ENGINEERING RESEARCH INSTITUTE
THE UNIVERSITY OF MICHIGAN
ANN ARBOR

Progress Report No. 7

WHOLESOMENESS OF A GAMMA-IRRADIATED DIET FED TO CHICKENS AND GAMMA-IRRADIATED POTATOES FED TO RATS

Period March 15, 1956 to June 15, 1956

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Project 2307
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY
CONTRACT NO. DA-49-007-MD-581

July 1956

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OBJECT

The object of the experiments reported on this project is to evaluate the wholesomeness of food and feed treated with gamma radiation.

ABSTRACT

The long-term experiment to test the wholesomeness for rats of three varieties of potatoes treated with two levels of gamma radiation three months prior to the start of the experiment has been in progress for three months. Data on growth rate are presented for the first seven weeks of life of all 150 albino rats and shows, if anything, a slightly favorable result with those diets containing the treated potatoes. Data on efficiency of food utilization are presented for the first six weeks of life of all animals. The six-week averages, expressed as grams gain per gram diet solid consumed, are .375 for controls, .394 for the diet containing potatoes given a normal (15,000 to 22,000 rep) dose of radiation, and .376 for the twice-normal dose of radiation. One male was lost owing to acute respiratory disease and was replaced by his brother. In addition to the normal occurrences of respiratory infection, there has been an occasional incidence of mild eye inflammation.

The second-generation chickens on the long-term chicken feeding experiment have nearly reached their mature weights. There appears to be a significant difference in body weight between the female but not the male groups. This was the result obtained with the first generation. An accidental exposure to chlorine gas resulted in the death of five of the 82 females in the experiment, but normal body-weight gains were resumed and egg production began when expected.

The breeding of the parent mice in the mouse reproduction study is still in progress in an effort to obtain sufficient numbers of second-generation females. Results on the breeding performance and body-weight changes of the parents are presented.

I. POTATO-FEEDING EXPERIMENT

A. PRESENT STATUS OF EXPERIMENT

The potato-feeding experiment has been in progress for fourteen weeks as of June 20, 1956. The animals in the first replicate are fourteen weeks of age and those in the fifth are ten weeks of age. Animals in the first replicate will have been mated starting June 27, 1956. Data on the growth of the animals have been calculated for all replicates through the seventh week. Calculations on efficiency of food utilization have been made through the sixth week for all replicates. In addition, data have been assembled on the total amount of food offered, total amount wasted, and net amount consumed. In the interests of logistics, these are expressed in terms of the total amount of whole potatoes and of the amount of each variety irradiated at each dosage.

Each replicate began with five litters of rats, each consisting of three males and three females. Each animal in a litter is placed in a different experimental group. Because the death or sickness of a single animal in transit from The Holtzman Rat Co., Madison, Wis., would have upset the design of the experiment, one extra male and one extra female littermate were requested in each of the twenty-five litters shipped. Since every one of the 200 animals arrived in good condition, it became possible to establish a fourth dietary group of animals. These are fed a standard laboratory "pellet" diet. The five extra animals of each sex belonging to each replicate are housed together in a large cage. These animals will enable laboratory personnel to observe "normal" pathological occurrences and will provide animals for practice autopsy at any stage of the experiment.

One animal became ill and died of severe respiratory failure after seven weeks of the experiment. This animal was replaced by its brother from the colony of extra littermates. Although this interfered with the quantitative data collected for the group as a whole, this replacement will be fed the experimental diet for nearly the entire duration of the experiment with respect to terminal pathology.

A tentative schedule has been set up as shown in Table I for the two-year experiment for breeding, making blood-cell counts, taking food-consumption data, and for sacrificing first- and fourth-generation animals at the termination of the experiment. The breeding schedule is based on fifteen weeks between weaning and start of breeding, a four-week mating period, and a minimum of one week of rest between successive breedings. The five replicates are staggered at one-week intervals. The fifth replicate follows the first by four weeks, and these two replicates are shown in Table I.

TABLE I

TENTATIVE SCHEDULE OF THE EXPERIMENT

		Replicate 1	Replicate 5
1. Weanlings received and started on experiment		14 Mar 56	11 Apr 56
2. Amount of food offered to and wasted by each animal recorded	start	14 Mar 56	11 Apr 56
	complete	6 Jun 56	4 Jul 56
3. First breeding of parent generation			
a. Mating period (males rotated each week)	start	27 Jun 56	25 Jul 56
	complete	25 Jul 56	22 Aug 56
b. Period of births	start	18 Jul 56	15 Aug 56
	complete	15 Aug 56	12 Sep 56
c. Period of weaning and sacrifice of weanlings	start	8 Aug 56	5 Sep 56
	complete	5 Sep 56	3 Oct 56
4. First set of blood-cell counts on parent animals		19 - 26 Jul 56	
5. Second breeding of parent generation			
a. Mating period	start	12 Sep 56	10 Oct 56
	complete	10 Oct 56	7 Nov 56
b. Period of births	start	3 Oct 56	31 Oct 56
	complete	31 Oct 56	28 Nov 56
c. Period of weaning and selection of second-generation animals	start	24 Oct 56	21 Nov 56
	complete	21 Nov 56	19 Dec 56
6. Amount of food offered to and wasted by each second-generation animal recorded	start	24 Oct 56	21 Nov 56
	complete	13 Feb 57	13 Mar 57
7. Second set of blood-cell counts on parent animals		12 Dec 56	9 Jan 56
8. First breeding of second generation			
a. Mating period	start	6 Mar 57	3 Apr 57
	complete	3 Apr 57	1 May 57
b. Period of births	start	27 Mar 57	24 Apr 57
	complete	24 Apr 57	22 May 57
c. Period of weaning and sacrificing of weanlings	start	17 Apr 57	15 May 57
	complete	15 May 57	12 Jun 57
9. Second breeding of second generation			
a. Mating period	start	22 May 57	19 Jun 57
	complete	19 Jun 57	17 Jul 57
b. Period of births	start	12 Jun 57	10 Jul 57
	complete	10 Jul 57	7 Aug 57
c. Period of weaning and selection of third-generation animals	start	3 Jul 57	31 Jul 57
	complete	31 Jul 57	28 Aug 57
10. Third set of blood-cell counts on parent animals		12 Jun 57	10 Jul 57
11. Amount of food offered to and wasted by each third-generation animal recorded	start	3 Jul 57	31 Jul 57
	complete	16 Oct 57	13 Nov 57
12. First breeding of third-generation animals			
a. Mating period	start	13 Nov 57	11 Dec 57
	complete	11 Dec 57	8 Jan 58
b. Period of births	start	4 Dec 57	1 Jan 58
	complete	1 Jan 58	29 Jan 58
c. Period of weaning and sacrifice of weanlings	start	25 Dec 57	22 Jan 58
	complete	22 Jan 58	19 Feb 58
13. Fourth set of blood-cell counts on parent animals		11 Dec 57	8 Jan 58
14. Second breeding of third-generation animals			
a. Mating period	start	29 Jan 58	26 Feb 58
	complete	26 Feb 58	26 Mar 58
b. Period of births	start	19 Feb 58	19 Mar 58
	complete	19 Mar 58	16 Apr 58
c. Period of weaning and autopsying of fourth-generation weanlings	start	12 Mar 58	9 Apr 58
	complete	9 Apr 58	7 May 58
15. Autopsying of surviving parent-generation animals		12 Mar 58	9 Apr 58

B. EXPERIMENTAL

1. Diet Composition.—Table II shows the composition of the diet in terms of the contribution of each ingredient to the several categories of nutrients, where data are available. Adequate allowances appear to have been provided for all nutrients; the amino acids contributed by the protein of potato are considered to bring the level of phenylalanine and threonine up to the level required by the rat.

The only major change made in the diet since the experiment began was the inclusion of U. S. P. Cod-Liver Oil at a level of 1.0% at the expense of the lard (reduced from 15 to 14%). This change was made at the suggestion of Dr. Eldon Rice as a means of assuring an adequate level of unsaturated fatty acids. This change was made on May 25.

The vitamin mixture used originally is the "Vitamin Diet Fortification Mixture" as compounded by the Nutritional Biochemicals Corporation, Cleveland, Ohio. On May 3, alpha-tocopherol acetate at a level of 5.0% of the mixture was substituted for alpha-tocopherol (alcohol) at 0.5% and ascorbic acid was omitted from the mixture (rats do not require this vitamin, and, besides, it is supplied by the potatoes). The use of cod-liver oil in the diet, which supplies adequate levels of vitamins A and D, made it unnecessary to continue to supply these vitamins in the mixture. Therefore, the 4.5 grams/kilo of vitamin A concentrate (200,000 I. U. per gram) and the 0.25 gram/kilo vitamin D concentrate (400,000 units per gram) were omitted. Because of past experience in mixing vitamin E into a diet containing irradiated food and cod-liver oil, it also was omitted from the vitamin mixture and is instead supplied to the rats individually. The vitamin mixture as it is now used (starting June 28) is shown in Table III, and is identical to the vitamin mixtures used previously, except for vitamins A, D, E, and C.

Table IV shows the composition of the salt mixture which has been used in the preparation of the potato diet since May 5. Until that time, "Salt Mixture U. S. P. XIV" was used. However, the potato component of the diet supplies all the necessary potassium, and in order to avoid an excess, it was necessary to devise a mixture in which potassium salts were excluded.

2. Potato Solids Content.—As a means of holding the content of potato solids to 35% of the total diet solids, the specific gravity of the potatoes was determined as the first step in preparation of the diet. However, the direct relationship which exists between solids content and specific gravity of potatoes of most any variety does not hold for potatoes which have even small amounts of rot or decay, whether inside or on the surface. Since nearly all nine lots had potatoes in this condition, the specific-gravity determinations were abandoned, and direct dry-weight determinations were made on individual potatoes at random. These values showed such a wide scattering for several potatoes in any one lot that it was not possible to determine a value for any one

TABLE II

COMPOSITION OF POTATO DIET

		Requirement by rat per 100 grams diet solids	Composition of Potatoes		Diet Composition, contribution to 100 grams diet solids by:									
			Percent, solids basis	Percent, wet basis	Potato solids, 35 grams	Casein purified, 15 grams	Lactalbumin, 10 grams	Corn starch 14 grams	Alphacel 5 grams	Lard, 14 grams	Cod-liver oil, 1 gram	Salt mixture, 4 grams	Vitamin mixture, 2 grams	Total
Protein	grams	30 (4)	9.0	2.0	3.2	13.2	7.9	—	—	—	—	—	—	24.3
Arginine	"	0.2 (5)	...	...	...	0.50	0.24	—	—	—	—	—	—	0.74
Histidine	"	0.4	...	...	...	0.33	0.17	—	—	—	—	—	—	0.40
Lysine	"	1.0	...	...	...	0.79	0.70	—	—	—	—	—	—	1.49
Valine	"	0.7	...	...	...	1.04	0.26	—	—	—	—	—	—	1.30
Leucine	"	0.9	...	...	...	} 1.28	} 1.11	—	—	—	—	—	—	} 2.39
Isoleucine	"	0.5	...	...	...			—	—	—	—	—	—	
Threonine	"	0.6	...	...	...	0.48	...	—	—	—	—	—	—	0.48
Phenylalanine	"	0.7	...	...	...	0.51	0.10	—	—	—	—	—	—	0.61
Tryotophane	"	0.2	...	...	...	0.29	0.21	—	—	—	—	—	—	0.50
Methionine and cystine	"	0.6	...	...	...	0.49	0.55	—	—	—	—	—	—	1.04
Fat	grams	(6)	0.45	0.1	.12	.23	.3-.4	—	—	14	1	—	—	15.7
Carbohydrate	grams	...	86	19.1	30	.038	...	14	—	—	—	—	1.8	46
Calories	kcal	...	384	85.5	143	60	36	63	—	133	9.0		8	452
Moisture (1)	grams					1.2	.5-.6	...	—	...	...	—	—	...
Ash	"					.315	.3-.4	—	—	—	—	—	—	...
Vitamins - fat soluble														
A	I.U.(2)	100	100-250	20-50	35-88	—	—	—	—	...	1000	—	—	1000
D	I.U.	(7)	...	...	...	—	—	—	—	...	100	—	—	100
E	mg	25	...	...	...	—	—	—	—	...	...	—	—	25 (10)
K	"	(8)	...	...	...	—	—	—	—	...	...	—	4.5	4.5
Vitamins - water soluble														
Choline	grams	.075	...	...	...	...	...	—	—	—	—	—	.15	.15
Inositol	"	...	...	...	...	...	...	—	—	—	—	—	.01	.01
p-Aminobenzoic acid	"	...	...	...	...	...	...	—	—	—	—	—	.01	.01
Thiamine HCl	mg	0.6	.60	.12	.20	...	...	—	—	—	—	—	2.0	2.2
Riboflavin	"	0.6	.20	.04	.07	...	...	—	—	—	—	—	2.0	2.1
Niacin	"	(8)	...	...	...	...	...	—	—	—	—	—	9.0	9.0
Ca pantothenate	"	...	...	...	...	...	...	—	—	—	—	—	6.0	6.0
Pyridoxine	"	0.15	...	...	...	...	...	—	—	—	—	—	2.0	2.0
Folic acid	"	...	...	...	...	...	...	—	—	—	—	—	0.18	0.18
Ascorbic acid	"	(8)	45-90	10-20	16-32	...	...	—	—	—	—	—	—	16-32
Biotin	µg	...	...	...	...	...	...	—	—	—	—	—	40	40
Cobalamin (3)	"	...	...	...	...	...	...	—	—	—	—	—	2.7	2.7
Mineral elements														
Calcium	grams	0.5	.05	.011	.017	.0045	...	—	—	—	—	0.69	—	0.71
Potassium	"	0.5	2.24	.496	.78	...	...	—	—	—	—	(0.00)	—	0.78
Sodium	"	0.25	.11	.024	.038	.003	...	—	—	—	—	.35	—	0.39
Magnesium	mg	40	120	27	43	...	...	—	—	—	—	70.	—	113.
Iron	"	25	3.2	0.7	1.1	.20	...	—	—	—	—	35.	—	36.
Manganese	"	4	.77	.17	.27	...	...	—	—	—	—	6.8	—	7.1
Copper	"	0.5	.72	.16	.25	...	...	—	—	—	—	.71	—	.96
Phosphorus	grams	0.4	.25	.056	.088	.12	.06	—	—	—	—	0.35	—	0.62
Chloride	"	...	.16	.035	.055	.015	...	—	—	—	—	0.53	—	.60
Sulfur	"	(9)	.13	.029	.046	...	...	—	—	—	—	(11)	—	0.1 (9)
Iodide	mg	...	...	...	...	...	...	—	—	—	—	3.2	—	3.2

— Known to be non-existent or infinitesimal.
... Data not available.
(1) Other than in potatoes.
(2) 1 I.U. is equivalent to 0.2 microgram vitamin A (alcohol) or 0.6 microgram beta-carotene.
(3) Vitamin B-12.
(4) There is evidence that rats can show slightly better growth with 40% protein, but 30% is considered adequate.
(5) Based on work of Rose et al.
(6) Rats require milligram quantities of unsaturated fatty acids (e.g., linoleic. arachadonic).
(7) Rats do not require vitamin D in their diet if the Ca:P ratio is from 1:1 to 2:1 and if the phosphorus level is 0.4%.
(8) There is no evidence that rats require an exogenous source of these vitamins.
(9) This is organic sulfur as supplied by sulfur-containing amino acid.
(10) Supplied orally as alpha-tocophenol acetate.
(11) All sulfur as sulfate.

TABLE III

VITAMIN MIXTURE USED IN PREPARATION OF POTATO DIET

	Grams/Kilo
Inositol	5.0
Choline chloride	75.0
Menadione	2.25
p-Aminobenzoic acid	5.0
Niacin	4.5
Riboflavin	1.0
Pyridoxine HCl	1.0
Thiamine HCl	1.0
Ca pantothenate	3.0
	mg/Kilo
Biotin	20
Folic acid	90
Vitamin B-12	1.35
	Grams/Kilo
Extender (dextrose)	902

TABLE IV

SALT MIXTURE USED IN PREPARATION OF POTATO DIET

	Percent
$CaHPO_4 2H_2O$	48.0
NaCl	22.1
$CaCO_3$	15.0
$MgSO_4 7H_2O$	8.7
Ferric Citrate $6H_2O$	5.5
$MnSO_4 1H_2O$	0.52
KI	.08
$CuSO_4 5H_2O$	.07
$ZnCl_2$	.025
$CoCl_2$	.005

lot. Starting June 2, 1956, and until it is possible to obtain more exact knowledge of the moisture content, an average value for the dry weight of 21% is used for all potatoes, regardless of condition. This requires 1300 grams of each of three varieties to make up each batch, whose solids content is 2355 grams, or whose total weight is 5435 grams. Thus, on the basis of total weight of diet, potatoes on a fresh basis constitute uniformly 73.6% of the diet.

3. Procedure of Preparation of Diet.—The procedure for preparation of the diet is as follows. First, 1300 grams of each of the three varieties of potatoes are weighed out and peeled. Any dry rot and other hard abnormalities are included with the skins. The peeled potatoes are then cut into pieces by a household-type French-fry potato cutter. The peelings, plus a small quantity of water, are placed in a Waring blendor and the mixture homogenized. This slurry is then added to the cut-up potatoes and the mixture steamed for 30 minutes at 10-lb pressure. The dry ingredients are weighed out, and the required amount of lard is melted in a hot-water bath. The steamed potatoes are transferred to a Hobart mixer and whipped for a short time to break up all lumps, the dry ingredients added, followed by the cod-liver oil and, finally, the melted lard. While still hot the diet is placed in flat pans, covered with a sheet of polyethylene, and allowed to cool. It is kept refrigerated until used. An attempt is made to prepare the diet on the day it is first fed. One batch of diet lasts two days.

4. Feeding Procedure.—In an effort to assure *ad libitum* feeding conditions, those rats having empty or nearly empty feed cups in the morning were given extra food. However, because the period between the morning and afternoon feedings usually turned out to be only four or five hours, and because the rats, which are nocturnal eaters, tended to waste most of the food given them in the morning, this practice was discontinued. In an effort to conserve diet, the amount of food to be given each rat each day is determined before feeding. This amount is based on that given the day before, plus 10 grams if the jar is empty, no change if there is a little left in the jar, and minus 5-15 grams if there is something left over. As a further means of conserving on diet fed, the diet left over from the previous day is transferred to the top of the new diet. We have not yet experienced rancidity in any of the feed jars.

5. Food-Efficiency Determination.—For the purpose of determining efficiency of food utilization, the solids content of each batch of diet is determined in duplicate each day that it is used. The solids content is based on the dry weight determined after drying the sample for 24 hours at about 70°C. The solids equivalent of all food given each rat each day is then computed and totaled for each week. The food wasted by each animal is caught by a screen (12 x 12 mesh) placed directly under each cage. At the end of each seven days of experiment (Wednesday noon), the food wasted plus that remaining in the jars is dried under the same conditions used in dry-weight determination of the diet samples. Since the screens under each cage catch all the fecal matter, this has to be separated from the waste food. This is done either mechanically or

by immersing the dried waste food and feces in water and floating off the dried feces. The value for food solids wasted is subtracted from the total food solids fed to give the net consumption of food solids for each rat per week. When the gain in weight for a particular rat for a given week is divided by the net consumption, a measure of the efficiency of food utilization is obtained.

6. Attempts to Reduce Wastage of Diet.—A number of materials were added to the diet on an experimental basis to make it more difficult for the rats to waste their food. Guar gum was added at a 1%, then at a 2%, level to the diet over a period of ten days without effect. For a two-day period, sucrose was added at a level of 10% at the expense of starch without reducing the wastage; for another two days, gelatin at a 4.2% level at expense of lactalbumin, again without effect. These additions are helpful for liquid diets, but serve to make a soft diet more friable.

When the morning feeding was omitted, wastage was reduced. Wastage was reduced further by restricting somewhat the amount of food offered without interfering with *ad libitum* feeding conditions. Wastage has now ceased to be a problem.

C. RESULTS

1. Growth Rate.—Table V presents the body-weight data for each group in each replicate from the initial ("zero") through seven weeks of the experiment. Each figure is the average for five animals. Averages are also shown for animals in each group in all five replicates for each week of the experiment, and the percent differences of the experimental (1X and 2X) groups from the control (0X) are calculated from these and averaged for seven weeks to provide an overall summary of group differences.

Figure 1 is a plot of the all-replicate averages. Calculations have not yet been made of mean weights and statistical probability of significance. Almost invariably the animals on the experimental (1X and 2X) diets show slightly greater percent gains than do those on the control diet. The males and females fed the diet containing potatoes treated with a normal dose (15,000 to 22,000 rep) of gamma radiation gained weight 2.4% more rapidly, and those fed the diet containing the twice-normal irradiated potatoes gained weight 1.3% more rapidly than the controls.

2. Efficiency of Food Utilization.—Table VI presents the data on efficiency of food utilization for each group in each replicate for the first six weeks. These data are to be determined for twelve weeks altogether, and the data for the remaining six weeks will be presented in the next report. The data are presented in terms of grams gain in body weight per gram diet solid consumed.

TABLE V

GROUP AVERAGE BODY WEIGHTS

Average body weight, grams, for each dietary group
(3 male and 3 female groups) in each replicate, for each of the first
seven weeks of the experiment. Each figure is an average for 5 animals.

Week	Replicate	Males			Females		
		0X	1X	2X	0X	1X	2X
0	1	47.8	47.2	46.4	46.4	47.2	46.4
	2	49.8	48.4	48.6	47.8	47.8	46.4
	3	49.6	48.4	49.4	47.8	47.2	48.2
	4	48.8	48.8	48.0	48.2	49.0	49.6
	5	48.8	48.0	49.0	48.4	47.2	46.8
	Avg	49.	48.2	48.3	47.7	47.7	47.5
1	1	91.0	88.8	80.0	83.4	86.8	82.2
	2	90.8	91.4	89.0	83.8	85.0	82.8
	3	93.8	91.8	91.4	85.4	86.0	87.0
	4	93.0	97.2	91.6	86.2	89.6	87.4
	5	93.2	90.8	92.8	84.2	85.0	81.6
	Avg	92.4	92.0	89.0	84.6	86.5	84.2
	Percent Diff.		-0.4	-3.7		+2.2	-0.5
2	1	135.6	133.0	144.6	121.2	124.8	124.6
	2	140.2	143.0	142.4	120.0	120.0	118.0
	3	145.6	145.2	141.2	122.4	125.6	124.8
	4	139.0	146.8	139.8	117.6	123.6	121.2
	5	141.4	139.6	146.0	120.0	120.2	117.0
	Avg	140.4	141.5	142.8	120.2	122.8	121.1
	Percent Diff.		+0.8	+1.7		+2.1	+0.8
3	1	185.8	183.2	193.6	149.8	157.4	153.8
	2	187.0	195.6	193.6	145.4	150.4	144.6
	3	193.0	193.4	188.0	149.2	152.2	153.0
	4	184.4	195.2	188.6	145.2	151.0	146.2
	5	185.8	185.0	196.6	147.4	151.4	144.0
	Avg	187.2	190.5	192.1	147.4	152.5	148.3
	Percent Diff.		+1.7	+2.6		+3.5	+0.6
4	1	230.8	232.0	243.6	168.4	178.0	181.4
	2	237.0	244.2	243.8	166.0	170.6	163.2
	3	243.0	242.8	230.4	171.2	173.6	176.4
	4	230.4	245.4	239.2	162.0	171.0	165.4
	5	234.8	237.8	250.2	170.0	177.0	170.6
	Avg	235.2	240.4	241.4	167.5	174.0	171.4
	Percent Diff.		+2.2	+2.6		+3.9	+2.3
5	1	274.4	275.2	236.2	188.0	198.2	198.8
	2	283.8	293.4	285.0	181.2	190.0	179.2
	3	287.2	283.2	275.4	187.4	190.4	192.6
	4	273.6	295.8	287.6	184.0	191.0	189.4
	5	281.8	288.8	302.2	189.4	204.0	189.6
	Avg	281.2	287.3	287.3	186.0	194.7	189.9
	Percent Diff.		+2.2	+2.2		+4.7	+2.1
6	1	311.0	309.2	327.2	205.4	213.6	214.6
	2	319.0	328.2	321.0	194.2	206.4	196.2
	3	324.4	321.4	314.4	208.4	211.4	213.8
	4	315.6	335.2	326.0	198.4	207.2	204.6
	5	322.6	325.6	346.0	207.8	221.6	203.0
	Avg	318.5	323.9	326.9	202.8	212.0	206.4
	Percent Diff.		+1.7	+2.7		+4.5	+1.8
7	1	336.6	337.6	356.4	214.4	227.6	223.6
	2	350.6	359.2	358.0	228.4	221.2	212.6
	3	355.4	351.8	342.2	224.4	228.6	221.4
	4	346.0	372.4	344.8	213.2	220.4	219.6
	5	355.8	345.0	381.0	216.0	230.0	216.4
	Avg	348.9	353.2	356.5	219.3	225.6	218.7
	Percent Diff.		+1.2	+2.2		+2.9	-0.3
	Average of weekly percent differences		+1.3	+1.5		+3.4	+1.0

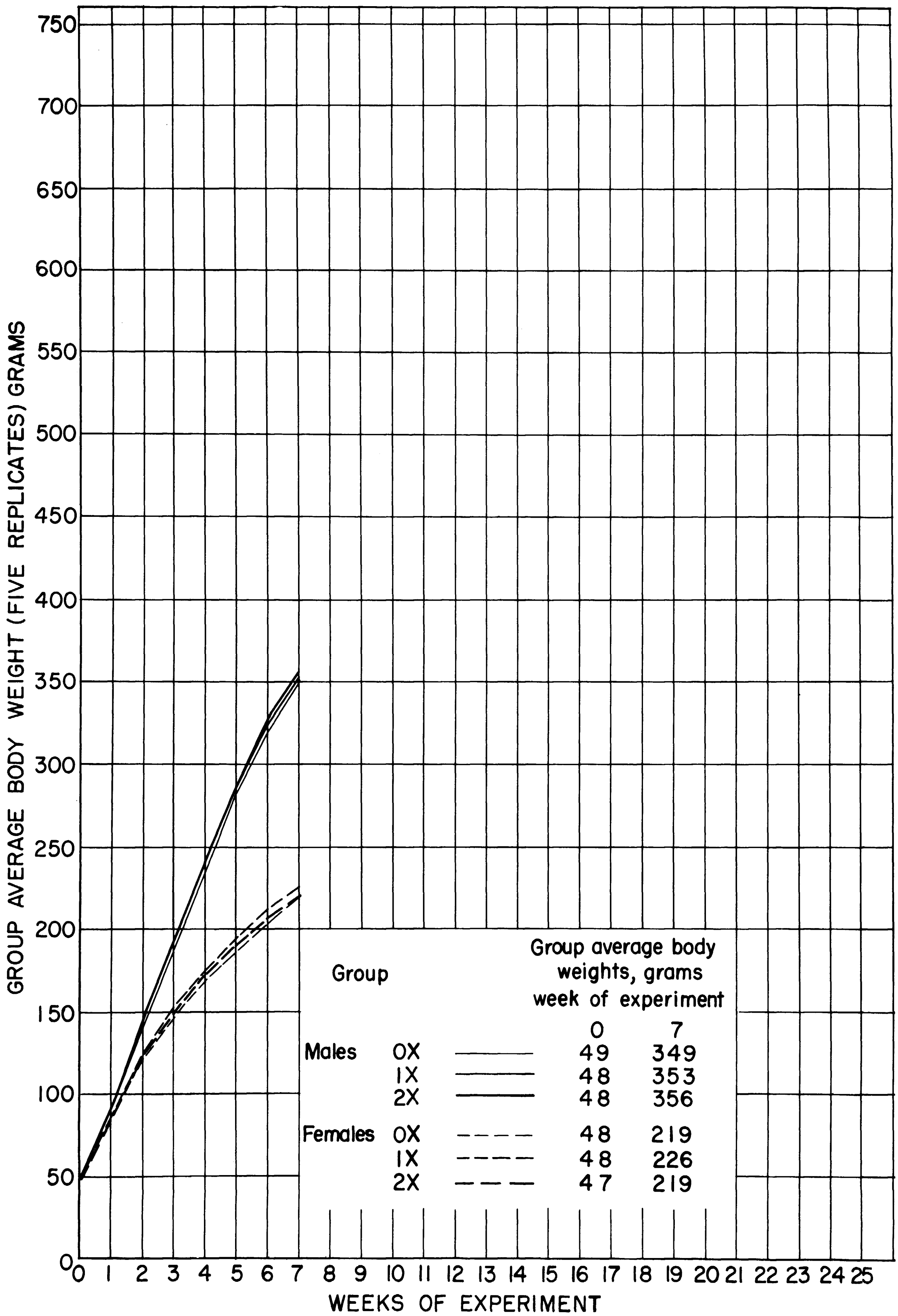

Fig. 1. Growth curves of males and females on the OX, 1X, and 2X potato diets for the first seven weeks of experiment.

TABLE VI

FOOD EFFICIENCY

Average body weight gain, grams, per gram diet solids consumed, for each dietary group (3 male and 3 female groups), in each replicate, for each of the first six weeks of the experiment. Each figure is an average for five animals.

Week	Replicate	Males 0X	Males 1X	Males 2X	Females 0X	Females 1X	Females 2X	Week	Replicate	Males 0X	Males 1X	Males 2X	Females 0X	Females 1X	Females 2X
1	1	.695	.646	.476	.653	.648	.624	4	1	.380	.397	.407	.210	.216	.252
	2	.650	.652	.653	.589	.639	.614		2	...	...	...	...	...	...
	3	.617	.623	.599	.572	.578	.603		3	.386	.361	.367	.226	.217	.238
	4	.669	.733	.634	.576	.626	.565		4	.351	.334	.357	.159	.176	.160
	5	...	...	...	...	...	...		5	.357	.384	.360	.211	.213	.242
	Avg(1)	.658	.664	.590	.598	.623	.602		Avg(1)	.369	.369	.373	.202	.206	.213
2	1	.503	.500	.735	.494	.483	.558	5	1	.331	.326	.335	.183	.198	.175
	2	.492	.561	.480	.426	.403	.379		2	.352	.358	.309	.161	.197	.156
	3	.562	.546	.552	.426	.442	.411		3	.282	.273	.317	.144	.151	.146
	4	...	...	...	...	...	...		4	.340	.334	.324	.219	.191	.229
	5	.652	.603	.686	.582	.520	.538		5	.345	.363	.373	.206	.271	.213
	Avg(1)	.552	.553	.613	.482	.462	.472		Avg	.330	.331	.332	.183	.202	.184
3	1	...	...	...	...	...	...	6	1	.268	.266	.305	.175	.154	.166
	2	.421	.455	.433	.269	.320	.267		2	.225	.226	.239	.131	.134	.157
	3	...	...	...	...	...	...		3	.236	.248	.263	.177	.181	.197
	4	.429	.419	.461	.308	.312	.277		4	.244	.275	.300	.167	.168	.165
	5	.438	.365	.377	.268	.253	.254		5	.283	.262	.294	.179	.172	.141
	Avg(2)	.426	.413	.424	.282	.295	.266		Avg	.251	.255	.280	.166	.162	.165

(1) Average of 4 replicates only.
(2) Average of 3 replicates only.

Many difficulties were encountered in obtaining these data, and a large number of weighings and calculations are involved in arriving at each of the figures in the table. For the most part, the all-replicate averages for the three male groups or the three female groups agree closely for any one week, although there are some glaring discrepancies. There is practically no difference in efficiency of food utilization between 0X and 1X males throughout the six-week period, but during three of the six weekly points, the 2X males differed widely from the other two groups. In the case of the females, there was good agreement between the 0X and 2X groups, but the 1X group differed considerably from the other two during two of the six weekly points. Although the variability of the data makes small differences statistically insignificant, it would appear that no marked differences in food efficiency were observed during the first six weeks between control and irradiated potatoes.

3. Total Diet Solids Fed, Wasted, and Consumed and the Equivalent in Whole Potatoes.—Tables VII, VIII, and IX present, respectively, the all-replicate totals for each week of diet solids offered, wasted, and, by difference, that consumed. The value shown for each week is actually the combination of the values for corresponding weeks for each replicate rather than the calenar week. Also shown is the equivalent in whole potatoes fed, wasted, or consumed of the total diet solids fed to each group for six weeks, and the average daily amount diet solids fed, wasted, or consumed per rat for the six-week period. Table VII, in addition, shows the amount of whole potatoes of all varieties and radiation doses used each week, and the amount of any one variety at any one radiation dose used for all six weeks.

These data will be discussed when data on the remaining six weeks are available.

4. Pathological Observations.—Routine pathological examination of each animal for any abnormality observable by external examination was begun on June 14 when the animals in the first replicate were 13 weeks old and those in the fifth were nine weeks old. Particular attention is given to rating the degree of respiratory infection, to the condition of eyes, and to swellings. Animals for which an abnormality is noted are checked again at semiweekly intervals.

Until the weekly checks were made, the only observations made were of eye inflammations and severe respiratory infections. Table X presents these observations and includes the pathological status of the animals as of the first routine check. Each of the fifteen blocks covers the five male and five females on each diet in each replicate. The eye inflammation ("EI") is characterized by an inflammation of the lids and a discharge at the corners of the eyes which becomes crusty. The eye also appears pale. Moderate respiratory infection ("MRI") indicates sniffling, shortness of breath, without evidence of fluid in the lungs, while severe respiratory infection ("SRI") indicates fluid accumulation. Animals sensitive to touch show profound irritation when handled and the majority do not have any other pathological condition. When the animals

TABLE VII

AMOUNT OF DIET OFFERED

		Diet Solids Offered, Totals Per Week, kilograms									Equivalent of total offered all groups in fresh potatoes, lb
		Per group of 25 males			Male groups	Per group of 25 females			Female groups	All groups	
Week		0X	1X	2X		0X	1X	2X			
1		2.91	2.86	2.82	8.59	2.84	2.76	2.74	8.34	16.9	62.1
2		3.27	3.15	3.31	9.73	3.58	3.20	3.57	10.35	20.1	73.6
3		3.46	3.46	3.54	10.46	3.86	3.52	3.59	10.97	21.4	78.6
4		3.66	3.99	3.78	11.43	3.92	3.75	3.67	11.34	22.8	83.5
5		3.80	4.10	4.05	11.95	4.18	3.84	3.86	11.88	23.8	87.5
6		4.12	4.16	4.14	12.42	4.00	3.92	4.01	11.93	24.4	89.3
Total		21.22	21.72	21.64	64.58	22.38	20.99	21.44	64.81	129.4	475
Equivalent of 6-week total in fresh potatoes, lb (1)		77.9	79.6	79.5	237.	82.1	77.0	78.6	238.	475	—
Equivalent in fresh potatoes of each variety, lb (2)	MeK(3)	27.6	28.0	28.0	83.5	28.9	27.1	27.7	83.8	167	Total 0X: 160
	MeR(4)	24.1	24.7	24.7	73.5	25.4	23.8	24.4	73.8	147	" 1X: 157
	IdR(5)	26.3	26.9	26.9	80.1	27.8	26.0	26.6	80.5	161	" 2X: 158
Average daily diet solids fed per rat for the 42-day period, grams		20.2	20.7	20.6	20.5	21.3	20.0	20.4	20.5	20.5	—

(1) Based on 21% solids content of all potatoes.
(2) Different amounts for each variety based on early values of specific gravity.
(3) Maine Katahdin variety.
(4) Maine Russet variety.
(5) Idaho Russet Burbank variety.

TABLE VIII

WASTE OF DIET

Week	Waste of Diet Solids, Totals per Week, Kilograms								
	Per group of 25 males			Male groups	Per group of 25 females			Female groups	All Groups
	0X	1X	2X		0X	1X	2X		
1	1.27	1.20	1.13	3.60	1.27	1.18	1.18	3.63	7.23
2	1.05	0.91	1.03	2.99	1.70	1.18	1.61	4.49	7.48
3	0.64	0.50	0.56	1.70	1.48	0.97	1.15	3.60	5.30
4	0.43	0.55	0.48	1.46	1.08	1.41	0.93	3.42	4.88
5	0.50	0.56	0.60	1.66	1.65	1.25	1.32	4.22	5.88
6	0.53	0.51	0.59	1.63	1.48	1.24	1.52	4.24	5.87
Total	4.42	4.23	4.39	13.04	8.66	7.23	7.71	23.60	36.64
Equiv. of 6-week total in fresh potatoes, lb.(1)	16.2	15.5	16.1	47.9	31.8	26.5	28.3	86.6	134.5
Average daily waste, diet solids per rat, for 6-week period, grams	4.21	4.04	4.19	4.15	8.26	6.90	7.35	7.50	5.83

(1) Based on 21% solids content of all potatoes.

TABLE IX

NET CONSUMPTION OF DIET

Week	Net Consumption of Diet Solids: Weekly total, per group of 25 males, kg.			Daily average per male each week, grams	Weekly total, per group of 25 females, kg.			Daily average per female each week, grams	Weekly total, all groups kgs.
	0X	1X	2X		0X	1X	2X		
1	1.64	1.66	1.69	9.50	1.57	1.58	1.56	8.9	9.70
2	2.22	2.24	2.28	12.8	1.88	2.02	1.96	11.2	12.60
3	2.82	2.96	2.98	16.7	2.38	2.55	2.44	14.0	16.13
4	3.23	3.44	3.30	19.0	2.84	2.34	2.74	15.1	17.89
5	3.30	3.54	3.45	19.6	2.53	2.59	2.54	14.6	17.95
6	3.59	3.65	3.55	20.6	2.52	2.68	2.49	14.6	18.48
Total	16.80	17.49	17.25		13.72	13.76	13.73		92.75
Equivalent in fresh potatoes, lb. (1)	61.6	64.1	63.3		50.4	50.1	50.4		340
Average daily net consumption diet solids per rat for the 42-day period, grams	16.0	16.7	16.4	16.4	13.1	13.0	13.1	13.1	

(1) Based on 21% solids content of all potatoes.

TABLE X

SUMMARY OF GROSS OBSERVATIONS OF DISEASE IN PARENT RATS DURING FIRST 13 WEEKS

Replicate	Dietary Group		
	0X	1X	2X
1	♂26 - EI(6) ♂27 - EI(9) MRI(14) ♂29 - EI(10, 14) 2 animals lost 5g or over at weighing on June 13 (14)	♀ 4 - EI(9)	♂51 - EI(10) 10 animals lost 5g or over at weighing on June 13 (14)
2	♀ 34 - EI(8, 10, 11, 12, 13) ♀ 8 - MRI(11) lost 9g (11) ♂ 7 - MRI(11) lost 25g (11) 3 animals lost 5g or over at weighing on June 13 (13)	♂7 - MRI(11, 12, 13) lost 25g (11) ♀ 8 - MRI (11) lost 9g (11) 3 animals lost 5g or over at weighing on June 13 (13)	10 animals lost 5g or over at weighing on June 13 (13)
3	♀ 38 - MRI(12) ♂39 - MRI(12) 2 animals lost 5g or over at weekly weighing on June 13 (12)		♀ 65 - EI(7, 10) ♂61 - MRI(12) 8 animals lost 5g or over at weekly weighing on June 13 (12)
4	♂41 - Sensitive to touch (11) ♂42 - EI(11) ♂44 - MRI(11) ♂45 - MRI(11)	♀ 18 - MRI(9) lost 11g (9) ♂19 - MRI(11) 1 animal lost 5g at weekly weighing on June 13 (11)	♂67 - lost 24g at weekly weighing and died following Monday (8) ♀ 68 - EI(9, 10, 11) ♀ 67 - MRI(9) lost 14g (9) ♂67*- SRI(9, 10, 11) Sensitive to touch (10,11) ♀ 70 - EI(11) 7 animals lost 5g or over at weekly weighing on June 13 (11)
5		♂23 - MRI(8, 9) lost 18g (8) SRI(10)	♀ 71 - EI(9, 10) ♀ 72 - MRI(10) ♀ 74 - MRI(10) ♂73 - Sensitive to touch (10) 8 animals lost 5g or over at weekly weighing on June 13 (10)

KEY:
MRI - moderate respiratory infection
SRI - severe respiratory infection
EI - inflammation of the eye
( ) - parenthesis equal number of weeks on experiment at time of occurrence
* - this was the replacement for 67 which previously died

were weighed on June 13, nearly all those fed the 2X diet lost weight. This was traced to rancidity and sourness in a particular batch of 2X diet due to prolonged storage. When fresh diet was used, the weight loss was restored.

Male rate No. 67, in the 2X group, came down with an acute respiratory infection infection. It could not eat for three days, and in an attempt to tube-feed the animal, it died. The observations made at autopsy and the histopathologist's report are shown in Table XI. The severe rattly breathing, the lung appearing as the only abnormal organ at autopsy, and the pathologist's finding of pneumonia and bronchiectasis indicate that this animal died of respiratory failure. Five other animals in the colony showed evidence of severe respiratory infection and loss of weight. They were isolated from the colony and recovered in a few weeks.

II. CHICKEN FEEDING EXPERIMENT

A. STATUS

The chickens of the second generation on the long-term chicken feeding experiment were 20 weeks of age on the 12th of June. The pullets have begun egg production, and all animals have been switched from the "Growena" mash to the "Breeding Mash" whose composition is given in Table I, page 2, Progress Report No. 5. A breeding program has been set up to test each of the 20 males in each group for fertility. This program will begin on July 30. Data on growth, food consumption, and pathology are presented herein.

On May 12 the colony was exposed to a dose of chlorine gas which resulted in the death of five adults and nearly all of a colony of young chicks maintained in the same room. The gas originated from a leaking cylinder stored in the laboratory used by chemists engaged in studying the effect of gamma radiation on chemical reactions. The exposure lasted for about four hours. The histopathological reports of some of the tissues of the birds lost are reported. We were advised by the pathologist that the direct cause of death was pneumonia and not toxicity as such, and that it was possible for the colony to recover from the exposure and to be of value to the experiment.

B. MANAGEMENT

The birds of the second generation were started in the chick battery and transferred to the rooster battery on March 1, 1956, each cage of which was provided with a raised wire bottom. After nine weeks of experiment, the 42 pullets of each group were transferred to individual cages in the laying battery. Insofar as possible, birds of each group are distributed in the various locations in the chicken room. The 21 roosters in each group were then spread

TABLE XI

AUTOPSY AND PATHOLOGIST'S REPORT ON 2X 4TH REPLICATE MALE RAT 67, DIED MAY 28, 1956

Autopsy: "Lungs enlarged (4.8 grams for floating portion, 1.2 grams for sinking portion; body weight of animal was 269 grams at death; average weight of group was 368 grams). Whitish enlargements on lung. Liver normal, weighed 14.3 grams. Lung only abnormally appearing organ."

Histopathological Report (9685-LBH)

Esophagus: Negative

Stomach: Negative

Testis: Spermatogenesis active. Postmortem change.

Voluntary Muscle: Negative.

Pancreas: Islet tissue abundant. Some very large islets. Essentially normal pancreas.

Heart: Subendocardial vacuolar change. Focal inflammatory infiltration of lymphocytes and eosinophils in the myocardium. No lipidosis.

Kidney: Congestion. Serous atrophy of subpelvic fat. No lipidosis.

Spleen: Intense acute passive congestion.

Liver: Intense acute passive congestion. Well-marked degenerative fatty infiltration.

Seminal Vesicles: Dilatation.

Prostate: Negative.

Vas Deferens: Negative.

Thyroid: Colloid in normal amount.

Trachea: Severe chronic catarrhal tracheitis.

Adrenal: Marked congestion.

Parotid, Submaxillary, and Sublingual Glands: Negative.

Small Intestine: Negative.

Lymph Nodes: Purulent lymphadenitis. Serous atrophy of regional fat.

Lungs: Congestion. Edema. Patchy emphysema. Severe chronic purulent fibroid pneumonia with dilatation of bronchi. Bronchiectasis and bronchiectatic abscesses. Organization of exudate in some of the bronchi. No fat emboli. Numerous lipophages shown by fat stain.

Vertebral Bodies: After decalcification, highly cellular bone marrow. Some evidence of endochondral bone formation.

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out individually in the rooster batteries, the raised bottoms having been removed.

The birds, both males and females, had been fed Startena mash for the first five weeks, Growena mash for 16 weeks, Layena mash one week, and since then have been fed the breeding mash. The composition of these mashes are all given in previous reports. Food consumption was recorded by recording the amount fed, inasmuch as the spillage was negligible and there was no spoilage. Ad libidum feeding conditions were permitted during the first two weeks of recording of food consumption, after which food intake was restricted slightly in an attempt to magnify any differences. However, after one week, the slightly restricted food intake was discontinued.

C. RESULTS

1. Growth.—Figure 2 shows the growth curves of the four groups of chickens to 20 weeks of age. The center of each band is the mean weight for that group, and the vertical thickness of the band represents the statistical deviation of the mean. A statistically significant difference between the mean weights of two groups exists if the bands do not overlap. It is apparent that there is a significant difference in mean weight between the two female groups, but this is only barely so with the males. This was exactly the case with the parent generation. The exposure to chlorine gas at the 14th week caused the depression in the growth rate of the females, more so with the control than with the experimental females. However, this retardation of growth rate was overcome and the statistically significant difference between the four groups reappeared. In the case of the males, the chlorine exposure increased the variation in weight among the birds in each group, so that there ceased to be a statistically significant difference between the mean weights of each group.

2. Food Consumption.—During a two-week period, which was the 11th and 12th weeks for the younger birds and the 12th and 13th for the older, the control males and females were fed 88.1 kg mash (dry weight) and the experimental males and females 81.0 kg mash (dry weight). During this period, the birds fed the control diet (42 females and 21 males) gained a total of 16.1 kg, while the same number of males and females fed the experimental diet gained 15.4 kg. This represents an efficiency of food utilization of 183 grams gain per kg dry mash consumed for the controls and 190 grams gain per kg dry mash for the experimental birds. This result is favorable with respect to irradiation of the diet. There is no difference in percent gain between the female groups, but there is a slightly higher percent gain by the control males than by the experimental males.

3. Pathology.—None of the 126 birds in the experiment were lost prior to the time when five (two control and three experimental) pullets were lost as a result of the exposure to chlorine. No males were lost at that time. A histopathology report of two of the five birds (no report was made on the

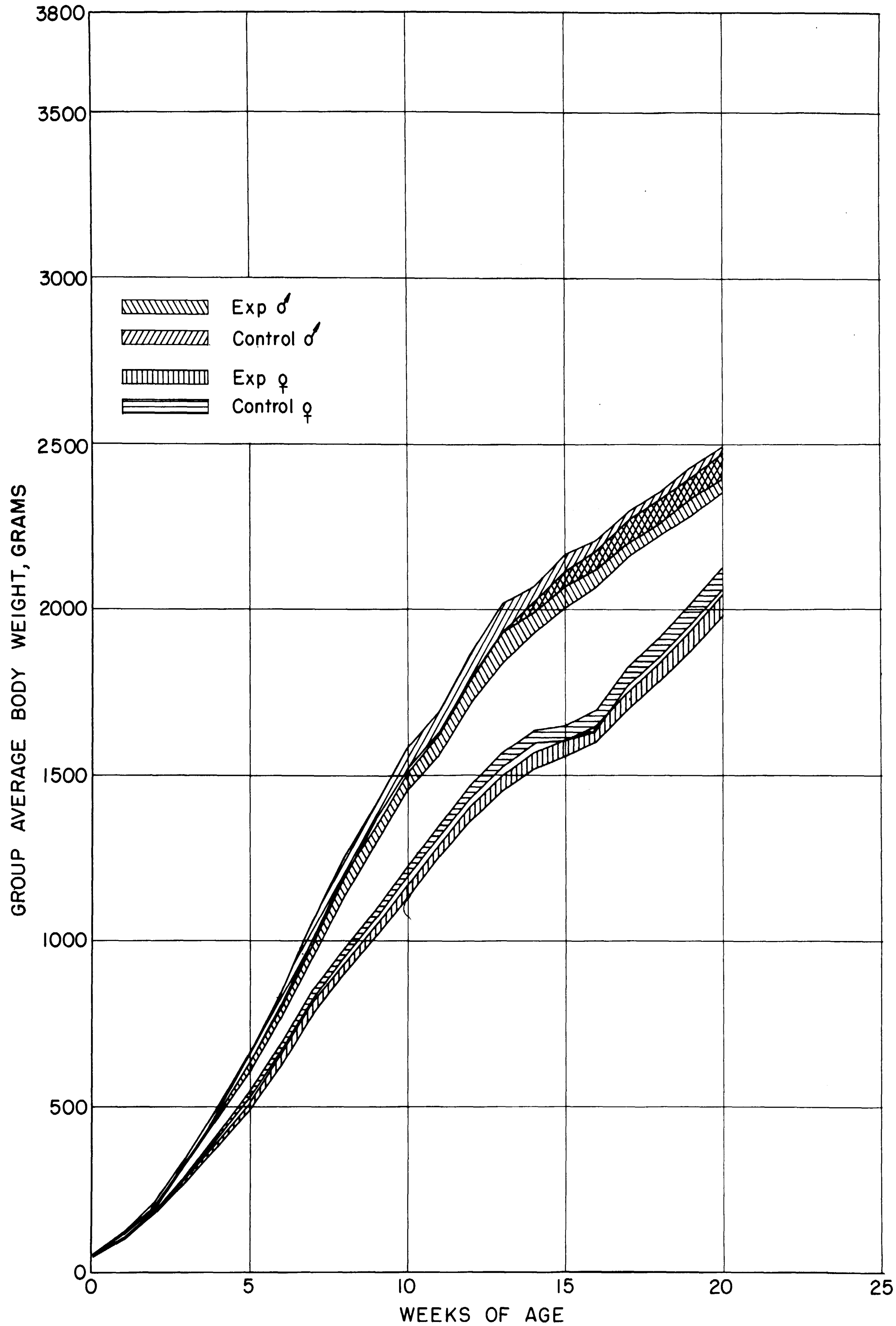

Fig. 2. Growth curves for second-generation male and female chickens on nonirradiated and irradiated wet mash for the first 20 weeks of life. The vertical distance across each band represents the statistical probability of significance.

others owing to post-mortem change) is given in Table XII. The report indicates that the birds died of acute pneumonia and not of poisoning by chlorine as such.

Since that time, one control pullet was sacrificed (June 12), another died (June 19), and one experimental rooster was sacrificed (June 12). The sacrificed pullet had difficulty in standing due to a twisted knee joint, and the sacrificed rooster has perosis. There was nothing grossly abnormal at autopsy of the pullet which died. The histopathological reports of these birds are not yet available.

III. MOUSE REPRODUCTION STUDY

A. STATUS

The parent-generation animals in this experiment are now approximately 27 weeks of age (June 15, 1956) and have been fed the experimental diets for 22 weeks. The breeding of the first group of ten females fed each diet has been completed. On May 8, 1956, the breeding of the second group of ten females fed each diet began and, owing to low male fertility, is still in progress. The yield of second-generation females from the breeding of the first group of animals was small. Male fertility has been very low, regardless of whether the males were raised on the control or irradiated beef diet or on the laboratory pellet ration. Because of the small number of males of proven fertility and because this fertility bears no relation to irradiation of the diet, these males have been combined into a single group and maintained on pellets when not used for breeding. With the limited number of fertile males available, breeding of the first group of parent females fed each diet will be repeated to increase, if possible, the number of second-generation daughters. Some of the fertile males will be used to start the breeding of the daughters from the first breeding as they reach about 14 weeks of age. It is the ability of the second-generation daughter mice to produce and raise young which is the criterion of this experiment.

B. RESULTS

1. Growth of the Parents.—Figure 3 shows the growth of the parent mice from the date they were first weighed. The females on the nonirradiated diet show steady weight gains throughout the period shown. Those on the irradiated diet show greater weight gains up to 15 weeks than the controls, then declined for seven weeks. Both groups of males showed more or less steady weight increases, except for those on the nonirradiated beef diet at the beginning of the experiment.

TABLE XII

PATHOLOGY REPORTS OF TWO OF THE FIVE PULLETS AND TWO THREE-WEEK-OLD CHICKS EXPOSED TO CHLORINE GAS MAY 12, 1956

Pullet "E" 14, our 9273-LBH.
Aorta: Negative.
Heart: Vacuolar change in the myocardium.
Liver: Congestion. Slight degenerative fatty infiltration.
Lung: Acute passive congestion. Hemorrhage. Atelectasis. Compensatory emphysema. Acute purulent bronchitis and lobular pneumonia. Dilatation of bronchi. No fat emboli.
Spleen: Congestion.

Comment: With early treatment this bird might possibly have survived.

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Four-month-old pullet "E" 258, our 9274-LBH.
Heart: Abundant subepicardial fat. No lipoidosis.
Lungs: Marked acute passive congestion. Hemorrhage into bronchi and alveolar spaces. Atelectasis and patchy emphysema. Acute purulent lobular pneumonia. Severe process.
Liver: Congestion. No lipoidosis. Slight increase in leukocytes in hepatic trinities.
Kidneys: Congestion. No lipoidosis.
Spleen: Congestion.

Comment: While one cannot say with certainty that early treatment might save the lives of these birds, it is certainly worth trying.

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Chicks, about three weeks old, our 9271-LBH.
Lung: Severe acute passive congestion. Hemorrhage. Atelectasis. Acute purulent bronchitis and lobular pneumonia. Many of the bronchi are dilated and filled with blood. No fat emboli.

Comment: The pneumonia is severe, but with treatment there might be a possibility of survival.

R. C. Wanstrom, M. D.

Three-week-old chick on "A" mash diet, our 9272-LBH.
Heart: Scattered foci of polymorphonuclear leukocytes.
Lung: Intense acute passive congestion. Hemorrhage. Acute purulent bronchitis and lobular pneumonia.

Comment: With prompt treatment there might be a possibility of survival.

R. C. Wanstrom, M. D.
(signed) Carl V. Weller, M. D.

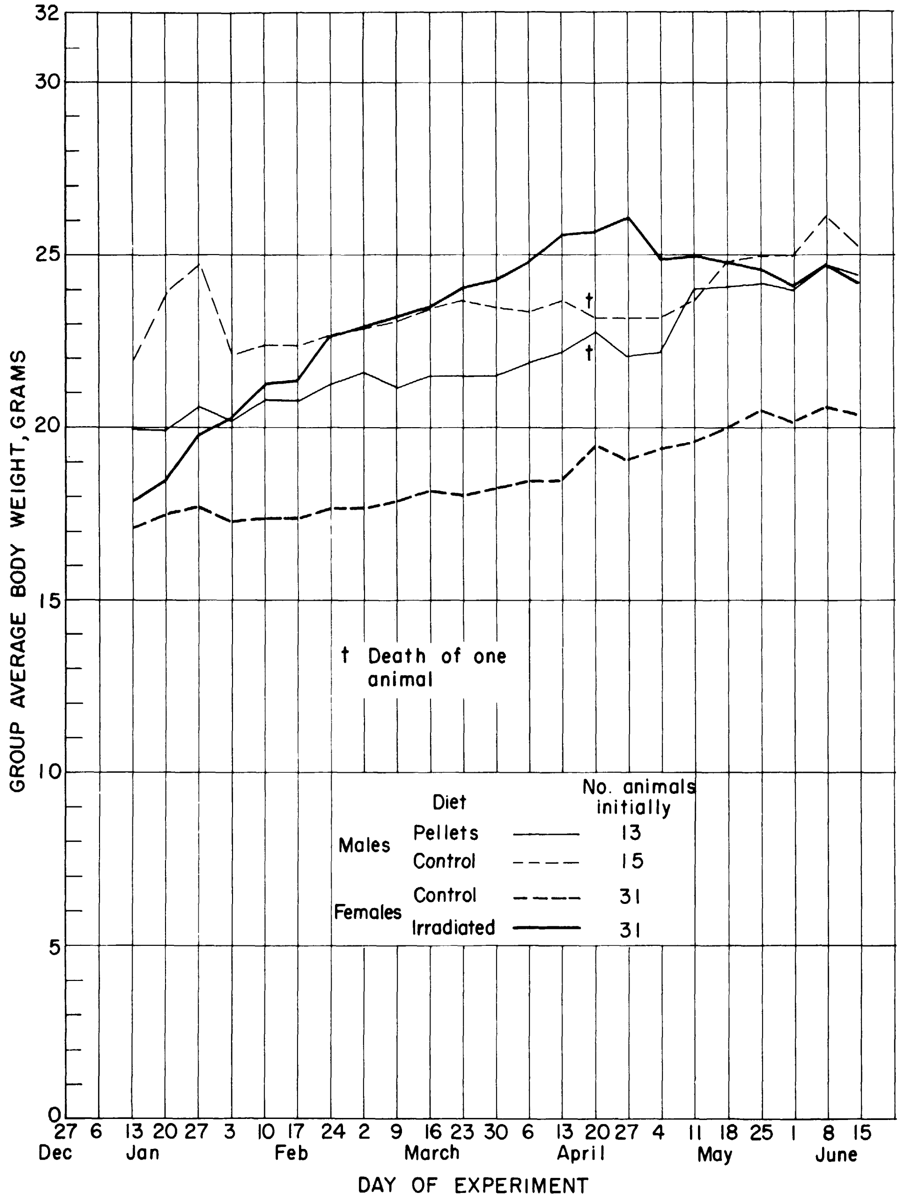

Fig. 3. Growth curves of male and female mice fed the control and experimental diets. The nonirradiated beef diet was fed to a male and a female group. Laboratory pellets were fed to the other male group and irradiated beef diet to the other female group.

2. Reproductive Performance.—Table XIII shows the performance of ten female mice from both the control and experimental group during their first breeding. The ten control females gave birth to about a third as many more pups as did the experimental females, and this difference in population of young between the two groups persisted to weaning age. This was probably due to the selection of females for the first breeding. They were selected on the basis of equal weight, but because the females on the irradiated diet had higher average body weights, it was necessary to pick ten light females from the experimental group to match the ten heaviest in the control group. In continuing the breeding, this consideration of equal body weight is being discarded.

TABLE XIII

REPRODUCTIVE PERFORMANCE OF TEN FEMALE MICE FROM BOTH THE CONTROL AND THE IRRADIATED DIETARY GROUP DURING THEIR FIRST BREEDING

	Control	Exper.
No. females bred	10	10
No. males used	5 (4)	5 (4)
No. females sterile (1)	3	3
No. males sterile (2)	4	3
Average week of conception (3)	2.65	1.95
No. females resorbing fetuses	0	1
Total no. litters born	7	6
Total no. litters born dead	0	0
Total no. litters born alive not surviving weaning	2	1
Average no. pups born per litter	4.7	4.2
Average no. pups born alive per litter	4.6	4.0
Average no. pups born dead per litter	0.1	0.0
% of pups born dead per litter	2.1	0.0
Total no. pups born	33	25
Total no. pups born alive not surviving weaning	7	6
Total no. pups alive at birth	32	25
Total no. pups alive at 5 days	31	25
% pups alive at birth surviving 5 days	96.9	100
Total no. pups alive at 14 days	26	20
% pups alive at 5 days surviving 14 days	83.9	80.0
Total no. pups alive at weaning (21 days)	26	19
% pups alive at 14 days surviving 21 days	100	95.0
% pups born alive which survived 21 days	81.3	76.0
Average weight of young at 21 days, grams	10.1	7.6 (9.1)(5)
Average no. young weaned per female bred	2.6	1.9

(1) Mated 6 times unsuccessfully.
(2) Mated unsuccessfully with at least one female which later became pregnant by another male.
(3) Based on 1 for conception in first week, 2 for conception in second week, etc.
(4) Both groups on control rat diet.
(5) Excludes one litter of six pups which averaged only 4.3 grams.