THE UNIVERSITY OF MICHIGAN

INDUSTRY PROGRAM OF THE COLLEGE OF ENGINEERING

THE NUTRITIONAL VALUE OF IRRADIATED WHEAT

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January, 1959

IP-347

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UMR1108

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INTRODUCTION

The exposure of wheat grain to mild doses of electron radiation (10,000 rep) has been demonstrated by Baker, Taboada, and Wiant to eliminate insect infestation[1,2], and Goldblith[3] has shown that gamma radiation will sterilize flour beetle and granary weevil eggs, and this same dosage will prevent the adults from reproducing. In view of the large losses of wheat each year due to insects, commercially feasible methods of reducing insect infestation are desirable. Brownell et al.[4] have shown that treatment of the wheat with gamma radiation for this purpose is a new process which can be adapted to existing wheat-handling methods.

A demonstration that the nutritional properties of wheat are unimpaired and that no toxic products are formed by this treatment is therefore in order. This is especially true with respect to the vitamin content of wheat because of the reported adverse effect of higher doses of ionizing radiation on the vitamin content of various foods.[5] Although the insect-deinfestation dose proposed for wheat is very low, one vitamin of principal importance in wheat, Vitamin E, is highly sensitive to radiation. Other sensitive vitamins, such as thiamine, would be protected because of the low level of moisture in wheat grain, whereas destruction of a fat-soluble vitamin such as Vitamin E is promoted in the presence of peroxides from irradiation of the wheat germ oil.

Because of the critical role Vitamin E plays in the reproductive process, it was felt that feeding experiments designed to test the reproductive performance of animals would serve as a means of indicating

the absence of toxic properties of irradiated wheat as well as indicating that Vitamin E, and presumably the other vitamins, had not been significantly reduced in content by the mild dose of gamma radiation used. The Vitamin E content of whole wheat varies from 1 to 6 mg/100 gm.[6,7] If wheat of the highest tocopherol content is fed at about 70% of the diet, the minimum requirement for this vitamin is just met for the most demanding phase of reproduction in rats, namely lactation, the requirement for which is 0.75 mg/day.[8] A feeding experiment designed to permit observation on the growth, reproduction performance, and pathology of four successive generations of rats was therefore undertaken.

EXPERIMENTAL

The wheat used was a mixture of soft white wheat and winter durum. It was irradiated in the form of the whole intact grain, inasmuch as this is the form which would be irradiated commercially. The wheat was exposed to a cobalt-60 source of gamma radiation having a flux of 180,000 rep/hr. It was stored at room temperature for a period of one to three months prior to feeding. Just before mixing into the diet, it was coarsely ground. The wheat fed to the fourth generation animals was stored for six months at 80-90F to test adverse storage conditions on the nutritional value of irradiated wheat.

Whole grain wheat does not by itself constitute an adequate diet for the rat, as it must be supplemented by additional protein, lysine, tryptophane calcium, salt, iron, Vitamin A, and riboflavin and other B vitamins. An excellent protein supplement which does not supply significant quantities of Vitamin E is skim milk powder[9] with added quantities of casein, which furnishes the B vitamin requirements, but

not in excessive amounts. The remaining requirements of minerals can be supplied as salts. The diet used thus had the following composition:

Coarsely ground wheat	70%
Purified Casein	14%
Skim Milk Powder (spray dried)	15%
Calcium carbonate	0.75%
Sodium chloride	0.25%
Ferric citrate	0.09%

To prepare the diet, 100 parts of this mixture were added to 35 parts of water, and the resulting thick paste, spread out in a 3/4-inch deep layer, is allowed to "set". It is then broken into small pieces and stored under refrigeration until fed. The only supplement is that of Vitamin A in 0.1/ml corn oil given orally to each rat each week, which supplies less than .08 mg Vitamin E.(10)

The diet containing irradiated wheat and a control diet, identical except for the irradiation step, were each fed to a group of 12 male and 20 female Holtzman strain albino rats. The rats were maintained individually in wire-bottom cages and given water and diet ad libitum. The rats were checked daily and weighed weekly. At 12 weeks of age, male and female rats were mated in weekly rotation. Pregnant females were isolated on shavings in cages until 14 days following the birth of a litter, when the family was transferred to a wire bottom cage. Litters over 10 were reduced to that number 7 days following birth. Animals were weaned at 21 days.

The second generation colony was composed of 20 females, at least one from each litter, and 12 males, selected from the 12 litters

with the most males. The first generation was bred again to obtain additional data on reproductive performance, but the offspring from the second breeding were discarded. The second and third generations were bred in the same manner as the first, and the third and fourth generations obtained in the same manner as the second. The fourth generation was bred only once.

Autopsies and histopathological examinations were made on nearly all rats when found dead or requiring sacrifice during the experimental period. Histopathological diagnoses were made by courtesy of members of the Department of Pathology, School of Medicine, University of Michigan.

Results

The growth curves of the male and female groups fed each diet for all four generations are shown in Figure 1. The vertical width of each curve represents the standard deviation above and below the mean for the group. In some cases, animals were not weighed after the beginning of the second breeding because pregnancy rendered comparisons between groups of little significance.

Good growth was obtained with all four generations of rats fed this diet. The 12-week mean weights for the males and females of each generation were fairly uniform, except that the growth rate of the fourth generation was somewhat higher. It was this generation which was fed the wheat stored for six months after irradiation at 80-90°F. This rate of growth compares favorably with that of rats fed standard laboratory chows. These 12-week mean weights are shown on the next page.

Generation	Males	Females
First	295	190
Second	305	190
Third	295	200
Fourth	335	215

There appears to be no consistent differences in growth rate between animals fed the irradiated and non-irradiated wheat. In most cases, the band of standard deviation overlaps, and where it does not, animals fed the irradiated wheat have the higher body weight. This is true of the first three generations of males, but not of the fourth. There appears to be no significant difference between the groups of females during the period of active growth.

Reproductive Performance

Table I is a summary of the data for all four generations of animals. The overall performance of the animals indicates that the diet as formulated is satisfactory in spite of poor survival of young from the second generation. The diet containing the irradiated wheat resulted in superior performance in nearly every case where a substantial difference in values was observed. This is especially true of the sub-optimum performance of the second generation animals. The paternity of males was established assuming a 22-day gestation period, and it is noteworthy that the percentages for all male groups were high.

As noted later, the members of the second and third generation were inadvertently given 100,000 IU dose of Vitamin A at one time, and this occurred between the first and second breeding of the second

generation and while the third generation was very young. It can be seen that with respect to percent pregnancy and to percent of pregnant females giving birth, the performance during the second breeding is sub-optimum, possibly as a result of the Vitamin A overdosage which is known even in doses as low as 10,000 IU to effect vaginal and uteral epithelium.(11) The survival and body weight of the pups appeared not to be affected. The reproductive performance of the third generation females was superior to that of the second generation in all respects other than rate of pregnancy, and this improved during the second breeding, an indication that any after-effects of the Vitamin A were dissipated.

Pathology and Mortality

Of the 248 animals involved in this experiment, death occurred or sacrifice became necessary for ten at random intervals throughout the 18-month experimental period. None of these animals were kept beyond 37 weeks of age; therefore, this mortality was not due to old age. During one four-week interval, however, an additional seventeen animals were lost following an accidental overdosage of Vitamin A followed in turn one week later by a temporary, but precipitous, drop in temperature of the animal quarters. The mortality from this cause was divided equally between control and experimental groups. Gross observations were recorded at autopsy for all but five of these 27 animals, and histopathological diagnoses were made on the heart, lung, liver, spleen, stomach, pancreas, small intestine, adrenal, kidney, and genital organs for all but seven in which post-mortem changes were too pronounced.

With regard to the ten animals, 7 males and 3 females, lost prior to or well after the period of Vitamin A overdosage, histopathological data is available on five males, the other 2 males and the 3 females having suffered post-mortem changes too extensive for histopathological examination.

There was nothing significantly abnormal at autopsy except for a small prostate hemorrhage in two of the five males, one of which had a hemorrhage behind the jaw and below the brain. There was no lipidosis in any heart tissue specimens, and acute passive congestion in only one (control). Atelectasis and/or emphysema were present in lung specimens from two controls and one experimental, and bronchial inflammation present in one control and one experimental. Lipid was absent in all specimens.

Hemosiderosis was reported in spleen specimens from two controls, and marked acute passive congestion in another. Decreased numbers of lymphocytes were reported in a specimen from an experimental animal, while the other was negative. The stomach, small intestine, and pancreas on all specimens were negative. Moderately heavy to marked lipidosis occurred in liver specimens from one control and two experimentals, the other two controls being negative. Acute passive congestion was present in two controls. Lipidosis was present only to a slight extent in one kidney specimen, while ischemic glomeruli was observed in one control and one experimental. Active spermatogenesis was reported in one testis specimen from a control animal, while spermatozoa were not present in specimens of seminal vesicles from two experimentals. The seminal vesicles of the control male with the hemorrhage behind the jaw was distended with fluid, and the epididymis had hemorrhage around the tubules.

The following is a summary of the pathological findings in the seventeen animals whose death was related to the Vitamin A overdosage in which they were accidentally given 100,000 units in place of the regular 100-unit weekly dose. Although this incident was not planned in the experiment, the results are of interest because sufficient stress was placed on the two colonies of animals to cause death to 17 animals. Subtle effects of feeding an irradiated diet might possibly appear as a result of such a level of stress. Of the seventeen, eight were from control groups, nine from experimental; eleven were from the second generation with an age range of 36 to 41 weeks, six with an age range of 12 to 14 weeks were from the second generation; thirteen were males, four were females.

Many of these animals, especially those which were most chilled when the thermostat failed on the air-conditioner, were found suffering from acute respiratory difficulty just before death. Various forms of hemorrhage were the most frequent observation at autopsy. Hemorrhage involving the uro-genital area was observed in six males (3 control, 3 experimental), one of which had testicular inflammation (experimental). Bleeding around the spleen was seen in two (both controls). The left lung in one male (control) was engorged with blood; in another (control) there was a large clot near aorta; in a third (control), there was thin bloody fluid in the peritoneal cavity. No abnormalities were noted in the four females at autopsy.

No lipidosis was reported in any heart tissue specimens; congestion was found in specimens from three males (2 controls, 1 experimental). Five specimens from males (2 controls, 3 experimental) were

reported entirely negative. Subepicardial purulent inflammation and hemorrhage was reported in one animal having testicular inflammation (experimental). One animal whose left lung and left testis was engorged with blood was found to have an interstitial subendocardial and subepicardial hemorrhage (control). Two females (1 control, 1 experimental) showed evidence of calcification in heart tissue.

Severe, acute, purulent bronchitis or pneumonitis was found in lung specimens from three males (1 control, 2 experimental). Acute edema and/or congestion was found in four (2 controls, 2 experimentals). Lipid was observed in three specimens, abundantly in one control specimen associated with acute purulent bronchitis and pneumonitis, moderately in another control with inactive bronchitis, and in phagocytes of an experimental female had atelectasis in the lung along with a focus of a chronic granulomatous inflammatory process. Another experimental female had emphysema and acute passive congestion of the lungs; the blood took on a heavy fat stain, which is unusual. A control female had chronic lobular pneumonitis with significant activity. There was no lipid, but acute passive congestion.

Hemosiderosis was observed in spleen specimens of seven males, (3 controls, 4 experimentals); hematopoiesis in one (control). Two were reported negative (one control, one experimental). Hemosiderosis was present in spleens from three females (1 control, 2 experimentals).

The stomach, small intestine, and pancreas were negative on all specimens submitted from this group, both males and females.

Liver specimens from all males were negative except for degrees of lipidosis observed in specimens from two controls and two

experimentals. Liver specimens from two females (both experimentals) showed acute passive congestion, with abundant lipid in Kupffer cells of one, and moderate lipid in liver cells near central veins.

Adrenal gland specimens showed decreased cortical lipid in three cases from controls and three from experimental; one specimen from an experimental animal had abundant cortical lipid. One female had a small, somewhat granulomatous inflammatory focus in adipose tissue adjacent to the adrenal gland.

No lipid was reported in any kidney tissue from males in the series receiving overdosage of Vitamin A. Three controls and three experimentals were reported entirely negative. Two experimentals had some protein in tubules; there was acute passive congestion of one control kidney, blood in the pelvis of another. There was no calcium deposition reported in any specimens from males, but three specimens from females were found to have moderately heavy calcium deposits in convoluted and collecting tubules. Lipidosis was slight in one control and one experimental female specimen, marked in another experimental which also had acute passive congestion. In one female, the renal lesions due to calcium deposition were extensive enough to cause death.

Spermatogenesis was active in testes from three control and three experimental males, but various forms of hemorrhage were also present in most of these specimens. One control and one experimental had interstitial hemorrhage; two controls and two experimentals had epididymal hemorrhage. In one control and one experimental with hemorrhage there was decreased spermatogenesis and retrogressive changes in the testes. No findings were reported on specimens of female reproductive organs.

It is abundantly apparent that the effect of the overdosage of Vitamin A did not reveal differences between controls and experimentals, inasmuch as the occurrence of nearly every pathological lesion was evenly divided between the two groups of animals. There has been considerable work on the effect of Vitamin A overdosage on rats, but most of this work involves doses considerably in excess of the 100,000 IU given in this experiment. In addition, most of the past work involves repeated doses to overcome the animal's ability to destroy the greater part of one large dose. Vedder[12] administered 25,000 IU daily for 100 days with no effect but some decrease in weight, and concluded from this and other data that if Vitamin A is toxic, it is in excess of 100,000 IU given daily. However, hemorrhage is one of the usual findings, especially in mucous-secreting tissue, such as lungs and reproductive tissue. Bone fractures, the other common finding, were not noted in these animals.

Conclusions

With so few animals lost during the experimental period, and with nearly all the pathological details appearing in both control and experimental animals, it is concluded that the irradiation of the wheat had no effect on growth, reproduction, and pathology when fed to rats for upwards of nine months. No difference between irradiated and non-irradiated wheat is revealed when both are stored under conditions promoting loss of vitamin content and increase in peroxide formation. The Vitamin E content of wheat given 10 kilorep irradiation is adequate to support good reproduction. It is significant that as many controls as experimental animals suffered from the Vitamin A overdosage, since alphatocopherol, among other forms of Vitamin E, according to Deuel et al[13] tends to mitigate the effect of hypervitaminosis A.

ACKNOWLEDGEMENT

This study was initiated with funds from Michigan Memorial Phoenix Project No. 41. Dr. Ruth Wanstrom, Dr. Robert Hendrix and Dr. A. James French of the Department of Pathology, School of Medicine, University of Michigan, performed the histological diagnoses and were most helpful in their advice during the experiment.

REFERENCES

1. Baker, V. H., Taboada, O. and Wiant, D. E., "Lethal Effect of Electrons on Insects Which Infest Wheat, Flour, and Beans - Part II, Agricultural Engineering, 34, No. 11, pp. 755-58, November, 1953.

2. Baker, V. H., Taboada, O. and Wiant, D. E., "Lethal Effect of Electrons on Insects Which Infest Wheat, Flour, and Beans - Part II", Agricultural Engineering, 35, No. 6, pp. 407-10, June, 1954.

3. Goldblith, S. A., et al., "Relative Bactericidal Efficiencies of Three Types of High Energy Ionizing Radiations", Food Res., 18, p. 659, 1953.

4. Brownell, L. E., et al., "The Design of a Gamma Irradiation Facility for the Control of Insect Infestation in Flour, Meal, or Grain", The Univ. of Mich., Ann Arbor, Eng. Res. Inst. Project 1943-7, May, 1955.

5. Brownell, L. E., et al., "Utilization of the Gross Fission Products", Progress Report No. 7, The Univ. of Mich., Ann Arbor, Eng. Res. Inst. Project 1943, December, 1954.

6. Binnington, D. S. and Andrews, J. S., "The Distribution of Vitamin E in Products of Cereal Milling", Cereal Chem., 18, p. 678, 1941.

7. Litzendorf, J. and Schneider, E., "Aneurine and Tocopherol Determinations in Flour and Bread", Ernahrung, 7, p. 203, 1942.

8. Evans, H. M. and Emerson, G. A., "The Prophylactic Requirement of the Rat for Alpha Tocopherol", J. Nutrition, 26, p. 555, 1943.

9. Abderhalden, R., "Vitamin E Content of Human Milk and Cow Milk", Biochem., 47, p. 318, 1947.

10. Verma, J. P., "Utilization of Oil Refinery Refuse", Indian Soap J., 18, p. 55, 1952.

11. Hedenberg, I., "Experiments with Large Doses of Vitamin A", Acta Chir. Scandinavia, Suppl. 192, pp. 1-87, 1945.

12. Vedder, E. B. and Rosenberg, C., "Concerning the Toxicity of Vitamin A", J. Nutr., 16, p. 57, 1948.

13. Deuel, J. J., Cox, P. O., et al.,"Comparative Effects of DPPD and Other Antioxidant on Hypervitaminosis A in the Rat", Fed. Proc., 15, p. 548, 1956.

TABLE I

DATA ON REPRODUCTIVE PERFORMANCE

	FIRST GENERATION				SECOND GENERATION				THIRD GENERATION				FOURTH GENERATION	
	FIRST BREEDING		SECOND BREEDING		FIRST BREEDING		SECOND BREEDING		FIRST BREEDING		SECOND BREEDING		FIRST BREEDING	
	CONTROL	EXPT'L	CONTROL	EXPT'L	CONTROL	EXPT'L	CONTROL	EXPT'L	CONTROL	EXPT'L	CONTROL	EXPT'L	CONTROL	EXPT'L
NO. OF FEMALES BRED	20	20	20	20	20	20	19	20	19	18	18	18	24	24
NO. OF MALES USED	12	12	12	12	10	10	10	10	8	9	7	9	12	12
% OF MALES WHOSE PATERNITY WAS ESTABLISHED	91.6	91.6	100	91.6	60	80	70	100	87.5	100	100	100	91.7	100
% OF FEMALES BRED WHICH APPEARED PREGNANT	100	100	100	100	100	100	89.4	85	89.4	94.4	83.3	88.8	100	100
% OF APPARENTLY PREGNANT FEMALES WHICH GAVE BIRTH	95	100	100	100	95	100	82.3	88.2	100	100	100	100	95.8	95.8
AVG. NO. PUPS BORN/LITTER	8.3	9.5	10.2	11.0	8.9	9.2	6.9	8.3	8.9	8.2	9.5	9.8	9.6	10.2
% OF PUPS BORN SURVIVING THE FIRST DAY AFTER BIRTH	92.7	87.8	98.0	92.7	63.9	76.1	64.5	70.1	95.4	95.7	97.1	95.5	82.3	88.0
% OF PUPS SURVIVING FIRST DAY WHICH SURVIVED 21 DAYS	79.7	85.5	75.5	79.4	55.5	82.1	69.3	68.9	88.2	79.1	77.5	81.3	77.3	85.0
AVG. BODY WT. AT WEANING (21 DAYS)	41.0	37.5	42.4	41.9	36.9	38.7	47.2	49.6	43.6	42.0	38.3	40.0	38.2	37.1

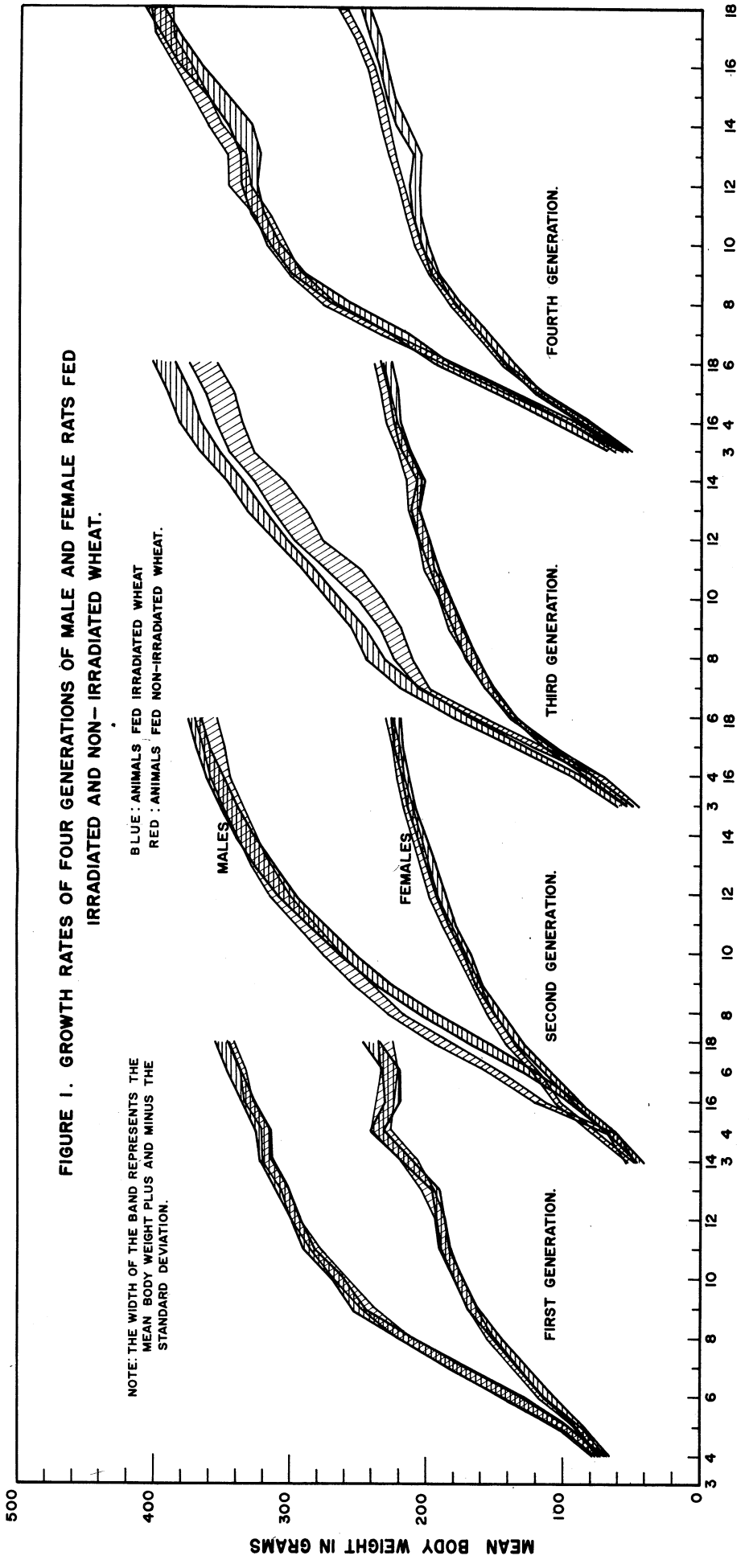
FIGURE I. GROWTH RATES OF FOUR GENERATIONS OF MALE AND FEMALE RATS FED IRRADIATED AND NON-IRRADIATED WHEAT.
BLUE: ANIMALS FED IRRADIATED WHEAT
RED: ANIMALS FED NON-IRRADIATED WHEAT.
NOTE: THE WIDTH OF THE BAND REPRESENTS THE MEAN BODY WEIGHT PLUS AND MINUS THE STANDARD DEVIATION.
MALES
FEMALES
FIRST GENERATION.
SECOND GENERATION.
THIRD GENERATION.
FOURTH GENERATION.
MEAN BODY WEIGHT IN GRAMS
AGE IN WEEKS