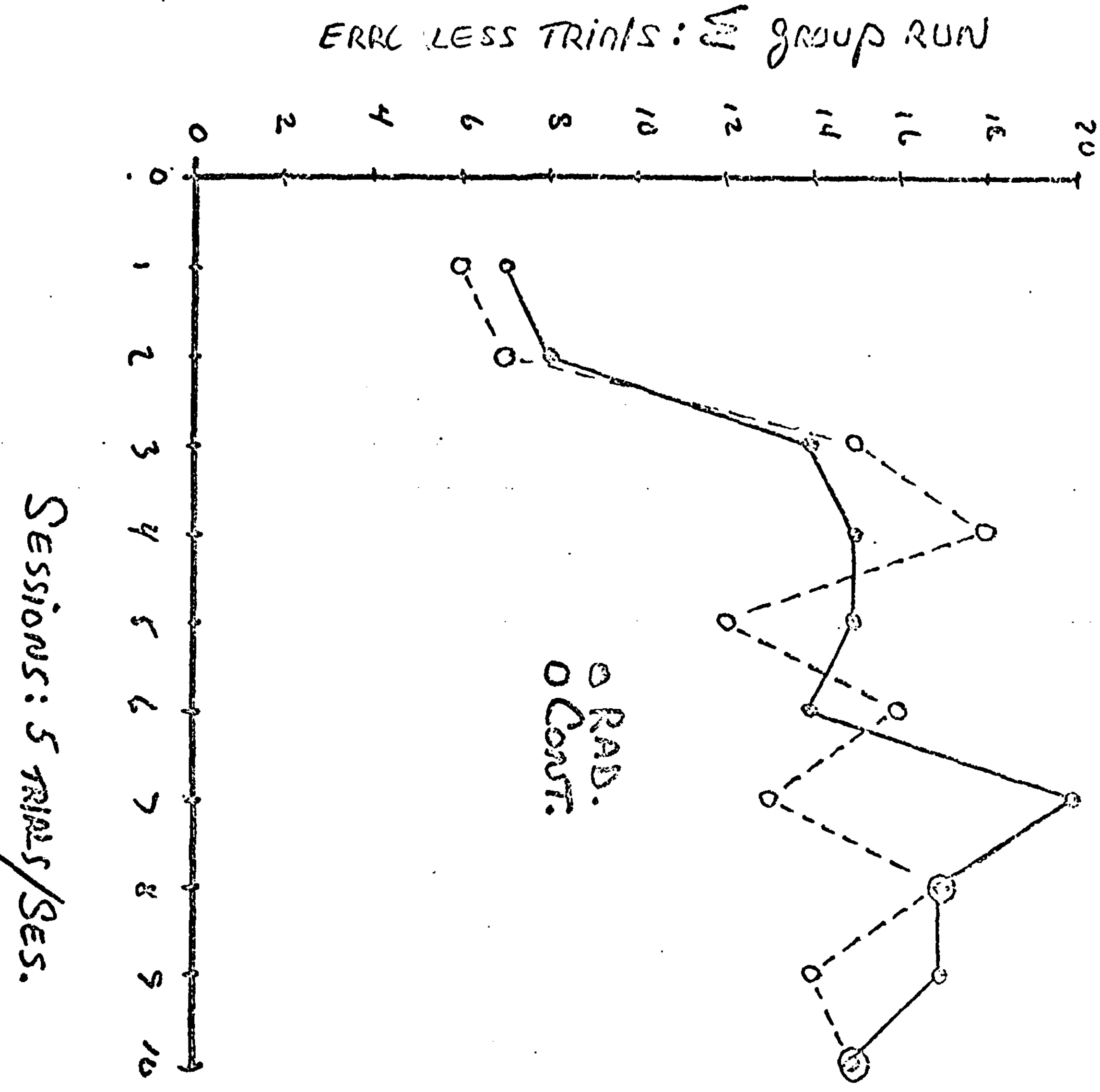
.004
.003
.002
.001
.0005
RADIATED
CONTROLS

Figure 6.


ERRC LESS TRIALS: Σ group RUN
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10
RAD.
CONT.
SESSIONS: 5 TRIALS/SES.

Figure 7.

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