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Progress Report No. 8

WHOLESOMENESS OF GAMMA-IRRADIATED DIETS

Period June 15, 1956 to September 15, 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 two-year subacute toxicity study of irradiated potatoes has passed the quarter-way mark with no evidence by growth, food consumption, reproductive performance, blood-cell counts, or by gross pathology of the presence of toxic substances or loss of nutrient value of potatoes exposed to gamma radiation up to 44,000 rep and stored for periods up to nine months. All the animals have been showing normal growth with low standard deviations in mean body weights. The three groups of males have shown approximately equal food consumption, and the same applies to the females; data on efficiency of food utilization has been somewhat erratic owing to a temporary retardation in rate of weight gain, especially among the females on the 2X diet. The breeding performance of the animals was good, especially with respect to lactation performance and weight of pups at weaning. Blood-cell counts were normal for all animals. There is no pattern to the incidences of abnormalities detectable by gross examination. Two males have been sacrificed to date, one (2X diet) for severe pneumonia, the other (0X diet) as a result of a middle-ear infection. One other male (1X diet) has a middle-ear infection, and a female on the 2X diet has a severe case of swelling of the joints. All other animals appear in good health.

In the chicken feeding experiment, there has ceased to be any statistically significant difference between the two groups of pullets with respect to body weight. Egg production has begun, and there does appear to be a significantly lower production on the part of the pullets fed the irradiated mash. A breeding program has begun which may be completed in December, wherein the fertility of each one of forty roosters (twenty in each group) will have been tested against eight pullets, using artificial insemination.

The mouse reproduction study has been progressing much more rapidly since a mite and louse infestation was brought under control. Approximately fifty experimental second-generation females and forty control second-generation females have been produced, with more anticipated. The breeding performance of these females should be known in about four months, and the experiment may be terminated at that time.

I. THE POTATO-FEEDING EXPERIMENT

A. PRESENT STATUS OF THE EXPERIMENT

The potato-feeding experiment has been underway for one-fourth of its intended duration as of September 12, and it is running smoothly. The food-consumption calculations for the first twelve weeks have been completed, and those for the latter six weeks are reported herein. The first breeding of the parent generation has been completed with good results and the second has begun. Preparations are being made to study food consumption of the second-generation animals when they are weaned.

Blood-cell counts were made on representative animals from each group and replicate during the third week in July. The counts were performed by Miss Florence Hartsuff, Supervisor of the University Hospital Outpatient Hematology Laboratory. At the tenth week of the experiment, routine individual examinations of each animal were begun on a weekly basis, with additional examinations of the animals with any disease process being made on a semi-weekly basis. The purpose of the examination is to furnish a case history of each animal to help in interpreting abnormalities observed at necropsy. This examination, although confined to abnormalities detectable by external observation and by palpation, is being done not only to detect differences among experimental groups but to increase the amount of information on the life history of the experimental rat. This examination has been made by K. A. Kleyn and R. S. Kruger, sophomore medical students, and is now being done with the additional help of a senior medical student, Paul C. Linnell. These three individuals, as a team, also will perform practice autopsies as preparation for autopsies of animals that may need to be sacrificed prior to termination of the experiment.

B. EXPERIMENTAL

1. Diet Composition.—As of 23 July 1956, wheat-germ oil was incorporated into the diet at a level of 1% in place of the oral supplementation of alpha-tocopherol acetate. The wheat-germ oil is furnished by the Nutritional Biochemicals Corporation, and is assayed to contain no less than 2 units of Vitamin E per gram. Oral supplementation of alpha-tocopherol acetate is continued with breeding animals. No other change has been made in diet composition.

2. Inclusion of Potato Rot in Diet.—In the interest of subjecting to wholesomeness testing every part of the potatoes which were irradiated and stored, all forms of rot as they occurred in the potatoes were to be included in the diet. Starting on 14 July 1956, the portion of rot from each variety of potato going into each batch of diet was recorded, not only to furnish data on the storage stability of irradiated potatoes, but also to keep a check on changes in the diet due to varying amounts of potato rot. Table I shows the average percent rot in the nine lots of potatoes used from 14 July to 22 September 1956 for the thirty-two to thirty-three batches of diet involved. The amount of rot is increased with increasing dosage of irradiation for all three varieties. There is little difference in this respect between the two Russet Burbank varieties, but the Maine Katahdin variety, which appeared to stand up better when not irradiated, was decidedly more susceptible to rot following irradiation. The susceptibility of other white-skin varieties such as Sebago to post-irradiation rot has been observed in other studies.

TABLE I

Percent of Rot by Weight Occurring in Potatoes and Included in Diet for the Period 14 July to 22 September 1956

Variety	Irradiation Dose		
	0X	1X	2X
Maine Katahdin	0.7	5.4	8.3
Maine Russet Burbank	1.4	2.6	3.8
Idaho Russet Burbank	1.5	1.9	4.4

The high amount of rot in the diet on one occasion did cause a marked weight loss in nearly all animals fed the 2X diet during one week, and the increasing amount of rot has on other occasions altered the diet composition.

Because this alone could obscure a subtle toxic effect or nutritional deficiency, the practice of including all the rot has been discontinued. Since 22 September 1956, 80-90% of the rot as found is being removed and replaced by an equal weight of unaffected potato. Records are still maintained on the amount of this rot as it occurs. The consistency of each diet is now more uniform and desirable from the standpoint of feeding.

3. Food-Consumption Data.—Following the first 12-week period for each replicate, when accurate food-consumption records were kept, records have been continued on the approximate amount of food fed to each rat daily. These records exist as a consequence of attempting to assure ad libitum feeding conditions with a minimum wastage of diet. The amount of food offered each day is based on the amount given the preceding day adjusted by the amount of it consumed. For convenience, the diet is parceled out on the basis of volume rather than weight. The diets are stored in a series of flat pans of uniform depth, having notches in the upper edges to guide the cutting of the diet into uniform cubes. Each cube is about 28 grams of diet. When accurate food-consumption records are desired, however, the actual weight to the nearest half gram of each portion of diet is determined.

4. Management of Breeding.—At 105 days of age, four of the five females in each group in each replicate were transferred to double cages and each was mated to its brother. The males were rotated weekly until pregnancy occurred, or until four weeks had elapsed, when each female had an opportunity to mate with each of the four males. When a female became pregnant, she was isolated in a single cage provided with shavings. At birth, the number and sex of all pups, dead or alive, were recorded. At five days of age, the mother and litter were transferred to a double cage provided with shredded paper. At fourteen days of age, the bedding was removed and food offered in a petri dish to encourage the young to start eating the diet. The numbers of pups in each litter were accounted for daily, and the mothers were weighed weekly as usual. At twenty-one days after birth, the young were weighed and all but one or two removed from the mother and discarded. The purpose of leaving one or two weanlings with the mother for a few extra days is to minimize inflammation of the breasts due to unrelieved milk supply following sudden withdrawal of the pups.

C. RESULTS AND DISCUSSION

1. Mean Body Weight.—Table II presents the mean body weights and standard deviations of the six groups of rats for all replicates at four-week intervals. For the difference in mean body weights between any two comparable groups to have a 2:1 probability of significance, they must differ by more than the sum of their standard deviations. None of the male groups differ to this degree. Among the female groups, those on the 1X diet appear to have a slight but consistently significant increase in body weight over the controls. This increase had appeared by the fourth week but did not become relatively

greater as the experiment progressed thereafter. The females on the 2X diet appear intermediate. With the second- and third-generation females, there will be two more opportunities to observe whether or not the 1X diet gives slightly better growth.

TABLE II

Mean Body Weights and Standard Deviations of the Six Groups of 25 Rats Each at Four-Week Intervals

Week	Mean Body Weight, Grams, and Standard Deviation					
	Male Groups			Female Groups		
	0X	1X	2X	0X	1X	2X
0	49.0 ± 0.4	48.4 ± 0.5	48.3 ± 0.5	47.7 ± 0.4	47.7 ± 0.5	47.5 ± 0.5
4	236 ± 4	241 ± 2	242 ± 3	168 ± 2	175 ± 2	172 ± 2
8	376 ± 6	377 ± 5	384 ± 6	227 ± 4	237 ± 3	231 ± 4
12	441 ± 7	447 ± 7	442 ± 7	257 ± 5	267 ± 4	258 ± 4
16	473 ± 7	480 ± 7	476 ± 9	275 ± 5	284 ± 4	274 ± 3
20	526 ± 8	513 ± 8	516 ± 9	307 ± 6	321 ± 5	311 ± 7
24	554 ± 8	546 ± 7	549 ± 10	314 ± 6	333 ± 5	312 ± 5

Figure 1 is a continuation of Fig. 1 in Progress Report No. 7, showing the average body weights graphically for the six groups of rats. The peaks in the curves for the females denote breeding periods.

2. Net Food Consumption and Efficiency of Food Utilization.— Progress Report No. 7 presented the results of food-efficiency determinations for the first six weeks of the 12-week period, and Table III below presents the values for the latter six weeks. It is seen that as the weeks progress, the differences between comparable groups become less and less consistent. There occurred during the 13th week of the experiment a loss of weight among nearly all animals, male and female, on the 2X diet, and a general retardation in growth rate among animals on the other two diets. This was followed during the succeeding week by a regaining of sufficient weight to restore the normal rate of gain for the entire period. This growth retardation was believed to be caused by the use of diets which had been frozen and thawed prior to use and which spoiled more rapidly as a result; this was more acute in the

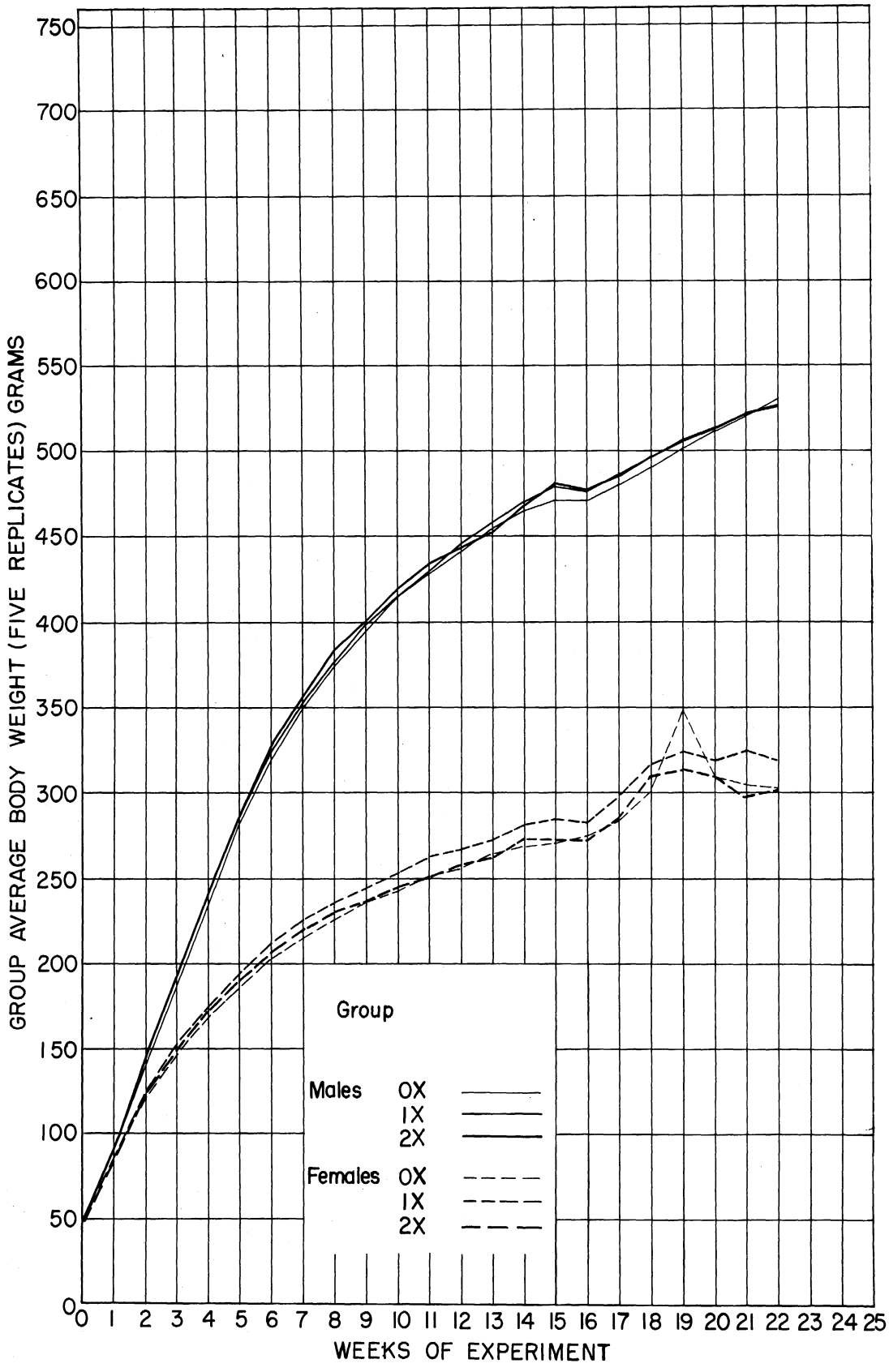

Fig. 1. Weekly average body weights of male and female rats on 0X, 1X, and 2X potato diets.

TABLE III

Efficiency of Food Utilization

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 week from the seventh through the twelfth week of the experiment. Each figure is an average for five animals.

Week	Replicate	Males			Females		
		0X	1X	2X	0X	1X	2X
7	1	.191	.181	.186	.089	.124	.078
	2	.193	.205	.235	.139	.128	.140
	3	.227	.219	.221	.143	.176	.127
	4	.217	.257	.228	.153	.134	.157
	5	.230	.123	.226	.083	.084	.139
	Avg	.212	.197	.219	.121	.129	.128
8	1	.177	.173	.178	.108	.101	.147
	2	.195	.209	.203	.089	.135	.153
	3	.186	.212	.158	.135	.124	.148
	4	.214	.131	.220	.053	.034	.009
	5	.185	.141	.210	.159	.165	.102
	Avg	.191	.173	.194	.109	.112	.112
9	1	.169	.170	.189	.156	.137	.131
	2	.186	.157	.199	.141	.115	.116
	3	.100	.162	.157	.105	.054	.054
	4	.185	.180	.133	.078	.127	.169
	5	.102	.135	-.045	.062	.003	-.134
	Avg	.148	.161	.127	.108	.087	.067
10	1	.156	.123	.133	.057	.037	.029
	2	.131	.054	.042	.026	.030	.042
	3	.155	.147	.161	.093	.113	.104
	4	.108	.067	-.109	.049	-.007	-.166
	5	.185	.190	.264	.102	.192	.322
	Avg	.147	.116	.098	.065	.076	.066
11	1	.119	.109	.147	.103	.097	.095
	2	.142	.169	.147	.121	.124	.110
	3	-.034	.038	-.109	.074	.074	-.299
	4	.147	.152	.228	.109	.187	.282
	5	.102	.059	.116	.048	.035	.012
	Avg	.095	.105	.106	.091	.103	.040
12	1	.110	.178	.110	.036	.116	.089
	2	-.012	.039	-.215	.014	-.054	-.282
	3	.125	.120	.234	.096	.091	.335
	4	.098	.105	.079	.062	.039	.059
	5	.129	.152	.091	.030	.006	.007
	Avg	.090	.119	.060	.048	.040	.042

case of the 2X diet because of the larger percentage of rot in the potatoes used. Since this occurred to all replicates at the same time, it occurred for individual replicates at different ages of animals. When the data are tabulated according to age, and the data for each week for a period of five weeks averaged for all animals in each group, irregularities caused by problems occurring on certain dates are difficult to interpret.

Added to the problem of masking as a result of averaging is the fact that the rate of gain of the animals is decreasing steadily and an increasing part of the food consumed is used for maintenance purposes. Thus, values for efficiency of food utilization, when based on body-weight gain, become quite erratic as a result of normal fluctuations in weekly body-weight increases. For this reason, net food-consumption values are also given as in Table IV. These values appear approximately equal for the comparable groups. The 1X females, which have had higher average body weights than the other two groups of females, also show a consistently higher net food consumption.

TABLE IV

Average Daily Net Consumption of Diet Solids Per Rat in Each Group for all Replicates for the First 12 Weeks of the Experiment

Week	Average Daily Net Consumption Diet Solids, grams per rat					
	Males			Females		
	0X	1X	2X	0X	1X	2X
1	9.4	9.5	9.7	9.0	9.0	8.9
2	12.7	12.8	13.0	10.7	11.5	11.2
3	16.1	16.9	17.0	13.6	14.6	13.9
4	18.5	19.7	18.9	16.2	13.4	15.7
5	18.9	20.2	19.7	14.5	14.8	14.5
6	20.5	20.8	20.3	14.4	15.3	14.2
7	20.7	20.8	20.7	14.1	15.2	14.6
8	19.8	19.9	20.4	14.2	14.4	13.6
9	19.0	19.5	18.6	13.5	14.1	13.5
10	19.1	18.7	18.8	13.5	13.8	13.1
11	18.9	18.6	18.6	13.6	14.1	12.8
12	18.6	18.4	17.9	13.2	13.6	13.2

3. Performance of First-Generation Animals During Their First Breeding.—Table V presents data from the first breeding of the parent rats. All other expressions for representing breeding performance, as found in previous reports from this laboratory, may be derived from these data.

TABLE V

Performance of Parent Rats on the Two-Year Potato-Feeding Experiment During the First of Two Breeding Periods

	Group		
	0X	1X	2X
1. Number of females bred	20	20	20
2. Number of males used	20	20	20
3. Percent of males whose paternity was established	60	55	70
4. Percent of females bred which became pregnant	90	90	90
5. Average number of days required for conception	11.6	9.5	9.5
6. Percent of pregnant females which gave birth	83	83	100
7. Average number of pups born per litter	11.2	9.6	10.7
8. Percent of pups born surviving birth and the first day after birth	80	78	77
9. Percent of pups surviving the first day which reached 21 days (weaning)*	93	86	92
10. Average body weight of pups at weaning, grams	51.4	53.7	52.9
11. Ratio of males to females born	1.27:1	0.96:1	1.06:1

*Any litter with over 10 pups was reduced to 10 on the fifth day. For purpose of calculation, these pups were not considered in deriving the percentage of pups born that reached 21 days (weaning). The numbers of pups disposed of were 10 in the 0X group, one in the 1X group, and 9 in the 2X group.

All but three of the criteria listed have some bearing on the adequacy of the diet, and in no case did there appear any wide differences in performance among the groups. In each case, only two of the twenty females bred did not become pregnant within four weeks. The resorption rate among females

in the 0X and 1X group appeared high (one out of six pregnant females), but there were no resorptions in the 2X group. The average number of pups born per litter appears good for all groups. Pups born dead and those born alive but not surviving one day often cannot be distinguished and the causes of death in each case are likely to be similar. For that reason, a single expression is given for those pups surviving the first day after birth. There were no differences here nor in percent survival to twenty-one days (weaning age). Average body weights of pups at weaning were uniformly quite high and indicate superior lactation by the mothers. As mentioned before, this high rate of lactation caused swelling and hardening of the breasts in mothers whose young were suddenly withdrawn at weaning. The present practice is to leave one or two young with the mother for a few days after weaning age.

Three of the criteria are listed mainly as reflections on the performance of the animals in general rather than distinctions among groups of them. One is the percent of males used whose paternity was established. This is not a reflection of male sterility since some males had no opportunity to mate with nonpregnant females of proven fertility. The second criterion is the number of days required for conception. This is governed by such factors as time of ovulation, compatibility of male and female, and male fertility. The third is the ratio of male to female pups born, which is primarily a genetic matter.

4. Blood-Cell Counts.—As directed in the "Procedural Guide for Coordinated Histopathological Studies Associated with Long Term Feeding Projects under the Irradiation Preservation of Foods Program," blood-cell counts were performed as close to the three-month point in the experiment as possible. Actually, the first replicate animals were eighteen weeks and the fifth replicate animals were fifteen weeks of age. Miss Florence Hartsuff was engaged to perform the counts. She was assisted by Mr. Kleyn and Mr. Kruger.

Blood was obtained by tail nick. No anaesthesia was employed. Four samples of blood were taken for the hematocrit determination, which was performed using heparinized capillary tubes and a centrifuge specially designed to accommodate them. Two samples were taken for the hemoglobin determination, which was carried out using a Klett spectrophotometer. Two samples were taken for the white-blood-cell count, and several smears on slides were made for the differential cell count.

One animal from each group in each replicate was used, and these identical animals will be used for each of the three forthcoming determinations of blood-cell counts (6, 12, and 18 months). The six animals in each replicate are littermates. These animals were not used for breeding, because at the time the first series of counts were requested all the other animals were being bred, and it was desirable not to create a trauma among the pregnant animals. Should it be desirable to get blood-cell counts on animals before and after breeding, this can easily be done with second-generation animals.

The results are given in Table VI. Besides the six experimental groups of animals, blood-cell counts were also made on extra male and female littermates being fed the stock laboratory diet, so actually five sets of littermates were subjected to the blood-cell counts, four brothers and four sisters in each set, each animal in a set from each of the eight groups.

Little if any difference among values is evident in the data, and what differences there are are not consistent with both sexes of animals used nor with the degree of irradiation of the potatoes in the diet. There appears to be the widest fluctuations in the white-cell count, especially in the percentage of polymorphonucleocytes, which is lowest in both males and females fed the 1X diet, the percentage for the males and females fed the 0X and 2X diets being about the same. The small differences in the hemoglobin values correlate with the hematocrits, as shown by the almost constant value for corpuscular hemoglobin, which is obtained by dividing the hemoglobin values by the hematocrit.

5. Gross Pathology.—Routine weekly examination of each animal individually began on June 14, when the first replicate animals were fourteen weeks of age and the fifth replicate ten weeks of age. In this examination, all abnormalities of each animal that can be detected by external examination, by palpation, and by listening are recorded. Undoubtedly, much of this information is superficial or otherwise of no consequence from the standpoint of this experiment. However, two reasons justify doing it: (1) it increases confidence in an interpretation of the findings at necropsy, and (2) it accumulates data on the abnormalities occurring throughout the life of individual animals which may, when there is enough of it, reveal certain patterns between abnormalities occurring in youth and those in old age. It would be interesting, for example, to correlate with some other disease process what appears to be a higher incidence of respiratory infection among males than females; another example is a correlation between high rate of lactation and the incidence of mammary tumors, a high incidence of which was noted in the other two-year experiment completed in this laboratory.

The observations made prior to the tenth to the 14th week, depending on the replicate, were listed in the previous progress report. From the tenth week onward, they will be listed as is shown in Table VII for the period covered by each report. Data for the tenth week were available in this manner only for fifth-replicate animals, for the 11th week for fourth and fifth and so on, as noted in the table. In future reports, the number of animals free of all observable symptoms will also be listed, and data for preceding periods summarized as averages over a ten-week interval.

The abnormalities or symptoms so far noted are listed on the left. The table gives the number of incidences of each abnormality regardless of whether two or more occurred on a single animal.

TABLE VI

Blood-Cell Counts of Representative Animals at Four Months of Age

Group	Hematocrit Percent	Hemoglobin Grams	Corpuscular Hemoglobin, Percent	White Blood Cells, Thousands per mm^3	Differential Count: Percent of White Cell Count					
					B(1)	E(2)	M(3)	LL(4)	SL(5)	P(6)
MALES										
0X	48.9	16.0	32.7	15.0	0	2	3	2	74	19
1X	47.9	15.9	33.2	13.3	0	1	3	3	79	14
2X	47.6	15.5	32.6	14.0	0	1	3	2	76	18
Pellet	48.3	16.2	33.5	14.7	0	1	3	3	80	13
FEMALES										
0X	46.1	14.6	31.6	10.4	0	1	3	3	74	19
1X	48.1	15.4	32.0	10.2	0	2	3	3	80	12
2X	47.9	15.3	31.9	9.0	0	2	2	2	77	17
Pellet	46.4	14.7	31.7	14.9	0	3	2	2	77	16

(1) Basophils
(2) Eosinophils
(3) Monocytes
(4) Large lymphocytes
(5) Small lymphocytes
(6) Polymorphonucleocytes

TABLE VII

Incidences of Grossly Observable Abnormalities in
First-Generation Rats, Period 10th-22nd Week of Experiment

	Age in Weeks - Males													Age in Weeks - Females											
	10	11	12	13	14	15	16	17	18	19	20	21	22	10	11	12	13	14	15	16	17	18	19	20	21	22
0X GROUP																										
Number of surviving animals*	5	10	15	20	25	25	25	25	25	25	25	24	24	5	10	15	20	25	25	25	25	25	25	25	25	25
Deaths												1	1													
Tumors and/or unidentified swellings																									1	1
Respiratory infection:																										
Severe					1		1	1	1																	
Moderate		2	1		3	2	3	3	1	3	3	4	2			1		1	1	1			2			
Infections and inflammations in:																										
Eyes		1		1	2		1	1	1				1				1		1	1	1	1	1	1	1	1
Middle ear																										
Nose													1													
Urino-genital system					1	2			2		1	1										1				
Abscesses and sores																										
Swelling of joints																										
Sensitivity of animal to touch		1				1																				
1X GROUP																										
Number of surviving animals*	5	10	15	20	25	25	25	25	25	25	25	25	25	5	10	15	20	25	25	25	25	25	25	25	25	25
Deaths																										
Tumors and/or unidentified swellings																						1	1	1	1	1
Respiratory infection:																										
Severe	1			1	1	2																		1		1
Moderate		1	2	2	2	3	3	3	3	1	2	4	2				1	2	1	1	1		1		2	
Infections and inflammations in:																										
Eyes												1	1													
Middle ear						1	1	1	1	1	1	1	1													
Nose									1	1	1	2												1		
Urino-genital system									1	1	1	2	1													
Abscesses and sores																										
Swelling of joints																										
Sensitivity of animal to touch												1														
2X GROUP																										
Number of surviving animals*	5	9	14	19	24	24	24	24	24	24	24	24	24	5	10	15	20	25	25	25	25	25	25	25	25	25
Deaths		1	1	1	1	1	1	1	1	1	1	1	1													
Tumors and/or unidentified swellings																										
Respiratory infection:																										
Severe		1	1			1		1			1													1	1	1
Moderate	1		1	1	2	1	2	1	3	4	1	3	3						1							
Infections and inflammations in:																										
Eyes							1			2				1	2		2	1	1	1	1	1	1	2	2	2
Middle ear																										
Nose																										
Urino-genital system				1		2		2	1	1																
Abscesses and sores																										
Swelling of joints																				1	1	1	1	1	1	1
Sensitivity of animal to touch	1	1		1	1	2	1			1	1	1	1													

*Data applicable to fifth replicate animals only during tenth week; to fourth and fifth replicate animals during 11th week, each succeeding week including one additional replicate until 14th week, when all animals are involved.

During the 18th week, one female (No. 21) in the 1X group was observed to have a very hard lump on the midline just anterior to the vagina. The animal had been mating for two weeks, and mating was continued even though the swelling increased in size. Four weeks later she gave birth to ten pups, one dead, but due to apparent failure in lactation, all pups were dead the following day. The swelling also started to regress from a maximum of 4 cm x 2 cm, to 1.5 x .5cm a week and a half later.

During the 21st week, another female (No. 37), in the 0X group, was observed to have a swelling approximately 2 cm in size and located just lateral to the scapula on the left side. It was subdermal and nodular in appearance. At this time, the female had a litter of ten fourteen-day-old pups. The swelling was still present one week later when the pups were weaned. Approximately a week and a half after weaning, the swelling began decreasing in size, and by three weeks after weaning, was gone altogether. This might have been a mammary cyst which became filled and swollen with milk while the female was lactating and which gradually disappeared after lactation was stopped. This female will be watched for any evidence of a mammary tumor later in life. To determine if there is any correlation between such swellings and mammary tumors, the second-generation females will not be sacrificed at the time of weaning, nor will any young be left to relieve an accumulation of milk. These animals will then be maintained for several months to observe any correlation between incidence of mammary tumor, if any, and swellings observed during and after lactation.

The sacrifice of a male in the 2X group (No. 67, fourth replicate) was reported earlier. In the 21st week, a male in the 0X group (No. 28, first replicate) was also sacrificed. One day after a routine pathology check, when no symptoms were noted, it developed a convulsive head-jerking motion. The animal rested on its haunches, lifted its front feet, and jerked its head back at approximately one-second intervals. His breathing and heart rate remained normal. The following day the jerking stopped, but one eye was inflamed and the other barely open. His food was untouched and he was listless. The animal was sacrificed and replaced by its brother from the colony of extra littermates. Autopsy showed nothing grossly abnormal except what appeared to be an excessive growth at the point where the trachea divides into the bronchi. The kidney also appeared slightly mottled. It is now suspected that the animal suffered from a middle-ear infection. The pathologist's report on the tissues is given in Table VIII.

Besides the two animals lost so far, two others appear to be useless for the purposes of the experiment. One is another male on the 1X diet (No. 21, fourth replicate) which has had a middle-ear infection not changing in severity for eight weeks. The other is a female on the 2X diet (No. 68, fourth replicate) which developed severe swelling of the first joint above each paw. For a period of seven weeks, the swellings have not abated, but this female has continued to maintain weight after a small initial loss. Injections of cortisone (four one-mg amounts daily) had no effect when given

when the swellings first appeared.

TABLE VIII

Histological Report of Male Rat on OX Diet, No. 28, First Replicate, Sacrificed August 4, 1956

Male rat, OX diet, No. 28, our 1133-LBI. "Autopsy:"
Heart: Negative.
Aorta: Negative.
Lung: Productive emphysema. No lipid in capillaries or alveoli.
Large Bronchi: Negative.
Trachea: Negative.
Esophagus: Negative.
Bronchial and Peritracheal Lymph Nodes: Lymphoid hyperplasia.
Thymus: Lymphoid hyperplasia.
Spleen: Hemosiderosis. Small foci of hematopoiesis.
Stomach: Negative.
Small Intestine: Negative.
Liver: Prominent Kupffer cells. Slight lipidosis.
Adrenal: Small cortical infarct.
Kidney: Glomerular capillaries have thickened walls. The tufts are shrunken and relatively ischemic. Tubules contain much precipitated protein. No significant lipidosis.
Testis: Active spermatogenesis.
Seminal Vesicles: Negative.
Summary: The thymus gland is hyperplastic. I doubt that it obstructed the airway, although the slight emphysema is suggestive of obstruction. Did this animal have otitis media? This is a common affliction of rats and the symptoms are suggestive.

— R. C. Hendrix, M.D.

II. THE CHICKEN FEEDING EXPERIMENT

A. PRESENT STATUS OF THE EXPERIMENT (18 SEPTEMBER 1956)

The second-generation birds have reached thirty-two weeks of age and have been producing eggs for fifteen weeks. Body-weight and egg-production data for these periods are presented.

Twenty pullets from each group have been assigned to a breeding program designed to furnish some measure of fertility of each of the twenty male chickens fed the irradiated diet and of an equal number fed the non-

irradiated diet. Additional data on hatchability of fertile eggs is furnished by the pullets not assigned to the breeding program. The next progress report should contain the results on fertility of males and on hatchability of fertile eggs.

B. BREEDING PROGRAM

The purpose of the breeding program is to conduct a number of inseminations between individual males and females sufficient to rule out that part of egg infertility due to faults in the technique. A schedule has been established in which each pullet, from a group of twenty control and twenty experimental pullets, is given, at successive biweekly intervals, the semen of four control and four experimental males. Inseminations are performed on alternate Mondays and are repeated the following day to minimize failures in technique. All eggs are incubated and are first candled after seven days of incubation. Fertility is checked by breaking open each egg which does not contain a living embryo. Male fertility is expressed by the percent fertility of the eggs laid during the period beginning with the second day (Wednesday) and ending with the 15th day (Tuesday) following the first insemination (Monday). A maximum of 320 matings are scheduled, eighty for each of the four combinations of the two male and two female groups. Should there be failure to obtain a sufficient number of semen samples or loss of egg production by many pullets, the program will be extended. At the present time, the program is one-fourth completed.

The eggs are counted starting two days after insemination because it was found with these pullets that, after a single insemination, not a single egg was fertile the following day, but a high percentage were fertile the second day. A single insemination may be capable of giving rise to fertile eggs beyond the 15th day. When semen from another rooster is introduced on the 14th day, and is capable of resulting in fertile eggs produced on the 16th day, confusion in establishing paternity would result were it not for the fact that semen recently introduced will almost completely replace any surviving semen from a prior insemination. Hence, one does not need to wait until the effect of a single insemination is exhausted before testing the semen of a second male. The question remains whether semen containing infertile sperm cells will inactivate old sperm cells, i.e., whether a low percentage of fertile eggs indicates low potency of the last male used or completely infertile semen which did not inactivate any surviving sperm cells from the earlier insemination. If such incidences arise, the males in question can be tested, using pullets which have not been inseminated for four weeks.

C. EXPERIMENTAL DATA AND DISCUSSION

1. Body Weight.—Figure 2 is a continuation of Fig. 2 in the

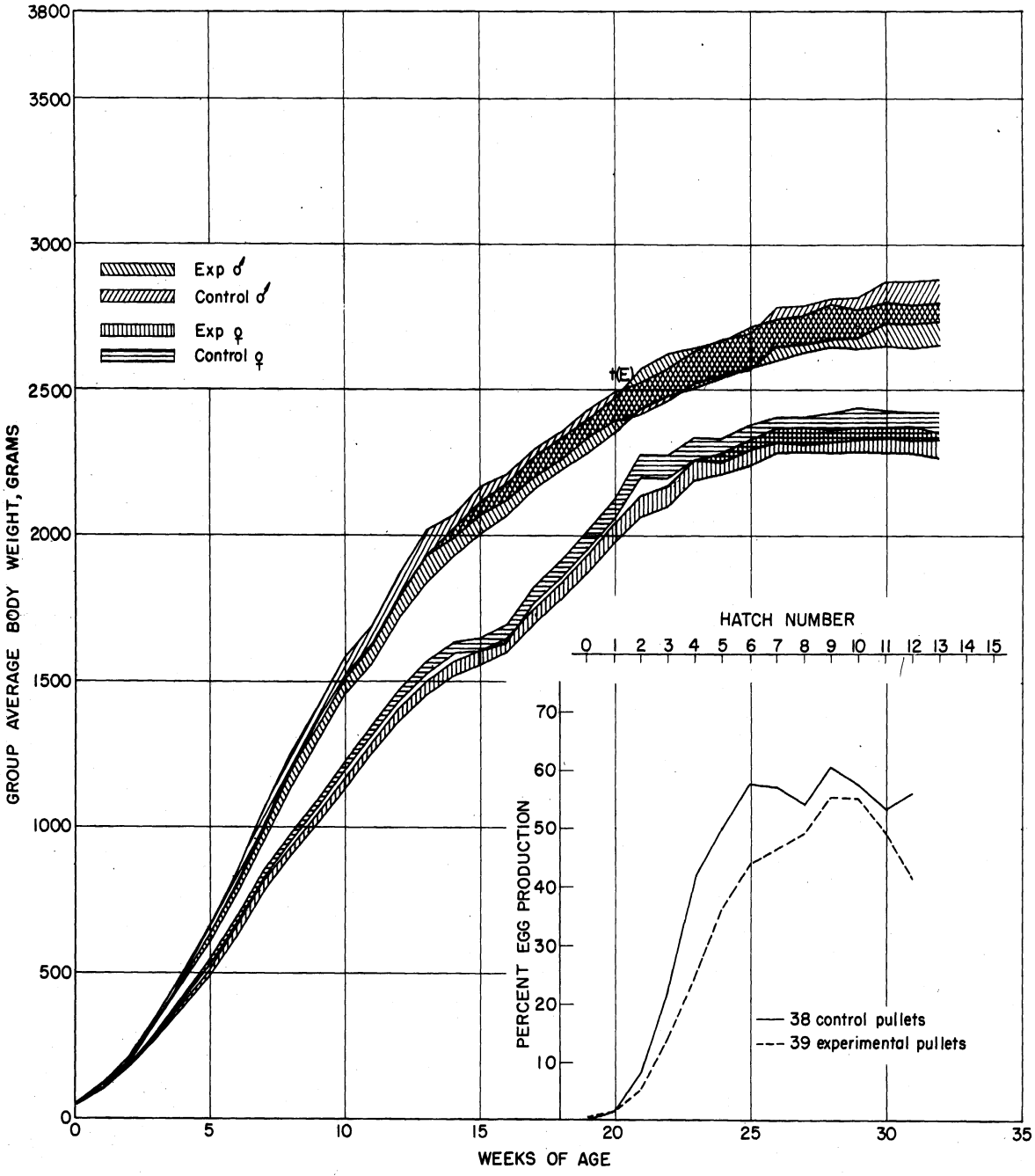

Fig. 2. Weekly mean body weight of male and female chickens on control and irradiated wet mash, including magnitude of standard deviation. Lower curves are the weekly percent egg production by control and experimental pullets.

previous progress report where the weekly mean body weights and standard deviations for each of the four groups are plotted as bands. The upper and lower edges of each band are fixed by the mean body weight plus and minus respectively the standard deviation. If any part of the two bands overlap, there is considered to be no statistical significance to a difference in mean weights. This is certainly the case with the two groups of male birds. If the bands just touch, there is considered to be a two-to-one probability that the difference in mean weights is significant or attributable to experimental treatment. Figure 2 shows that this was true of the two female groups, but the probability that the difference in mean weights was related to irradiation of the diet disappeared with time.

2. Egg Production.—Figure 2 also shows percent egg production for the two groups of pullets plotted on the same time scale. The control pullets appear to be doing somewhat better. If the mean egg production by control and experimental birds is compared during the first five weeks, when the pullets were coming into full production, and again during the seven-week period following, the results shown in Table IX are obtained. The standard deviations also shown indicate that there is a greater than two-to-one probability that the differences are atrributable to the experimental treatment. Since the difference is less during the second period, it is possible that the experimental birds, as a result of a slightly diminished rate of body-weight gain, relative to the control birds, noted frequently for animals fed irradiated diets, required a slightly longer time to come into full egg production.

TABLE IX

Comparative Egg Production by Control and Experimental Pullets, First-Fifth Week and Sixth-Twelfth Week

Week Since Egg Production Began	Mean Number of Eggs Produced Per Pullet and Standard Deviation	
	Control	Experimental
First through fifth	9.5 ± 1.0	6.6 ± 1.0
Sixth through twelfth	28.6 ± 1.6	24.7 ± 2.0

III. THE MOUSE REPRODUCTION STUDY

As described in earlier reports, a group of about thirty female mice

fed the control and thirty fed the irradiated-canned-beef diet have been on a more or less continuous breeding program. This experiment has been underway with a colony of about sixty female Bagg-strain albino mice, half fed the irradiated-canned-beef diet and half fed the nonirradiated control diet. The purpose has been to produce and raise a large colony of second-generation females in order that their breeding performance, in turn, may be evaluated properly. However, the breeding results with the parent females have been poor. It has been necessary to breed some parent females twice to make up for others in the group which, because of low body weights and poor appearance, are not believed capable of breeding. The task has been further complicated by a low incidence of virility or fertility among the males. As a result of this, all males were placed on the laboratory pellet ration, except when breeding.

In August it was discovered that lice and mites were infesting the mice and that this might be a possible cause of the poor breeding performance. Mr. Mitch Yudelevich, an entomologist investigating the feasibility of exposing grapefruit to low doses of gamma rays as a means of controlling fruit-fly infestation, recommended treating the mice with rotenone, which is a relatively innocuous insecticide and is especially suitable for treating animals directly. Since then, all mice over fourteen days of age (this includes young with mothers prior to weaning) have been sprayed once a week with an aqueous solution of .002% rotenone, containing also .087% pine oil, .012% camphor oil, .015% para-dichlorobenzene, and a few inert ingredients. The treatment has resulted in considerably improved appearance of the mice, a much higher rate of conception, and at the present time it is anticipated that the breeding program will yield sufficient second-generation daughters.

Breeding of part of the second-generation females began on 15 August, but data are not yet available. Since females of this generation are being born at present and will continue to be born for a month or so, it is anticipated that complete breeding data for these animals will be available in February, 1957. All second-generation males are being raised on the laboratory pellet ration. Males from both the first and second generations will be used for mating with the second-generation females.

Table X shows the progress to 27 September in regard to the production of second-generation animals. The eighteen litters born in each group include the seven and six litters born from control and experimental parent females, respectively, which were reported in Table XIII of the previous progress report. Table X also includes second as well as first litters of those females being bred for a second time. The next progress report should contain complete data on the breeding of the parent females.

TABLE X

Production of Second-Generation Animals by
Parent Control and Experimental Female Mice

	Control	Experimental
Total no. of litters born	18	18
Avg no. of pups born alive/litter	4.2	3.6
Avg no. of pups weaned/litter	2.8	3.2
Total no. pups weaned	51	58
Total no. of female pups weaned	27	32
Avg wt of pups at weaning (21 days)	9.4 gm	7.9 gm