

RADIATION-INDUCED MICRENCEPHALY IN GUINEA PIGS

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ABSTRACT

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The effect of x rays on brain weight of guinea pig pups at birth was studied for 21-day old embryos exposed in utero to doses of 75 and 100 mGy. When compared to controls and when corrected for body weight, gestation time, litter size, sex, and examiner differences the brains of irradiated pups weighed approximately 46 mg less than those of controls ($p < 0.001$) for the 75-mGy group and about 55 mg less for the 100-mGy group. Brains of females weighed 51 mg less than those of males of the same body weight. Dam weight and caging conditions had no observed effect on brain weight.

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INTRODUCTION

The potential morphogenic effects of low doses of ionizing radiation on the development of the human brain are an important concern. The morphogenic effects most frequently observed among those exposed in utero to atomic-bomb radiation at Hiroshima and Nagasaki were small head size (SHS) and mental retardation (MR)(1-5). The data for each effect are statistically consistent with a no-threshold dose-response relationship, but a threshold at conceptus doses in excess of 100 mGy is also statistically consistent with the data (3,6). Only individuals at Hiroshima are affected at doses less than 500 mGy, of which neutrons were a factor. Animal studies could prove useful in determining whether an effect on brain size is possible at doses less than 100 mGy of low linear-energy-transfer (LET) radiation and, if so, may help ascertain the mechanism for both effects (SHS and MR).

Cell depletion is commonly invoked as a mechanism for the effects of SHS and MR (7, 8) and is likely a predominant mechanism at high doses (e.g. >200 mGy). However, at low doses other mechanisms may be at work (8-11). Otake and Schull (4,5) found that MR was induced during the interval of 8 to 25 weeks postconception and that there was no radiation-induced MR in individuals exposed in utero prior to the 8th week post-conception. This differs from the temporal patterns observed in the SHS data (2,3), wherein SHS occurred in individuals exposed during the 2 to 15 week post-conception interval. If the finding of radiation-induced SHS in individuals exposed prior to 8 weeks post-conception is a valid finding, then it is likely that the mechanism or the cells affected are different from the mechanism for radiation-induced MR alone or SHS associated with MR.

Morphogenic effects of ionizing radiation in animals at doses of 100 mGy or less when delivered in utero have been reported (12-15). We are not aware of independent corroboration of some of these findings (14,15) and confounding factors, such as litter size or gestation time are not always considered as potential biasing factors (14). Wanner and Edwards (16) demonstrated a linear regression technique that is sensitive to detect radiation-induced micrencephaly in guinea pigs. (Micrencephaly as used here means an abnormal smallness of the brain). Their data showed a consistently decreasing effect with decreasing radiation dose when radiation was delivered on the 21st day after conception in guinea pigs. This corresponds embryologically with a developmental stage in the human between the 5th and 6th weeks post-conception (17,18), when small head size is induced but not mental retardation. However, the precise phase of neuronal development is uncertain. The data of Wanner and Edwards (16) also suggested an effect at doses less than 100 mGy and that a threshold in the vicinity of 50 mGy might exist. The purposes of the experiments in our study were to use the technique proposed by Wanner and Edwards to a) provide independent information at the 100 mGy level, b) determine whether micrencephaly might be induced at a dose on the order of 75 mGy, and c) to study potentially confounding factors such as sex, gestation time, litter size, dam weight, and others. The data reported here were acquired in a blind study in which one investigator was responsible for irradiation of pregnant guinea pigs while three other investigators were responsible for data on the brain weight of guinea pig pups without prior knowledge of the irradiation history. A fourth investigator examined 31 brains, or 5% of the total, and was involved in the irradiation process. However, the overall conclusions of the study are the same when these data are excluded from the analysis. Our data demonstrate that irradiation at 75 mGy and 100 mGy has a statistically significant effect on brain weight independent of body weight, gestation time, litter size, or sex. Additionally, brain weight relative to body weight, gestation time, and litter size is different for males and females.

MATERIALS AND METHODS

Data Acquisition

A vendor (Hilltop Lab Animals, Inc., Scottsdale, PA) supplied timed-pregnant Hartley albino guinea pigs in twenty-five shipments of 8 to 12 guinea pigs per shipment. The dams were from the breeding stock of the vendor and were mated during postpartum estrus. Mating occurred on a Wednesday and 13 days later the dams were shipped by same-day express to the housing facility at our institution. To ensure that no irradiation of the dams took place during shipment, thermoluminescent dosimeters (TLD) were included in the shipping crates with the dams. The TLD indicated an exposure of approximately 0.02 mGy per crate, consistent with the doses expected from air travel.

Upon arrival at our institution, animals were weighed to the nearest gram on a triple beam balance and assigned to cages with an identification number. Guinea pigs from the first nine shipments had cages with wire bottoms, and the remainder had cages with solid bottoms and hardwood chips as bedding. Animals were maintained *ad libitum* on a diet of water and guinea pig chow (Purina Lab Chow, Purina Mills, Inc., Richmond, Indiana). Animals were observed daily and any unusual conditions or behavior were brought to the attention of a staff veterinarian. On the 21st day after conception, animals were transferred in individual cages to a fluoroscopic x-ray room where they were divided into three groups: a control group, a 75-mGy irradiation group, and a 100-mGy irradiation group. Typically, four animals were assigned to the control group, four to the 75-mGy group, and two to the 100-mGy group.

Individually, each animal was constrained in a plexiglas cylinder with outside and inside diameters of 100 mm and 88 mm. One end of the cylinder was sealed with a conically shaped attachment that had a 25 mm opening at the end. The animal's head fit snugly into the cone with the snout positioned in the opening. To ensure uniform exposure across the abdomen of the

animal, it was necessary to seal the other end with a device that compressed the hind end of the animal into the cylinder. This assured that the abdomen fully occupied the spaces of the cylinder.

A General Electric LU fluoroscopic system with MPX generator (General Electric Co., Medical Systems Division, Milwaukee, Wisconsin) was used to irradiate the dams. The x rays were generated at 110 kVp, the half-value layer was 3.85 mm of 1100-type aluminum and the tube current was about 4 mA. The image intensifier was positioned at 110 cm from the focal spot and a 1-mm thick lead plate was placed in front of the image intensifier to reduce the x-ray intensity at the input phosphor of the image intensifier. This ensured that the output fluoroscopic image was within the brightness range of the TV pick-up tube. The animal was positioned laterally in the beam with the center of the abdomen approximately 75 cm from the focal spot. An ionization chamber was placed at the output of the x-ray tube to monitor the amount of radiation delivered in each case. The system was set up in an identical manner each time and was calibrated for radiation output prior to irradiations.

Irradiation of the full abdomen was verified on the fluoroscopic image. Calibration employed the use of the same constraint cylinder as was used for the animals, but a cylinder of water was used to simulate the abdomen of the guinea pig. The insert cylinder was constructed of polystyrene in such a way that an 0.6 cm³ Farmer ionization chamber (Model 2505/3B, Nuclear Enterprises, Ltd., Brookshire, England) fit into the cylinder to measure the internally administered dose. The Farmer chamber and electrometer (Model 602 dosimeter, Keithley Instruments, Inc., Cleveland, Ohio) interfaced to a digital multimeter were calibrated by the M.D. Anderson Accredited Dosimetry Calibration Laboratory, Houston, Texas and at beam qualities similar to those used in this test. Doses at the center of the abdomen and around the periphery of the abdomen were examined. The calibration involved normalizing the dose delivered internally in the water to the monitor chamber reading. In order to achieve a

relatively uniform dose distribution across the abdomen of the animal, the radiation was delivered in a parallel-opposed manner in which the x-ray tube was first positioned laterally on the left side of the animal with half the radiation dose delivered. The tube was then positioned on the other side of the animal and the remaining dose delivered. In this manner, the uniformity of the dose across the entire volume was measured and found to spatially vary by no more than $\pm 6\%$. To verify the accuracy of the dosimetry, TLD were placed in the water insert and other TLD were placed in the cylinder along side one of the animals that was irradiated. The dose was determined at the Radiation Physics Dosimetry Laboratory of the M.D. Anderson Cancer Center, Houston, Texas. The doses as determined by TLD were 8% lower than the ionization chamber measurement for the cylinder calibration and 4% higher for the animal test. A higher value for the animal test is expected because the TLD measures the external dose, not the internal dose.

The total constraint time to irradiate animals in the 100-mGy group was approximately four minutes. In order to assure that the stress imposed by this procedure was the same for all animals, all animals were constrained in the device for four minutes and placed on the irradiation table simulating the same procedures in all cases. The x rays were not engaged for the control group and were engaged for the appropriate duration for animals in the 75-mGy and 100-mGy groups.

Following this procedure, the dams were weighed on a triple beam balance (Ohaus, Florham Park, NY) to the nearest gram and marked with a color that identified them according to their dose group. The dams were then returned in their cages to the animal care facility until parturition.

Approximately 1 week prior to parturition, the dams were transferred to large solid bottom cages with a bedding of hardwood chips. The cages were checked daily for pups.

Within 24-hours after delivery, information was recorded regarding the total litter size, date of delivery, and the number of live pups. The live pups were identified by cage

number only (no color identification) and transferred to a laboratory where they were weighed to the nearest gram and euthanized with an overdose of sodium pentobarbital (approximately 0.3 ml at 50 mg/ml). No dosimetry information was available to the examiners. The animals were then decapitated at low cervical levels with a large scissors. The cervical spinal cord and cranial contents were exposed using fine ronguers. The brain was separated from the spinal cord using a sharp scalpel introduced normally to the neural axis at the atlantoepistrophic articulation. The brain and olfactory bulbs were then carefully removed with a fine spatula and immediately weighed to within the nearest milligram on an electronic balance (Sartorius Instruments, Ltd., Surrey, England). The accuracies of both scales were checked with a set of calibration weights (Ohaus, Florham Park, NJ) and periodically verified for consistency over the course of the experiment. The electronic scales did not vary in reading by more than 1 milligram and the balance was consistent to within 1 gram. The sex of each animal and the person performing the cerebral excision were recorded in most cases.

RESULTS

Characteristics of caging, dam weight, dam weight change, gestation time, litter size, pup weight, examiners processing the pup brains, and sex distribution of the pups were studied to determine their possible effects on the results. The data from pups of one animal were discarded because the animal developed an ear infection which was treated with antibiotics. Data from pups of another dam were discarded because the dam was too large (>1 kg) to fit into the constraint device.

Caging and Weight Change

As a function of shipment number the data demonstrate systematically different changes in dam weight during the 8-day interval between arrival at our facility and the time of irradiation (Table I). This suggests a systematic improvement in our ability to accommodate the adaptation of the guinea pigs to their new environment.

Gestation Days, Litter Size, and Dam Weight

The average gestation time (defined as the time from the day of mating to the day that live pups were observed in the cage) was 69.7 days per dam with a standard deviation of 1.9 days. In one case, the gestation lasted for 83 days, well outside the norm, and the data from pups of this animal were discarded.

There were no significant trends in gestation time with shipment number or dam weight (Tables I and II). No significant differences in average gestation time were observed for the dams in individual dose groups (Table III). There was a significant correlation between gestation time and litter size with smaller litters having an average gestation slightly longer than those of larger litters (Table IV), consistent with trends previously observed (19). The litter size distributions among dose groups were similar (Table III) and the distribution of numbers of dams producing live pups in each dose group was very similar to the distribution of numbers of dams assigned to each dose group. These latter two observations do not suggest any differences in early mortality among the different dose groups. Dam weight at shipment was correlated with litter size (Table II), consistent with previous findings (20).

Examiner and Sex of Pups

A summary of the distributions of pups by examiner, dose and sex is given in Table V. In our analysis of factors that influence brain weight of pups, only data of examiners A and B were included, even though data from examiners C and D were consistent with results of A and B, because of previously discussed problems associated with the small numbers of animals examined by C and D and the potential for bias on the part of examiner D. The distribution of sexes of the pups for animals processed by examiners A and B is not the same. Examiner A processed proportionately more females than males while the reverse was true for examiner B.

Regression Analysis

The previous results suggest that the caging conditions of the dam, the weight of the dam, gestation time, litter size, sexes of pups, the examiner processing the brain weight data, and the dose administered should be examined as potential factors that might influence brain weight. To test in order of priority the effect of each of these factors, multiple linear regression analysis was performed with each factor as an independent variable using the SPSS/PC+ 4.0 statistical package (SPSS Inc., Chicago, Illinois). Shipment number and the weight change of the dam were variables used to analyze the impact of caging conditions. Each sex and each of the two examiners (A and B) were assigned integer values and linear regression performed using these values. The significance of the T-value for the slope of each variable when compared to zero value was examined and a step-wise analysis was used to identify the important factors. After identification of the most important factor in the group of independent variables, the data were corrected according to the slope for that factor and the remaining variables analyzed again by regression analysis for their significance. This iterative process was continued until the significance of all remaining T-values was greater than 0.05. Those factors with significance

greater than 0.05 were disregarded in future analyses, except for the case of litter size. Litter size, being strongly correlated with gestation days, was found to compete with gestation days for significance and correcting for one tended to eliminate or markedly reduce the significance of the other. The factors identified as having an important influence on brain weight in the order of their significance were: weight of the pup, gestation days (litter size), dose, sex, and examiner. The weight of the dams at shipment, the weight change of the dams after shipment, and the shipment number did not demonstrate a significant influence on brain weight.

In the second phase of analysis, the data were separated into 12 groups by dose, sex, and examiner. In each group linear regression was performed using pup weight, gestation days, and litter size as adjustment variables. The formula used to adjust brain weight in each of the 12 groups was:

$$B' = B + p(92.1 - P) + g (69.4 - G) + l(4.7 - L)$$

where B' is the adjusted brain weight, B is the measured brain weight, p is the slope for pup weight, P is the measured pup weight, g is the slope for gestation days, G is the observed gestation days, l is the slope for litter size, L is the actual litter size. The 92.1 is the mean weight in grams of all pups in this analysis, 69.4 is the mean gestation days per pup in this analysis, and 4.7 is the mean litter size per pup. The mean gestation days per dam was 69.7 and the mean litter size per dam was 4.2. We performed the analysis with both sets of means and found only minor changes in the adjusted data with no change in the overall conclusions.

An analysis of variance on the adjusted mean values was performed and the data are given in Table VI. The mean brain weight by dose when corrected for sex and examiner differences are given in Table VII. Also given are the mean brain weight and variances by sex when corrected for dose and examiner, and the mean weight and variances by examiner when corrected for dose and sex. The raw data for examiner B are given in Fig. 1a as a function of pup weight. The

adjusted data are given in Fig. 1b for the same examiner. Note that both graphs demonstrate a preponderance of control data points in the upper part of the plot, while data for the dose groups dominate in the lower portion, visually suggesting the dose effect. We conclude that the 75-mGy dose group had a mean brain deficit of 46 mg when compared to controls with a 95% confidence range on the deficit of 24 to 68 mg. For the 100-mGy group the mean deficit was 55 mg with a 95% confidence interval of 22 to 88 mg. Male brains were on the average 51 mg greater than dose of females with a 95% confidence interval of 31 to 71 mg. Brains excised by examiner B were on the average 23 mg heavier than those of examiner A with a 95% confidence range of 2 to 44 mg.

Our data affirm the conclusions of Wanner and Edwards (16) that a brain weight deficit is induced at 100 mGy and that a deficit is also induced at less than 100 mGy. Despite the facts that our data represent a different strain of guinea pig and have been corrected for gestation time, litter size, sex, and other factors not addressed by Wanner and Edwards, the two sets of data yield comparable results (Fig. 2). Using linear regression, each set renders an intercept of 1.0 (the normalized control value) and the slopes are not statistically distinguishable ($p>0.2$). However, the data are not sufficient to speculate about a threshold or about a preferred functional form of the dose-response relationship.

Inter- and Intralitter Variance

Jensh and Brent (21) demonstrated that variance of fetal weight, placental weight and placental/fetal weight ratio among litters of rats is an important factor to consider in the design of teratological investigations in polytocous animals. The principal potential confounding effect of interlitter variance is bias if the numbers of litters are small. Table III shows the numbers of litters in each dose group and that the distributions of litter sizes representing dose groups

are similar. A concerted effort to avoid litter bias was made in the assignments of dams to each dose category by assuring that weight categories of dams were equally distributed among the dose groups.

We have also separately analyzed the inter- and the intralitter variances for brain weight in our control populations, after correcting for body weight, gestation time, litter size, sex and examiner, to determine the magnitude of the independent variances and how they might affect the interpretation of the results of this study. The variance in adjusted brain weight due to intralitter variations was substantially greater than variance due to interlitter variations, but interlitter variance was significant, confirming the applicability of the findings of Jensch and Brent (21) to guinea pigs. The consistency of the results regarding the effects of dose for both sexes, for both examiners, and for all dose categories (Table VI) do not suggest a bias due to interlitter variance that might invalidate the conclusions.

CONCLUSIONS

The confirmation of a micrencephalic effect induced by x rays at doses of 75 mGy during this embryonic stage of development of the guinea pig is consistent with the findings of small head size induced in humans exposed prior to the eighth week of conception at Hiroshima. This lends credence to a causal interpretation of the relation observed between radiation and small head size in humans at very low conceptus doses (3), but it is uncertain whether the guinea pig brain at conception day 21 is homologous to that of the human prior to 8 weeks conception age. Migration of neurons in humans, rats and mice begins in the very late embryonic or very early fetal stages, which corresponds to about day 28 in the guinea pig (18). By day 30 in the guinea pig the cortical plate is well defined and three zones of the cerebral hemisphere are present (22), homologous to about 10 weeks post-conception in the human (23). Based on this information, it is likely that the induced micrencephaly in 21-day gestation guinea pigs is a

result of cell depletion of proliferating and maturing neurons, but because of uncertainty in the phase of development an effect on the early phase of postmitotic migrating neurons and subsequent connectivity cannot be ruled out. In addition, whereas small head size after embryonic irradiation in humans appears to be correlated with an overall growth retardation (3), our guinea pig data are corrected for body weight and the effect of micrencephaly appears to be independent of a general growth retardation effect.

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TABLE I

Weight change of dams one week
after shipment and gestation time as a function of
shipment number

| SHIPMENT NO. | 1-5 | 6-10 | 11-15 | 16-20 | 21-25 |
|--|---------------|----------------------------|---------------|---------------|---------------------------|
| No. of dams in group | 28 | 38 | 31 | 33 | 41 |
| Mean % weight change (standard deviation) | -1.3 (3.8) | -1.6 ^a (4.7) | 0.2 (5.2) | 0.8 (5.3) | 1.8 ^a (5.1) |
| Mean gestation time ^b in days (standard deviation) | 69.5 (2.1) | 69.8 (1.5) | 69.1 (2.5) | 70.1 (1.7) | 70.0 (1.6) |

^a Difference in these two groups is significant ($p < 0.05$).

^b No two groups are significantly different ($p > 0.05$).

TABLE II

Gestation time and litter size by dam weight

| Dam weight at shipment | ≤800 g | >800 g |
|---|------------------------|------------------------|
| Number of dams | 120 | 50 |
| Gestation time in days (standard deviation) | 69.8 ± 1.9 | 69.5 ± 1.9 |
| Litter size ^a (standard deviation) | 4.0 ^a ± 1.7 | 4.7 ^a ± 1.8 |

a Difference in litter size between groups is significant ($p < 0.05$)

TABLE III

Gestation time, number of dams and litter size distribution
by dose group

| DOSE GROUP | CONTROLS | 75-mGy | 100-mGy |
|---|------------|------------|------------|
| Gestation in days (standard deviation) ^a | 69.8 (1.9) | 69.5 (1.9) | 70.0 (1.7) |
| No. of dams with small litters (1 to 3 pups) ^b | 23 (26.6) | 24 (23.2) | 11 (8.2) |
| No. of dams with medium litters (4 to 6 pups) ^b | 44 (43.1) | 40 (37.6) | 10 (13.3) |
| No. of dams with large litters (7 to 9 pups) ^b | 11 (8.3) | 4 (7.2) | 3 (2.5) |
| Total number of dams delivering live pups | 78 | 68 | 24 |

^a No two groups are significantly different ($p > 0.05$).

^b Values in parentheses are expected values. There are no significant differences in the distributions of litter sizes with dose ($p > 0.1$) based on a chi-square contingency table analysis.

TABLE IV
Characteristics of Litters

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | ALL LITTERS |
|---|---------------|---------------|---------------|---------------|---------------|---------------|---------------|----------------------------|-------------|------------------|
| Number of pups in litter | | | | | | | | | | |
| Mean gestation time ^a in days (standard deviation) | 71.4 (2.5) | 70.8 (1.5) | 70.7 (2.2) | 69.9 (0.9) | 69.0 (1.1) | 68.0 (2.1) | 69.3 (1.7) | 69.3 ^b (0.6) | 67.0 (-) | 69.7 (1.9) |
| No. of dams with live pups | 10 | 24 | 24 | 39 | 33 | 22 | 14 | 3 | 1 | 170 |
| No. of live pups | 10 | 46 | 67 | 145 | 142 | 106 | 70 | 13 | 4 | 603 ^c |
| % Survival at birth | 100 | 96 | 93 | 93 | 86 | 86 | 71 | 54 | 44 | 84 |

^a Gestation times for a litter size of 1, 2, and 3 are significantly different from 5 and 6; 4 is significantly different from 6; and 1 is significantly different from 7 ($p < 0.05$).

^b Dam with 83-day gestation excluded from these data.

^c One pup in this group was excluded from brain weight analysis because brain weight was not recorded.

TABLE U

Distribution by Sex and Dose Groups for Examiners Performing Excisions

| EXAMINER | MALES | | | | FEMALES | | | | BOTH SEXES |
|---------------|----------|----------------|-----------------|--------------------|----------|----------------|-----------------|--------------------|---------------|
| | Controls | 75-mg group | 100-mg group | All dose groups | Controls | 75-mg group | 100-mg group | All dose groups | |
| | | | | | | | | | |
| A | 64 | 61 | 19 | 144 | 70 | 72 | 22 | 164 | 308 |
| B | 49 | 55 | 11 | 115 | 51 | 36 | 17 | 104 | 219 |
| C | 5 | 4 | 0 | 9 | 12 | 3 | 0 | 15 | 24 |
| D | 10 | 5 | 1 | 16 | 8 | 5 | 2 | 15 | 31 |
| TOTALS | 128 | 125 | 31 | 284 | 141 | 116 | 41 | 298 | 582* |

* Twenty pups were excluded from this categorization because either sex or examiner was not recorded.

TABLE VI

Mean adjusted brain weight in grams by
dose, sex, and examiner

| DOSE | SEX | EXAMINER A | EXAMINER B |
|---------|-----|--------------------|--------------------|
| Control | M | 2.758 ± 0.113 (64) | 2.792 ± 0.099 (49) |
| | F | 2.689 ± 0.127 (70) | 2.718 ± 0.113 (51) |
| 75-mGy | M | 2.709 ± 0.109 (61) | 2.724 ± 0.119 (55) |
| | F | 2.657 ± 0.126 (72) | 2.680 ± 0.111 (36) |
| 100-mGy | M | 2.689 ± 0.085 (19) | 2.641 ± 0.063 (11) |
| | F | 2.663 ± 0.097 (22) | 2.710 ± 0.065 (17) |

TABLE VII

Results of analysis of variance for dose group, sex, and examiners taking into account the effects of pup weight, gestation time, litter size, sex of the pups, examiner, and dose. Provided are the mean adjusted brain weights in grams for each item and the number of pups involved. The standard deviation in all cases is about 0.12 grams.

| | Controls | 75 - mGy | 100 - mGy |
|-----------------------------------|--------------|----------------|------------|
| Dose effects ^a | 2.736 (234) | 2.690 (224) | 2.681 (69) |
| | Males | Females | |
| Sex differences ^a | 2.735 (259) | 2.684 (268) | |
| | A | B | |
| Examiner differences ^b | 2.700 (308) | 2.723 (219) | |

^a p<0.001 for difference between controls and each dose group and for difference between sexes.

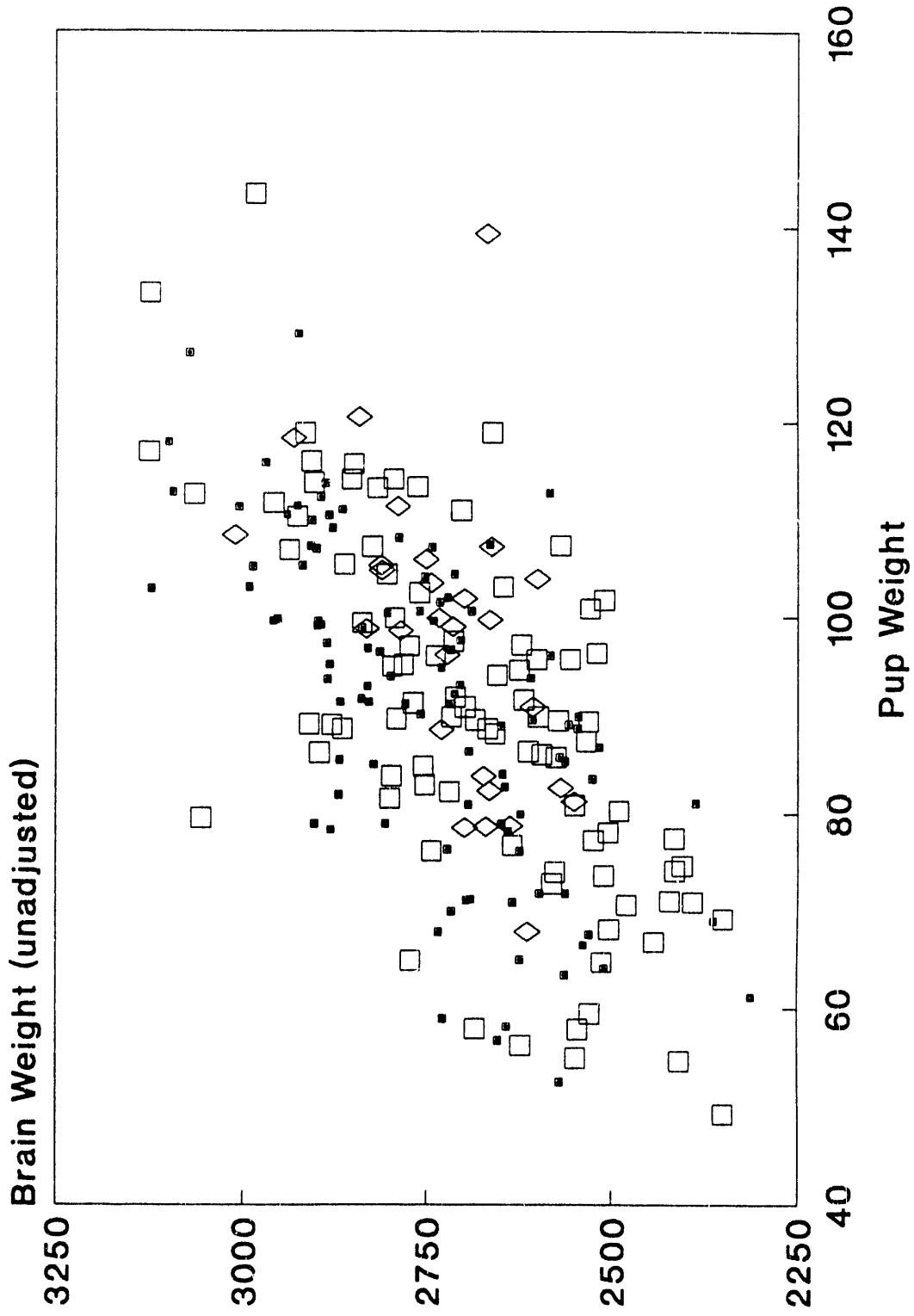
^b p<0.05

FIGURE CAPTION

Figure 1. Scatter plots of brain weight data in milligrams versus pup weight data in grams for examiner B. Small filled squares are control data, open squares represent the 75-mGy group, and open diamonds the 100-mGy group. A) Raw data. B) Data adjusted for pup weight, gestation days, litter size, and sex.

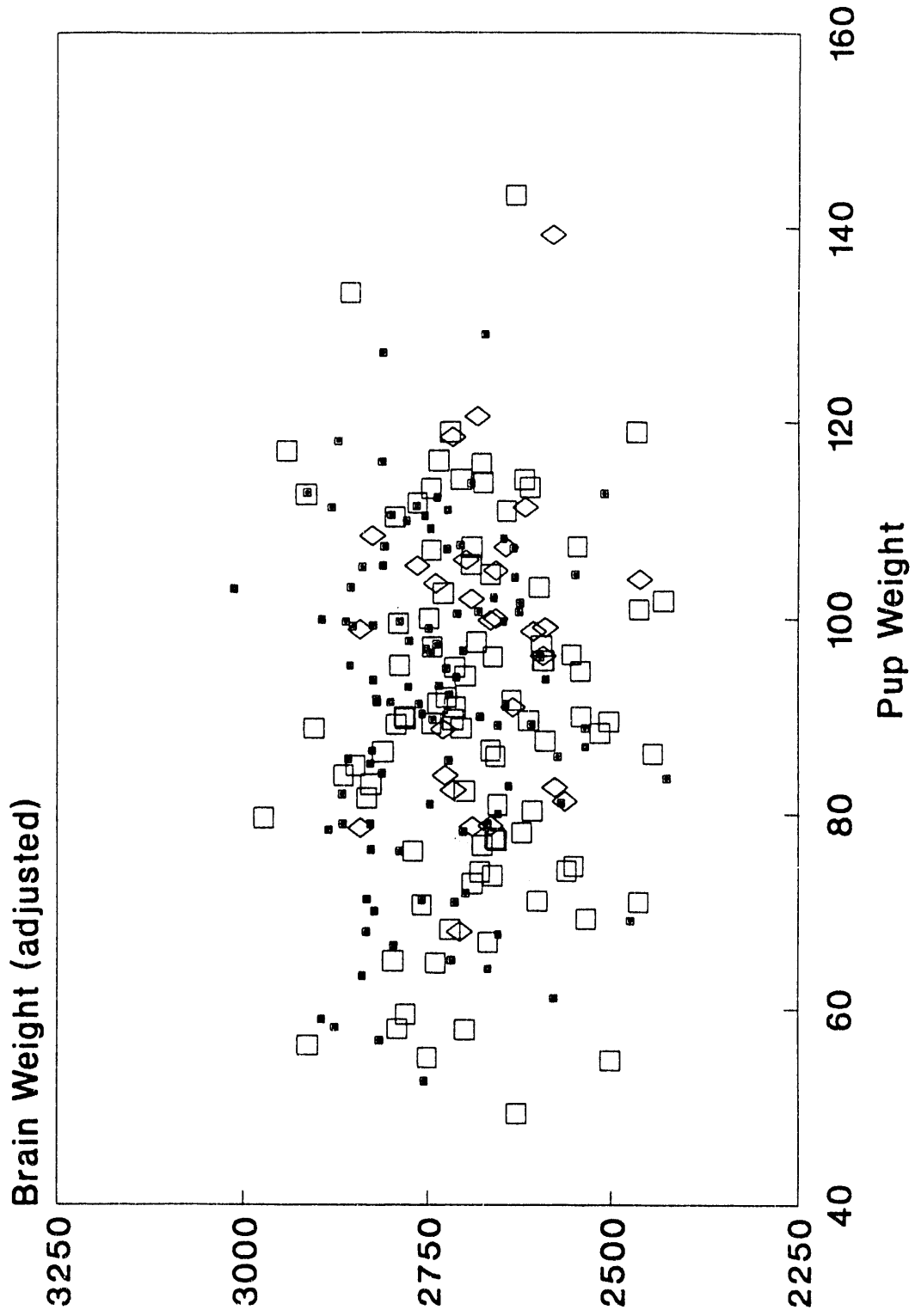
Figure 2. Normalized and adjusted brain weights of guinea pig pups as a function of dose delivered on day 21 post-conception. The ordinate is the proportion of adjusted brain weight relative to the controls as 1.0. Data from ref. 16 and Table VII of this report are compared. Arrows indicate our data. Data of ref.16 have been shifted slightly to avoid overlapping data points. All brain weights from both studies have been adjusted for 92 g pups. Data of Fig. 1 in ref. 16 were used to adjust their data. Our data are adjusted for gestation time, sex, litter size, and examiner differences, also. Error bars represent +/- two standard errors about the mean. The dashed line is the 1.0 normalized brain weight value, providing easy reference for the significance of the data when compared to controls.

EFFECTS ON GUINEA PIG BRAIN WEIGHT



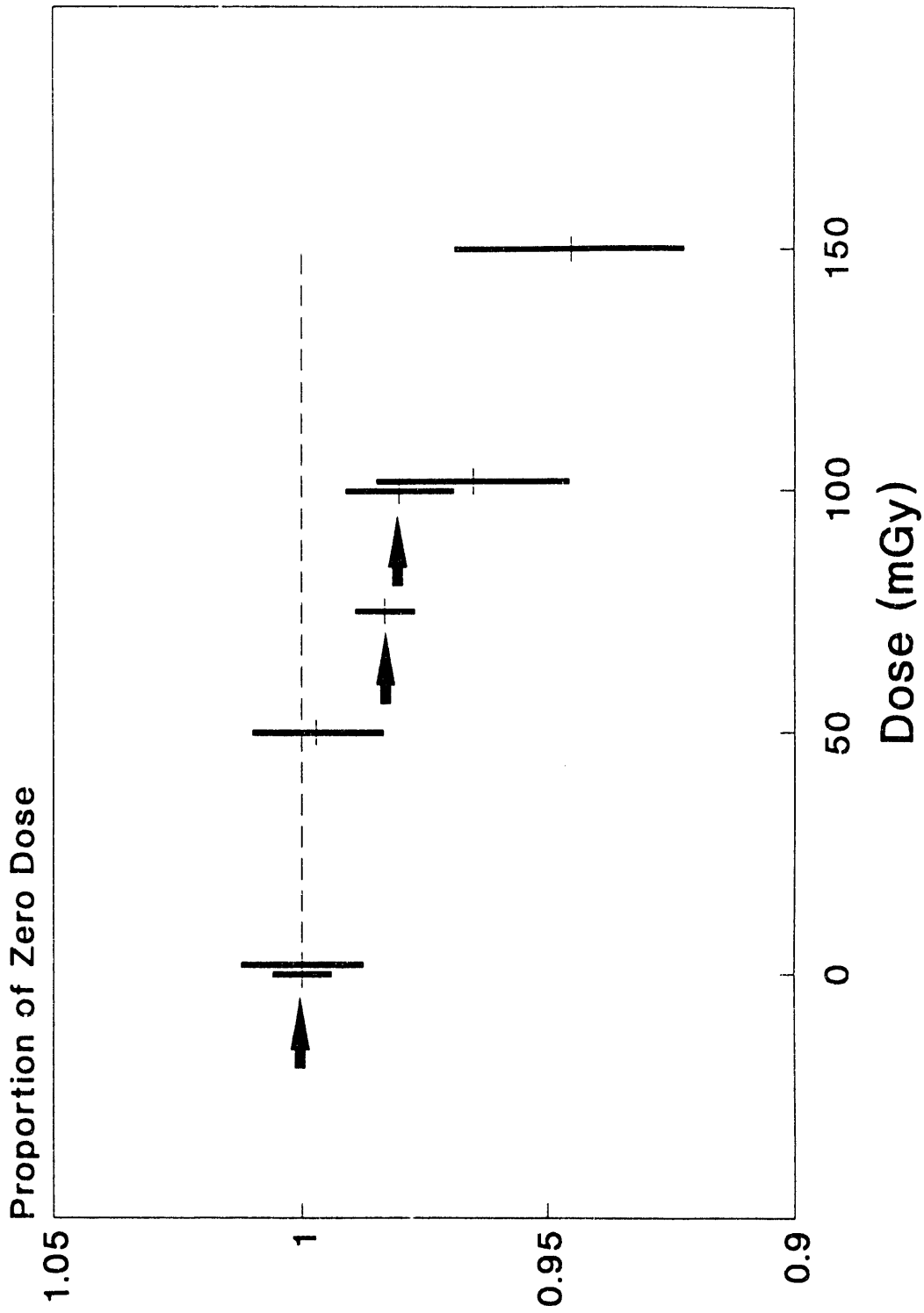
For Examiner B

EFFECTS ON GUINEA PIG BRAIN WEIGHT



For Examiner B

RADIATION EFFECTS ON BRAIN WEIGHT



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