EFFECT OF PATERNAL IRRADIATION ON 154 DAY WEIGHT IN SWINE¹

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Weight at 154 days of age in swine is a common measure of growth rate from conception to near puberty. Weights between 170 and 180 pounds are average. This represents about a 60-fold increase in weight from birth. The interaction of many genes with the developmental environment produce both the general pattern and the variation in growth. Information concerning the genetic effect of irradiation on growth in mammals is limited (CHARLES, TIHEN OTIS and GROBMAN 1961). This paper presents evidence concerning the effect of paternal irradiation on 154 day weight. The effect favored offspring by control males in the first experiment while in the second experiment offspring by irradiated males were heavier at 154 days. Possible causes for this reversal of the effect are examined.

MATERIALS AND METHODS

The two largest noninterbreeding groups of domestic swine in the United States, the Duroc and Hampshire breeds, were sampled equally for the foundation animals. These were purchased near six months of age, the usual age for the sale of breeding stock. Pairs of nonpregnant females that were born in the same litter were purchased from farms of breeders who raise swine that are registered with the breed associations. Several pairs of females were obtained from each of 39 farms. Forty pairs were purchased in the spring of 1959 and 90 in the fall. These will be called group A and B respectively. Most females farrowed two litters. Daughters from the first litters of females in groups A and B are designated as A' and B', respectively.

Males were also purchased in littermate pairs from breeder farms. The males having offspring in this study were obtained in four groups of 12, 20, 28 and 28 individuals, respectively. Males of a group were exposed to X rays at approximately the same time, but groups were separated by six months. The same machine and operator irradiated all the animals. One male, chosen at random from each full-sib pair, received 300r of X rays directed locally to his testicles in one exposure (250 kvp; 15 ma; external filtration = 0.5 mm Cu, 1 mm Al; HVL 1.3 mm Cu; X-ray target to center of testicles 35 cm; average testicle diameter 6 cm; exposure dose rate 100r/min; measured by a Victoreen dosimeter 35 cm

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from the X-ray target and in the center of a rice phantom 6 cm in diameter). The males at the time of exposure were past puberty which usually occurs before 200 days of age in swine. The time from exposure to first breeding averaged 212 days. Even the shortest recovery time of 159 days assures that the sperm sampled at breeding originated from irradiated spermatogonia, not spermatids or mature sperm (WILLHAM and COX 1961). Fertility, based on the percent conception to first service, and size of the resulting litters were both comparable between control and exposed sires. No large differences in age at exposure, recovery time, fertility, or litter size occurred among the four male groups. Males of the first three groups were used in two breeding seasons while males of group four were used in only one season. No females were irradiated at any time.

The females obtained from the farms of breeders were bred to males of the first three groups. Their offspring comprise what is designated as experiment I. The offspring produced later by females born in experiment I are designated as experiment II. These offspring were sired by males of the last two groups. This division into two experiments was made because the females born in experiment I were sired by both control and exposed males. Table 1 presents some statistics concerning experiments I and II. Each experiment was divided into replicates based on the season of the year and the parity of the females having the litters. Parity and environmental factors which differ from one season to the next are known to influence the weight of the offspring.

When a female showed estrus she was bred to a randomly chosen male of the same breed. Then, since both males and females were obtained in littermate pairs, her sister was mated to the brother whenever possible. The resulting pair of litters are related as double first cousins. Fifty-two percent of the 712 litters were obtained in such pairs.

Each litter was born and reared to 154 days of age in an individual pen, 8 × 16 feet. Feeding and management practices were as uniform in a particular season as possible. All growing rations were self-fed. Litters were weaned at 42 days of age by removing the dam. Males were castrated before 28 days of age.

TABLE 1

Statistics concerning the four replicates of experiment I and the three replicates of experiment II

Exp.	Rep.	Year	Season of year	No. of litters		Group*	Parity	Age†	At farrowing Weight‡	
				Duroc	Hamp.				Duroc	Hamp.
I	(1)	1960	May-Jun.	33	35	A	1	1.7	(not weighed)	
	(2)	1960	Sept.-Nov.	76	72	B	1	1.1	425	415
	(3)	1960	Oct.-Nov.	25	29	A	2	2.2	654	580
	(4)	1961	Mar.-May	51	41	B	2	1.6	505	498
II	(1)	1961	Mar.-May	70	62	A§	1	1.0	357	308
	(2)	1961	Sept.-Nov.	41	36	A'	2	1.5	466	411
	(3)	1961	Sept.-Nov.	64	77	B'	1	1.0	399	359

* Purchase group.

† Average age in years.

‡ Average weight in pounds.

§ Offspring of the designated purchase group in experiment I.

ANALYSIS AND RESULTS

Figure 1 presents the frequency distributions of 154 day weight for the offspring of purchased females, experiment I, and for the offspring of replacement females, experiment II. Offspring by control males are shown by solid lines while those by exposed males appear as dashed lines. The breeds and replicates are combined in each distribution. Litters by control and exposed males are about equally represented in the breeds and replicates so neither should bias the comparison between treatments. All distributions are skewed toward the lighter weights, which is typical of 154 day weight distributions. All are of similar shape. No large differences occurred between the variance within litters sired by control males and the variance within litters sired by exposed males. For experiment I the variance within litters sired by control males was 701 with 1131 degrees of freedom while the variance within litters sired by exposed males was 740 with 1051 degrees of freedom. For experiment II these variances were 658 with 933 degrees of freedom and 575 with 808 degrees of freedom, respectively.

FIGURE 1.—Frequency distributions of 154 day weight in experiment I and II. Solid lines represent the weight distribution of offspring by control males while dotted lines represent the weight distribution of offspring by exposed males.

The largest difference among the distributions is between the mean of the first and second experiment. However, the most striking difference is the fact that for experiment I the offspring by control males have a higher mean than the offspring by exposed males while for experiment II the offspring by exposed males had a higher mean than those by control males.

Individual pig weights were analyzed to find the average difference between offspring by control and exposed males and to test the statistical significance of this difference. Offspring by a male pair within a replicate formed the blocks, and the interaction between these blocks and the male treatment was used to test the significance of the treatment difference. Least square procedures for data with unequal subclass numbers (KEMPTHORNE 1952, p. 79) were used since males had different numbers of pigs and in some male pairs only one male left offspring. The analysis of variance for each experiment appears in Table 2 along with the average treatment difference (control minus irradiated) and the mean for the experiments. Both treatment effects were significantly different from zero. In experiment I, offspring by control males were eight pounds heavier than those by exposed males while in experiment II offspring by exposed males were nine pounds heavier than those by control males. The difference in outcome of the two experiments seems far too large to be due merely to chance.

Individuals by control males were heavier than those by exposed males for each replicate in experiment I and lighter than offspring by exposed males for the three replicates of experiment II. Further, all but one of the eight separate breed-replicate effects of experiment I favored control offspring while all six breed-replicates of experiment II favored the offspring by exposed males. Thus, a substantial degree of consistency exists within the two experiments.

Most difficulties arising from the inherent imbalances in the data can be avoided by using only the data from complete double first cousin pairs. Analyses like those of Table 2 were conducted using only double first cousin pairs. The difference between offspring by control males and exposed males in experiment I was $+12.1 \pm 3.4$ and in experiment II it was -11.6 ± 3.3 . Using only the complete data (52 percent of all the data) strengthens the result.

The data were examined for possible causes of this treatment reversal. No

TABLE 2

Analyses of variance for the treatment effect on individual pig weights at 154 days of age

Source	Experiment I		Experiment II	
	df	MS	df	MS
Male pairs	47	22,054	46	11,127
Treatment	1	30,267**	1	35,806**
Male pair $\times$ treatment interaction	22†	3,372	41	3,265
Difference (control-irradiated)	$+8.3$ pounds ± 2.7		-8.8 pounds ± 2.7	
Average weight	174.2 pounds		152.1 pounds	

** $P < .01$.

† In a balanced experiment the pair and pair $\times$ treatment interaction should have the same number of degrees of freedom. The reduction in degrees of freedom occurs because six males in the first purchase had no full-sib pair and some males of a pair left no offspring.

large differences between the two experiments occurred in sex ratio, survival percentage to 154 days, average litter size, average difference in litter size between offspring by control and exposed males, inbreeding of the offspring, or inbreeding of the dams. However, several differences did exist between the two experiments. First, most males had offspring either in one experiments or the other, but some overlap existed. Second, females born in experiment I and having offspring in experiment II were sired by either control or exposed males used in experiment I. All females having offspring in experiment I were sired by control males. Third, the average 154 day weight was 22 pounds less in experiment II than in experiment I. See Table 2 or Figure 1. Each of these differences will be examined in turn.

First, the same males had offspring in replication four of experiment I and in replication one of experiment II. Bred to purchased females the difference was two pounds in favor of the offspring sired by control males. Bred to females born in experiment I the difference was five pounds in favor of the offspring sired by exposed males. Thus, the same males bred to two different groups of females gave contrasting estimates of the effect of paternal irradiation. This suggests that differences between the female groups in experiment I and II are responsible not the male groups.

Second, the fact that half the females born in experiment I were by exposed sires makes experiment II different from experiment I. Separate analyses for experiment II similar to those of Table 2 were made using in one only females by control sires and in the other only females by exposed sires. The difference between offspring by control and exposed sires from females sired by control males and thus directly comparable to experiment I was -6.3 ± 3.8 pounds. The same difference using females by exposed males was -9.7 ± 3.5 pounds. The offspring by exposed males in both cases are heavier at 154 days than those from control sires. Thus, it seems unlikely that the sires of the females used in experiment II, being both control and exposed, are responsible for the offspring of exposed males exceeding those of control males in weight at 154 days in experiment II.

Since the females used in experiment II were sired by both control and exposed sires, it is possible to inquire about the effect of treating the maternal grandsires. The effect of treating the maternal grandsire was examined separately for offspring of control males and for offspring of exposed sires. The sire groups within treatment were used as blocks in analyses similar to those of Table 2. For control males, the offspring from females who were themselves sired by control males averaged $+4.3 \pm 3.3$ pounds heavier than the offspring of females who were themselves sired by exposed males. For exposed males, the corresponding difference was $+3.9 \pm 3.2$ pounds. Although not statistically significant the differences are consistent in showing lighter pigs when the maternal grandsires were irradiated. Moreover, these differences are roughly half as large as the difference between offspring sired by control and exposed males in the first experiment. Since the difference is roughly one half the effect observed in the first generation, it suggests that the treatment difference observed in experiment I

was genetic. However, females by control males averaged 18 pounds heavier than those by exposed males at the time they farrowed the offspring of experiment II. This weight difference to some extent will be reflected in their offspring. Thus, part of the difference between maternal grandsire treatment may be environmental.

Third, several factors are responsible for the 22 pound difference between the average 154 day weights of experiment I and II. The females saved from experiment I were younger and lighter than the purchased females when they farrowed. See Table 1. Part of this lighter weight at farrowing comes from half of the females having irradiated sires as indicated in the preceding paragraph. Experiment II had a higher percentage of litters from dams farrowing for the first time than did experiment I. Several management problems, that would reduce growth rate, occurred during experiment II. Also some differences in the selection of females occurred between the two experiments. These several factors are confounded making distinct separation impossible. Thus, the two experiments must be considered as two sets of circumstances which differ in several respects.

Differences in the selection procedures for the two groups of females deserve some comment. The purchased females were doubtless selected for characters of economic importance which includes weight for a given age. Although little conscious selection was practiced by the purchaser, the breeders would have offered only animals which they thought would be a credit to their herd. Obviously, no way exists to measure this selection. In contrast, every effort was made to choose the replacement females at random. This effort resulted in small selection differentials for 154 day weight, indicating that little if any selection was practiced for weight. However, selection for the ability to conceive at about seven to ten months and to farrow a litter at one year of age was probably more intense for the replacement females than for purchased females. For example, 54 percent of the B' females saved from replicate (2) of experiment I actually farrowed a litter in replicate (3) of experiment II (see Table 1). This gives a selection differential of 7/10 of a standard deviation. But the selection differential for purchased females is not known. The extent to which the difference in selection procedures between experiments could influence the treatment reversal is not known.

DISCUSSION

The data presented indicate that paternal irradiation produced a mean reduction in weight of swine under one set of circumstances (experiment I) and a mean increase in weight under a slightly different set of circumstances (experiment II). The mortality rate in these data is consistently higher in offspring sired by exposed males in both experiments (Cox and WILLHAM 1962). Each experiment shows a statistically significant effect of paternal exposure on the growth rate of the offspring. What is actually demonstrated in these data is not a consistent effect of irradiation but instead an interaction of the irradiation effect with combinations of circumstances, which may be of both genetic and environmental origin. Similar results have been indicated for growth rate in yeast by

MÜLLER and JAMES (1961). However, (CHARLES, *et al.* 1961) indicated no significant mean changes due to paternal irradiation on growth of mice.

In the first experiment average weight at 154 days of age is reduced by about five percent among the offspring of exposed males when compared with contemporary controls. This indicates that the effect of paternal irradiation is to reduce the rate of growth to 154 days, at least under the set of circumstances prevailing in experiment I. To the extent that normal growth rate can be considered to measure the adaptation of the individual to its particular environmental situation, the adaptation of offspring sired by exposed males is reduced by about five percent. This result is what would be expected on the grounds that most mutations are harmful when dealing with a relatively well adapted population. The purchased males and females used to produce the offspring in experiment I can be considered a sample from a population well adapted to the farm environments of the midwest.

In the second experiment average weight was increased by about six percent among offspring sired by exposed males when compared with contemporary controls. This suggests that the effect of paternal irradiation is to increase the growth rate under the set of circumstances occurring in experiment II. This is a complete reversal of the treatment effect seen in experiment I. As previously mentioned several differences exist between experiments. However, none of the differences seem adequate to explain the treatment reversal.

At present no plausible explanation of these contradicting treatment effects is evident. The differences in selection procedure do not seem important enough to account for the change. The lower average weight of experiment II suggests that a less desirable environment existed for the expression of growth than in experiment I. This poorer environment might be expected to increase or decrease the phenotypic effect but hardly to reverse it unless one is willing to suppose either that a slower rate of growth is more adaptive under the conditions of experiment II or that the changes induced by radiation allowed the offspring by exposed sires to exploit the poorer environment more effectively than control offspring. Neither argument is particularly compelling. The reasons for the results must remain in the area of conjecture since it seems impossible with the data at hand to untangle the various circumstances surrounding experiments I and II anymore than has been done already. Additional data now being collected will serve to distinguish between whether there is actually only a small effect and we have encountered improbably extreme and opposite sampling errors or that an interaction between treatment effect and conditions exists for growth as is indicated by these experiments.

SUMMARY

The testes of male swine were exposed once to 300r of X rays. The males were allowed to recover before they were used for breeding so that the sperm sampled were irradiated as spermatogonia. Exposed males, when bred to females purchased from breeders, sired offspring that had a significantly lower weight at 154 days of age than the comparable controls. Exposed males, bred to females that

were from litters produced by the purchased animals, sired offspring having a significantly higher weight at 154 days than the controls. These data suggest that the genetic effect of irradiation may interact with combinations of circumstances, perhaps of both genetic and environmental origin.

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