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Progress Report No. 2

WHOLESOMENESS OF A GAMMA-IRRADIATED DIET FED TO CHICKENS

Period January 15, 1955, to March 30, 1955

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ABSTRACT

This report describes the progress of the animal feeding studies at the Fission Products Laboratory from the first week in January to the last week in March, 1955. The long-term chicken feeding experiment supported by the Office of the Surgeon General of the U.S. Army is now well established and in its fifteenth week. Data on the growth rate, feed consumption, and blood cell counts of the chickens are presented. The long-term rat feeding and breeding experiment, supported by Michigan Memorial-Phoenix Project No. 41, is now in its fifteenth month. Third-generation rats consisting of the second litters from the animals of the first filial generation have been weaned, and a comparative summary of the breeding performance of the parents and first filial generations is presented. Data on the blood of the first filial generation were obtained at the time of sacrifice. Finally, a mouse feeding and breeding experiment has been commenced to check the reproductive performance of Bagg-strain albino mice fed a diet of irradiated food.

SECTION I. CHICKEN FEEDING EXPERIMENT

A. INTRODUCTION

Of the original 190 chicks obtained to start this experiment, none were lost after the first week. However, in accordance with the original plan of the experiment as described in Progress Report No. 1, the number was reduced to 120 chicks which were selected as being representative of the group. These birds are now caged individually in the new chicken room, which was completed about February 18. Body weights of the birds have been determined weekly and weight records have been kept for each bird. Laboratory assistants have been engaged to assist in the feeding of the birds, keeping feed and weight records, maintaining sanitary conditions in the chick room, weighing, etc. Facilities for the irradiation of mash at a rate of approximately 35 lb per day have been completed. The services of an experienced hematologist were enlisted for the purpose of determining cell counts on the blood of the chickens.

B. MANAGEMENT OF EXPERIMENT

1. Chicken Quarters.—For the first 2 weeks the chicks were maintained in the electrically heated brooder (see Progress Report No. 1, p. 46) which was divided into two compartments. The temperature was maintained at 95°F during the first week and lowered approximately 5°F each week for the next two weeks. Then the brooder was removed and the chicks were maintained in two pens with raised wire floors at a temperature which varied between 80 and 85°F until 8-1/2 weeks of age (see Fig. 1). At this time the new chicken room had been completed, and the chicks were shifted to individual cages. For the first few days there were two in each cage; thereafter they were caged singly. The birds on the control diet and those on the irradiated diet were kept in alternate batteries. It was not possible to distribute control and experimental birds in alternate cages in one battery because all birds in each battery have access to a common water trough. A view of the chicken room is shown in Fig. 2. The temperature in the new chicken room was maintained around 75°F initially, in order to enable gradual lowering of the temperature. It is now maintained between 68 and 70°F, and in a month it will be lowered to 64°F which is considered the optimum temperature for chickens. Thermostatically controlled fans force air in from the adjoining room to control the temperature within 2 degrees of the desired temperature. The relative humidity measured with a wet-bulb thermometer is about 50 per-

Fig. 1. View of the control and experimental chickens in pens at 8 weeks of age.

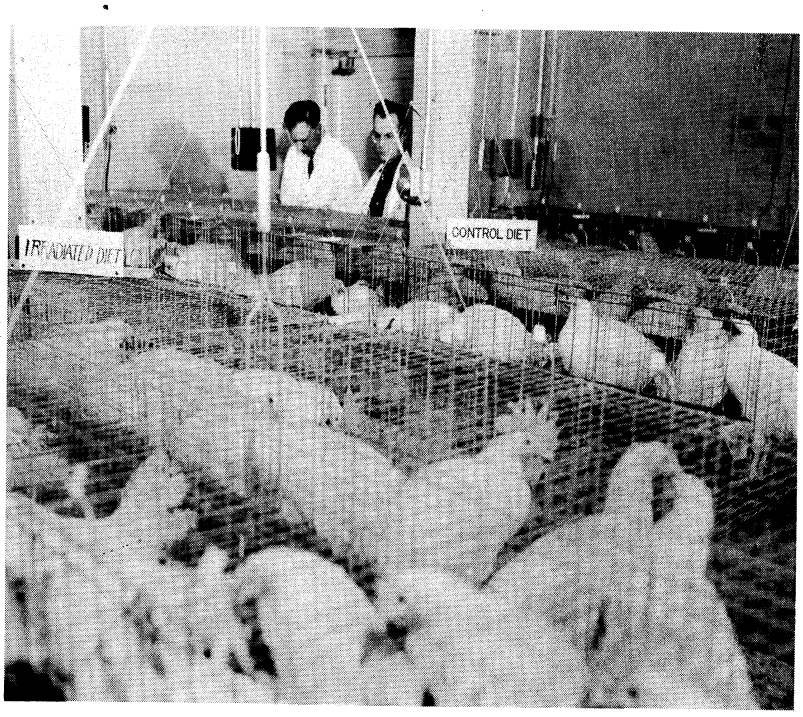

Fig. 2. View of chicken room, showing control and experimental chickens in separate batteries. The males are alternated with two females, since there are twice as many of the latter.

cent. In spite of the presence of 120 birds and their excreta, the ventilation is adequate to keep the environment free of odor and oppressiveness. Even though the birds are not free to run, since space in their cages is limited, they produce the characteristic sounds of contented fowl.

A time switch which operates the lights is set to turn off at 8 p.m. and on at 8:30 a.m. The windows have been covered in order to maintain controlled lighting conditions. This time cycle has been selected to minimize disturbance of the persons in the dormitory adjoining the laboratory.

2. Culling the Chickens.—The experimental plan calls for 60 birds in each of two groups, or 120 altogether. It was decided to divide each group into 40 females and 20 males, because in addition to the growth, fertility, and longevity data available from both groups, the females would provide data on egg production and hatchability.

In order to make it possible to select a uniform population, 190 chicks were obtained initially. The early weight data were collected twice weekly, and percentage gains were computed for each chick for several semiweekly periods. For each of the four groups, the percentage gain was plotted vs body weight for each chick for two separate semiweekly periods, and those chicks in each group whose position on the graph was nearest to the average for both periods were chosen for the experiment. When the averages of the weights of the original and reduced flock were compared, very little difference was found, indicating that this method of culling had introduced greater uniformity without changing the average weight of each group.

At the time of culling, the possibility of using the culled chicks for pathological examination was considered, but it was felt that it was too early in the experiment to expect significant information. Histological examination of the organs of the birds will be made when external symptons, e.g., occurrence of tumors and disease, make this necessary.

3. Irradiation and Handling of the Diet.—The chicks were switched from a commercial starting mash to a commercial growing mash when they were 4 weeks old. The growing mash is Purina "Chick Growena" obtained from the Ralston Purina Company. It is the standard "Growena" except that it does not contain the antibiotic feed supplement. The mash has the "guaranteed" analysis as given in Table I.

TABLE I. ANALYSIS OF GROWING MASH

Crude protein (min.)	17.0%
Crude fat (min.)	3.0%
Crude fibre (max.)	7.0%
N.F.E. (min.)	48.0%

This mash, according to the manufacturer, consists of a selected mixture of meat scraps, soybean-oil meal, cottonseed meal, ground yellow corn, ground oats, fish meal, dehydrated alfalfa meal, ground barley, ground-grain sorghums, wheat standard middlings, animal fat preserved with butylated hydroxyanisole, vitamin B_{12}, vitamin A feeding oil, riboflavin supplement, D-activated animal sterol, 1% low-fluorine rock phosphate, 1.5% calcium carbonate, 0.5% iodized salt, and 0.02% manganese sulfate.

Because irradiation destroys a large portion of vitamins in the commercial mash, a nonirradiated vitamin supplement is added to both the irradiated and control mash shortly before feeding. (See Table 17 of Progress Report No. 1 for analysis of vitamin supplement.) The mash to be irradiated is mixed with an equal weight of water prior to irradiation; the same proportion of water is used with the control mash.

In general, the procedures described in the first progress report of this series are still being used.

The mash for the experimental group receives a 3-megarep sterilizing dose of gamma radiation in one of three containers: an elongated No. 10 tin can placed in the center (high-flux position) of the multikilocurie gamma source for 15 hours, a galvanized sheet-steel "cheese-box" placed above the source for 96 hours (and turned over after 48 hours), and a bank of No. 10 tin cans placed around the source for 192 hours (and rotated after 96 hours). The temperature in the radiation cave varies between 4 and 8°C in the winter and rises to 20°C in the summer. The mash which is fed to the control group is not prepared at the same time as the mash to be irradiated because bacterial activity in the control mash would add a variable. For this reason, the control mash is prepared just prior to use and is stored in a refrigerator. A bank of irradiated mash is kept in a deep-freeze held at -10°F.

The amount of mash given to each group of birds is controlled to keep mash always before the birds, with only a small amount of carry-over to the next day. The fresh mash is mixed thoroughly with the old mash in the feed troughs. Very little feed is wasted by the birds. This enables feed-consumption records to be kept simply on the basis of the amount of mash supplied to each group every morning.

C. RESULTS

1. Growth Curves.—The mean weights of each of the four groups were calculated after the chickens were weighed each Monday morning. Significant differences between the means of the control and experimental groups were determined statistically by the "Overlapping Test." The weekly mean body weights for the four groups are presented graphically in Fig. 3.

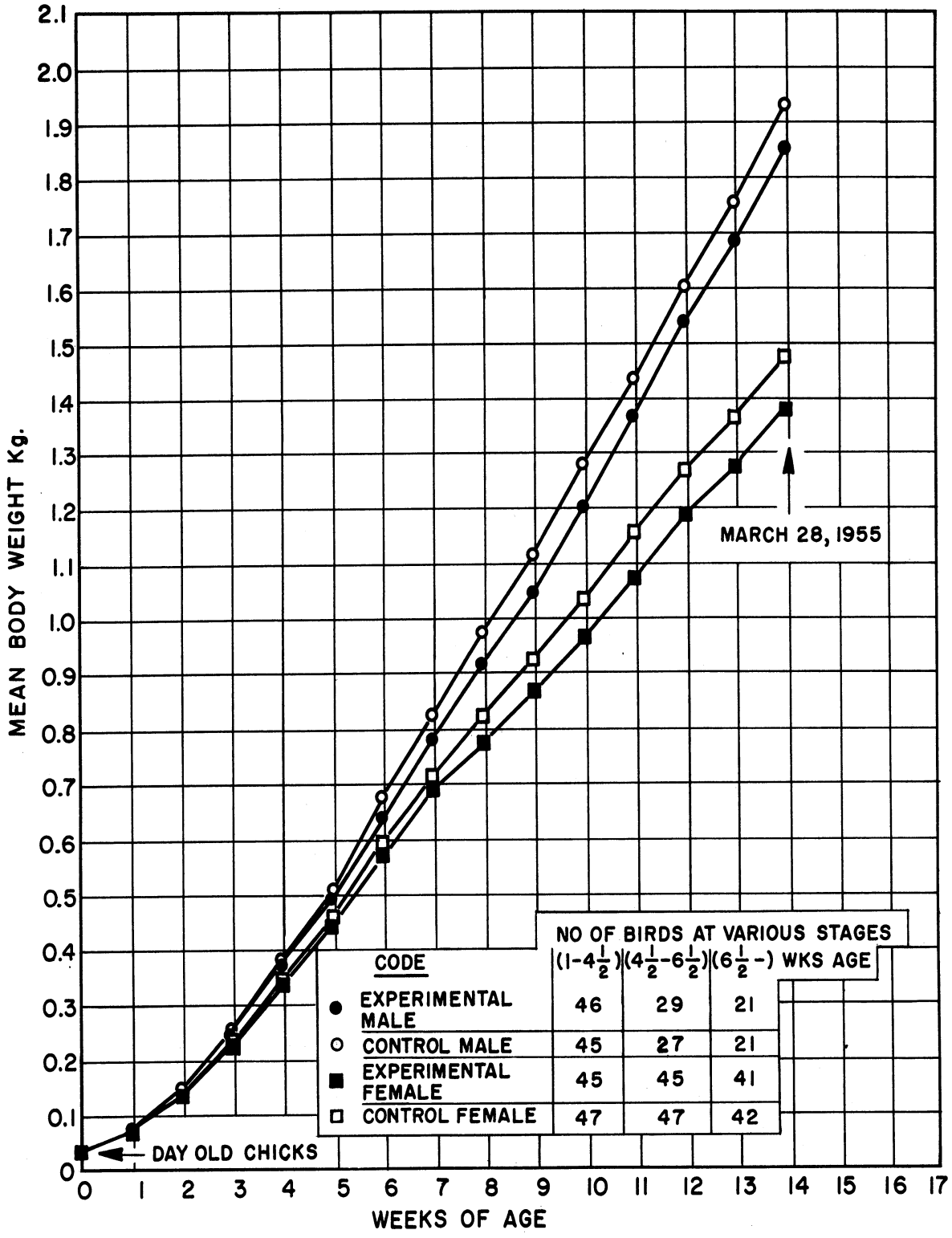

Fig. 3. Growth curves of the four groups of chickens from one day to 14 weeks of age. The numbers of chickens were reduced from the original 190 to the present 125 in 3 stages.

The small difference in growth rate between birds on irradiated and control diets became statistically significant for the males by 2 weeks and for the females shortly thereafter. For the latter half of the period (see Fig. 3), there seems to be a constant difference in weight between the control and experimental males, and hence a diminishing percentage difference, but with the females the percentage difference is remaining quite constant. The chickens were culled in two stages, and the figures in parentheses are the respective numbers of chickens included in each mean weight.

Comparison of the result of the rat experiment with data reported by other workers using different diets suggests that some fundamental component of food and feed is the target of gamma-radiation effects. Inasmuch as the intestinal microbiological flora in animals plays an important role in the nutrition of the host, and inasmuch as gamma irradiated food would contain only those organisms acquired after irradiation, the small lag in growth rate may be due to the absence of organisms that are indigenous to foods in the native state. A more likely possibility is that essential amino acids are destroyed, as their distribution would be somewhat similar in different foods and feeds, and all animals are more or less equally dependent upon them. It is much more difficult to argue for the presence of toxic substances or of lack of palatability in the light of the evidence.

2. Mash Consumption.—Daily and cumulative records were kept of mash supplied to each group. Since spillage or wastage is at a minimum and since the mash from the previous day is mixed with the new mash, feed consumption and efficiency of utilization can be closely estimated. Table II presents the data necessary to calculate the feed efficiency for both groups.

TABLE II. FEED EFFICIENCY IN CHICKEN EXPERIMENT

Group	Gain in Total Weight of Each Group (7th to 13th week), kg	Mash Consumed (7th to 13th week), kg	Ratio (Wt Gain/Weight Wet Mash Consumed)	Relative Efficiency, %
Controls 21 males 41 females	46.14	392	0.118	100
Experimental 21 males 42 females	43.72	391	0.112	94.7

It is possible that the feed-efficiency figures are affected by a difference in water content of the irradiated mash owing to some evaporation

and leakage of juice during irradiation. However, the occasional checks that were made have shown no difference in percentage of dry weight in each group of mash. Checks of moisture content of the mashes are now being made daily. Since the feeding method is ad libitum, any slight difference in moisture content cannot account for the significant difference in body weight between control and experimental birds.

3. Gross Appearance of Birds.—There is nothing in the appearance or general behavior that would distinguish the two groups. The appearance of both groups is considered very satisfactory by those who have experience with birds. The only abnormality seems to be the appearance of crooked toes in some of the birds. This condition is believed to be caused by walking on wire, or perhaps is due to the avian "leukosis" complex, but it is no more prevalent among the experimental birds than the controls.

A typical cockerel and pullet of the control and experimental groups are shown in Figs. 4, 5, 6, and 7, respectively. The poor appearance of the feathers results from incomplete feathering of the birds at this age and from broken tail feathers caused by caging.

4. Blood Analyses.—Determination of the numbers of the various cellular elements in the blood of experimental animals affords valuable evidence for the interpretation of the effects of experimental diets. Periodic blood analyses are believed to be warranted. It is hoped that these can be made be made every 3 months on at least one-fourth of the chickens in each group. The same chickens will be used for the blood analyses each time to confine any possible jeopardy to the experiment by the taking of blood samples.

Table III presents the first series of blood analyses for 4 males and 8 females from the two groups. Blood was obtained by puncture of a wing vein (vena metatarsas). The red-cell volume was estimated by triplicate hematocrits. Hemoglobin was determined photometrically in duplicate at 545 millimicrons in a 1:250 dilution of blood in 0.0625 normal aqueous ammonium hydroxide. White cells were counted in duplicate by standard counting methods after the erythrocytes are lysed with 0.1 N HCl. Differential counts (on the basis of which eosinophils, reticulocytes, polymorphonucleocytes, basophils, monocytes, and lymphocytes are estimated) are made using Wright's stain. An experienced hematologist was engaged to make all but the hematocrit and hemoglobin determinations.

In the comparisons between control and experimental animals no differences were found beyond normal fluctuations in the blood-cell values. The white-cell count in individual animals may exhibit a fivefold fluctuation in one day without being considered abnormal. Hence the difference in the white-cell count of control and experimental females is probably not significant statistically, considering the number of animals used. The difference in whole-blood hemoglobin between males and females appears to be a

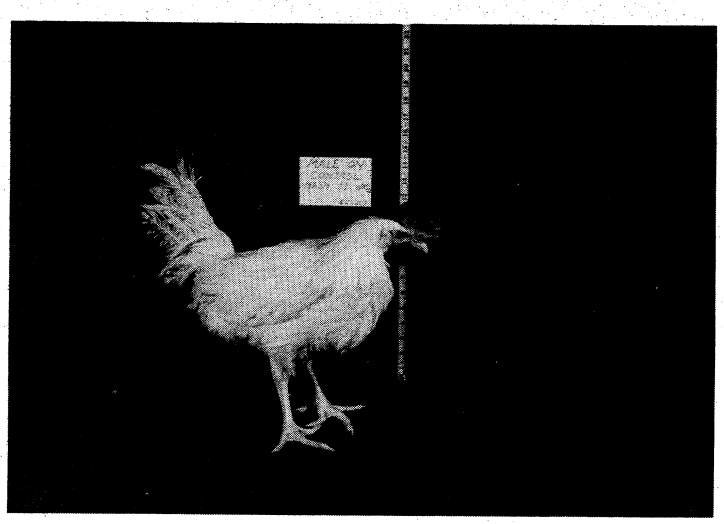

Fig. 4. Typical cockerel in the control group, age 11 weeks.

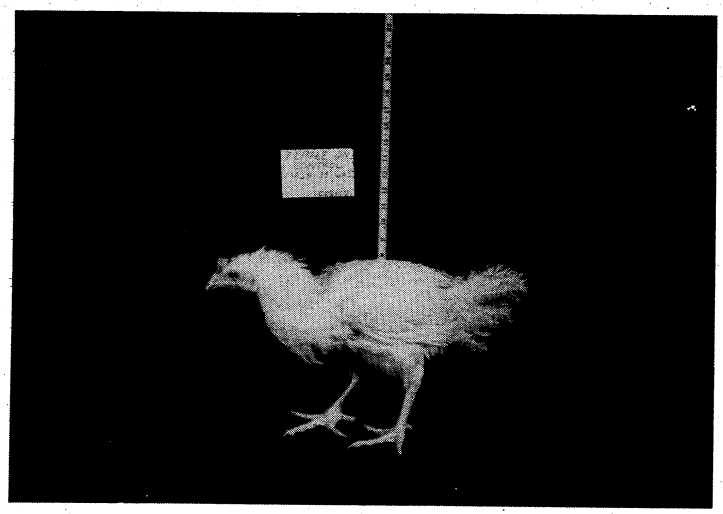

Fig. 5. Typical pullet in the control group, age 11 weeks.

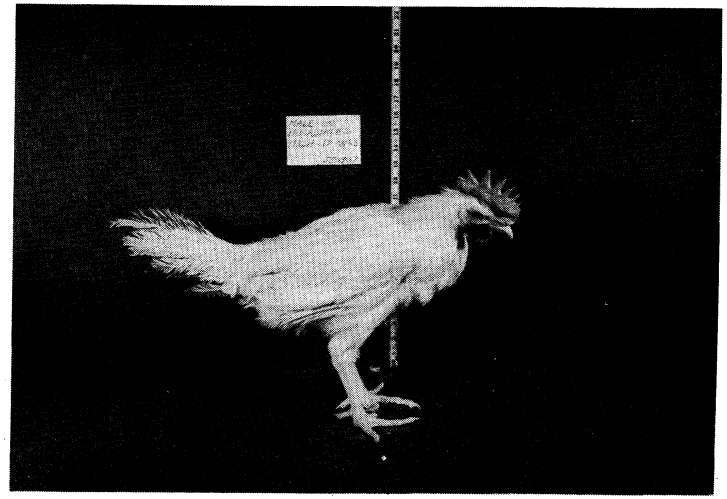

Fig. 6. Typical cockerel in experimental group, age 11 weeks.

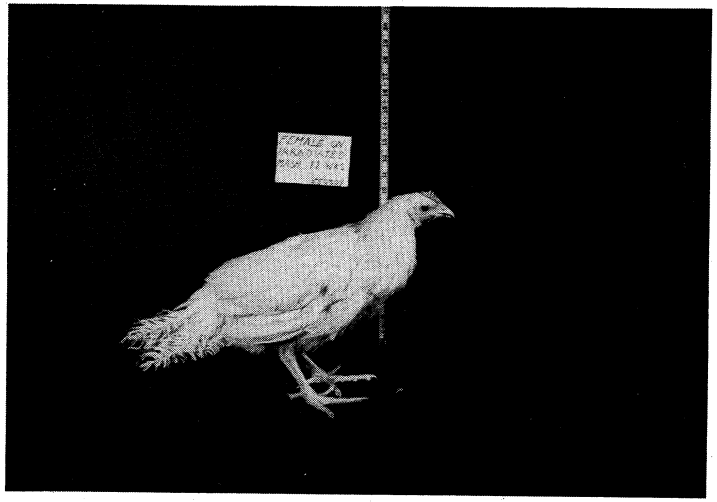

Fig. 7. Typical pullet in experimental group, age 11 weeks.

TABLE III. BLOOD-CELL COUNTS OF CHICKENS ON LONG-TERM EXPERIMENT (March, 1955)*

Group	No. of Animals	Percent Hematocrit	Percent Hemoglobin in Whole Blood	Percent Corpuscular Hemoglobin	White-Cell Count	Percent Polymorphonucleocytes	Percent Monocytes	Percent Basophils	Percent Eosinophils	Percent Lymphocytes
Control Males	4	33.1 (28.9-37.0)	10.8 (9.7-12.0)	33.2 (28.6-35.6)	24,000 (7,500-42,500)	4.7 (3-6)	3 (2-4)	2.7 (1-5)	10.2 (8-12)	80.7 (78-84)
Experimental Males	4	32.4 (31.4-32.5)	10.5 (9.8-11.1)	32.7 (30.4-35.3)	24,000 (13,000-30,000)	3.7 (2-6)	1.7 (1-2)	2 (1-3)	12.7 (7-19)	79.7 (75-87)
Control Females	8	31.1 (28.3-33.3)	9.0 (8.4-10.0)	28.9 (27.5-30.2)	43,000 (22,250-62,000)	2.9 (1-10)	1.2 (1-2)	4 (1-10)	12.5 (5-19)	81.6 (69-94)
Experimental Females	8	31.6 (28.9-35.0)	9.0 (8.5-10.3)	28.6 (27.7-29.4)	37,000 (25,000-40,000)	3.9 (1-11)	1.9 (1-4)	4.3 (1-9)	16.4 (6-23)	74.4 (59-91)

*Values in parentheses are the ranges of the four or eight determinations; the other value is the average. The reticulocyte count never exceeded 2% of the red-cell values in any of the samples.

difference in corpuscular hemoglobin rather than in red-cell count, but there is no difference between control and experimental animals. It is particularly interesting that the eosinophil count is not greatly elevated in experimental animals; although the functions of the eosinophils is not well understood, it is suspected that they act as a detoxifying power. In some birds they are increased in certain allergic states (5).

D. SUMMARY AND DISCUSSION

At this early stage of the chicken experiment, there is no evidence for the presence of toxic substances or for serious lack of wholesomeness in the diet. In the effort to make this chicken feeding and breeding experiment definitive, every criterion within reach will be used to evaluate the wholesomeness of a gamma-irradiated diet. In this respect, attention is focused on egg production, fertility of eggs, and hatchability—sensitive measures of dietary irregularities. In view of the failure of the second generation of Bagg-strain albino mice to reproduce in the pilot experiment performed on Phoenix Project No. 41, the reproduction studies using chickens may have particular significance.

The pullets are expected to begin laying in late May. To obtain reliable data on male and female sterility, it is planned to perform artificial insemination. Fertility data become much more significant by virtue of this technique (rather than by natural mating), which has become standard practice in poultry experiments.

The maximum number of eggs from 80 pullets is around 400 per week, and a Jamesway incubator handling this capacity will be installed. This type of incubator is preferred for both commercial and experimental use. Temperature and humidity are controlled rigorously. The eggs are turned automatically every 2 hours and are transferred at 18 days to a special hatching compartment.

Each egg will be numbered, dated, and weighed, and the eggs collected each week will be set at the end of the week. Eggs will be candled after 7 days of incubation to obtain data on fertility. They will be candled again at 12 and 18 days to determine survival of embryos, and those which are dead at these times will be examined as well as those which fail to hatch. It is possible to determine the age of death of an embryo by the morphological features developed.

To the extent that facilities permit, a representative fraction of the chicks which hatch can be raised to 4 weeks of age in order to compare growth rates of the second generation chicks; a selection of these, in turn, can be maintained for production of third generation chicks.

SECTION II. REPRODUCTION STUDY WITH BAGG-STRAIN ALBINO MICE

A. REVIEW OF PILOT EXPERIMENT

Under support of Phoenix Project No. 41 (1,2) reproduction studies and other observations were made on two generations of mice fed the same diets that the rats received in the initial phases of the long-term experiment conducted by Phoenix Project No. 41. Two groups of mice, one composed of highly inbred animals of the Bagg albino strain and the other of mixed-strain, pigmented animals, were transferred from Purina Laboratory chow to the experimental diets at the time they were bred. Six Bagg albino and 16 mixed-strain female offspring of the animals on irradiated and like numbers of the offspring of the animals on the nonirradiated diet were continued on the diets that their parents received.

The breeding potential of the albino mice was too low to permit setting up groups of adequate size, and a high incidence of sterility among the albino males of the first filial generation necessitated the replacement of the albino males with mixed-strain males of proven fertility.

None of the albino females on the irradiated ration had produced young by the time they were 6 months old, and both the male and female animals on this diet developed skin lesions with loss of hair and inflammation of the eyes. Reproduction performance of the albino females fed the nonirradiated rations appeared to be normal. These data are important despite the small numbers of animals because the results suggest that the Bagg albino mice may be highly sensitive to dietary changes produced by irradiation, at least on the experimental ration fed.

B. PLAN OF PRESENT EXPERIMENT

Since the data of the pilot study described above are the only evidence to date of any harmful effects of gamma radiation on food other than flavor changes and partial destruction of vitamins, it is important to check these preliminary results. A new pilot study was initiated March 28, 1955, with 18 female and 10 male Bagg-strain albino weanling mice. The females were divided into two groups of nine each, the control and the experimental groups. The experimental group was placed on the 4-megarep irradiated diet presently being fed to the rats in the experiment being conducted by Phoenix Project No. 41. The control group will receive the same but nonirradiated

diet. The 10 male mice will be kept on stock diet to avoid the complication of male sterility. The experiment is being commenced with a small number of animals because in the previous pilot study difficulties in reproduction were not observed until the second generation. Estimating about 5 offspring per litter from the breeding of the parent generation would result in about 45 animals in each group for continuation of the experiment with breeding of the first filial generation. It should be pointed out that a slight change was made in the diet being fed the rats, which has slightly improved both the growth and reproduction of the rats in the first filial generation as compared to the rats in the parent generation. Since the Bagg-strain albino mice in the original pilot study were fed the same ration as used with the parent generation of rats, some differences in reproduction may be expected if the modified diet is used. If this is true, this pilot study may be repeated using the original ration. However, if there is a toxic substance in the original irradiated diet which interfered with the reproduction of this strain of mice it would be expected to produce similar results with the slightly modified diet.

EXPLANATION OF SECTION III

Although the experimental study on the albino rats described in Section III of this report has been supported entirely by Michigan Memorial-Phoenix Project No. 41, the progress of the experiment is described in this report because it is considered to be of interest to the Office of the Surgeon General and to those participating in the study of the irradiation of foods.

SECTION III. LONG TERM RAT FEEDING EXPERIMENT
(Conducted by Michigan Memorial-Phoenix Project No. 41)

A. INTRODUCTION

The plan of this experiment has been previously described (1,2,4,5). Briefly, it is to maintain two groups of sixty rats, one on a control diet and the other on an irradiated diet, for a period of at least two years to furnish data on the effects of irradiation on growth and longevity. In addition, the parent generation was to be bred twice, the litters from the second breeding to be raised on the experimental and control diets and then bred twice, and so on for four generations to furnish data on the effects of irradiation on growth and on reproduction. As of the present date, the third-generation animals consisting of the second litters from the first filial generation have been weaned and the first filial generation has been sacrificed. This section of the report presents the data on the first filial generation with respect to growth rate, reproduction, and red-blood-cell count. At the time of sacrifice, tissue samples were taken from nearly all the animals. The findings of the pathologists will be included in the next quarterly report.

B. GROWTH OF FIRST FILIAL GENERATION

From the second litters of each group of parent rats, 12 males and 20 females were chosen at random to represent the first filial generation. Figure 8 shows the average weights of the four groups from the time they were weaned until they were sacrificed. The average weights of both the males and females on the irradiated diet lagged somewhat behind the corresponding animals on the control ration. With respect to the males, the result is similar to that obtained with the parent generation; in both cases there was about a 5% difference in weight between control and experimental males. The difference in growth of the first filial generation females, however, showed about the same difference as the males, whereas the parent females at this age did not.

The "humps" in the curve indicating the two breeding periods were not as distinct as in the case of the parent females because the ages of the first filial females were more scattered than were those of the original parent generation.

A typical male and female of the control and experimental groups are shown in Figs. 9, 10, 11, and 12, respectively.

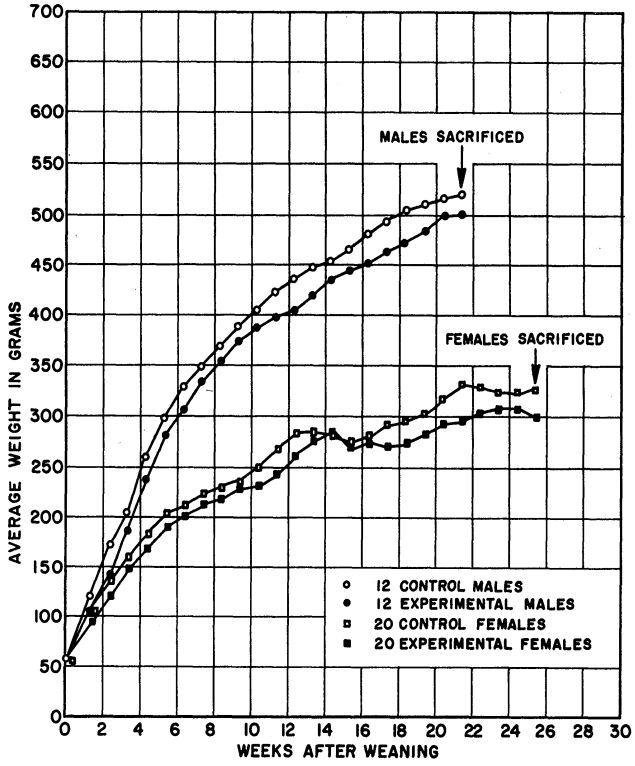

Fig. 8. Growth curves of the first filial generation of rats.

C. BREEDING PERFORMANCE OF FIRST FILIAL GENERATION

Table IV is a summary of the breeding performance of both the parent and first filial generation rats through both of their breeding periods. (The animals are bred twice because the results of the first breeding are usually irregular; also, sterile females can be eliminated. When the females are bred a second time, their litters are larger and more uniform and the breeding data become more reliable as a criterion of the wholesomeness of the diet.) The performance of the control and experimental animals is compared through each of the four breeding periods.

With respect to the incidence of female sterility, the experimental animals during both breeding periods of the parent generation showed a three-fold greater incidence than did the controls, but this difference did not occur during the breeding of the first filial generation. There was no significant difference in the incidence of male sterility between controls and experimental animals. The data do not indicate that irradiation of the diet causes a delay in the time of conception. Two control females in each of the two breeding periods of the parent generation resorbed their fetuses.

There was no significant difference in the number of litters born. The control groups altogether gave five dead litters compared with two dead litters from the experimental groups, but five litters from the experimental

Fig. 9. Typical male in control group.

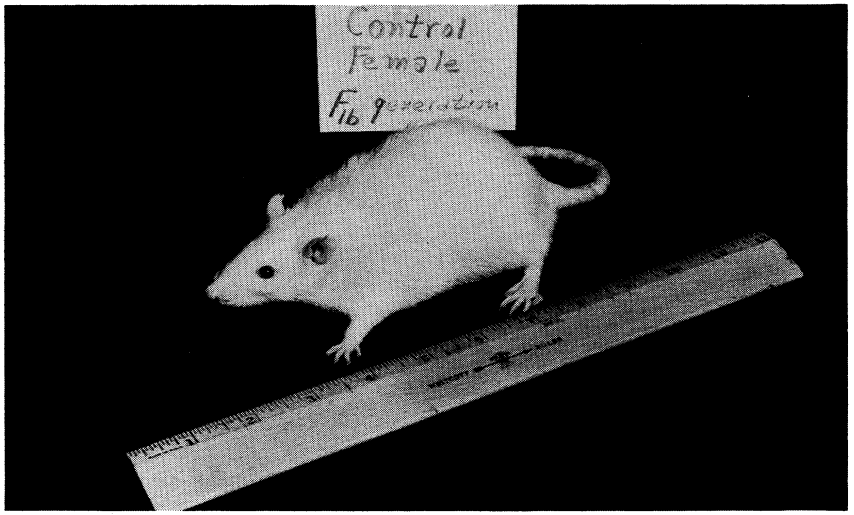

Fig. 10. Typical female in control group.

Fig. 11. Typical male in experimental group.

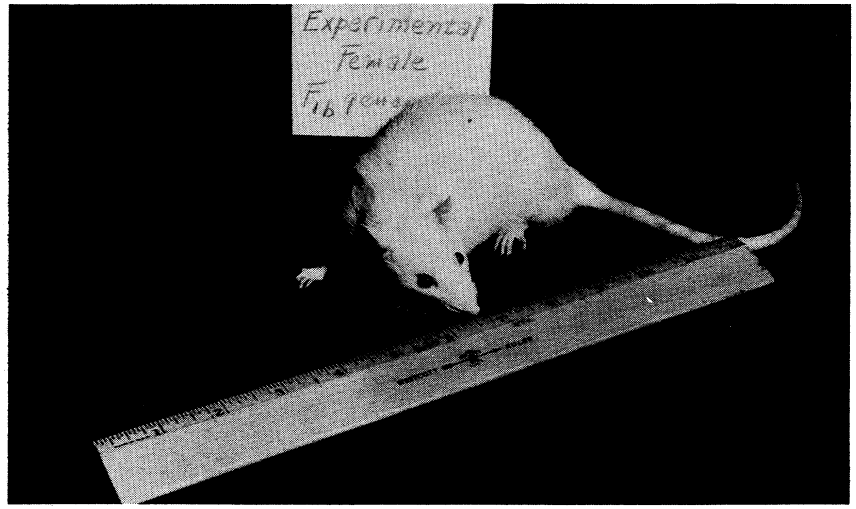

Fig. 12. Typical female in experimental group.

TABLE IV. DATA ON THE FIRST AND SECOND BREEDING OF THE PARENTS AND FIRST-FILIAL-GENERATION RATS

	1st Breeding of Parent Gener.		1st Breeding of First Filial Gener.		2nd Breeding of Parent Gener.		2nd Breeding of First Filial Gener.		
	Cont. Grp	Exp. Grp	Cont. Grp	Exp. Grp	Cont. Grp	Exp. Grp	Cont. Grp	Exp. Grp	
No. females bred	20	20	20	20	20	20	20	20	
No. males used	20	20	12	12	20	20	12	12	
No. females sterile (1)	2	5	2	1	2	6	3	2	
No. males sterile (2)	8	9	4	3	10	5	3	3	
No. males not proven (3)	2	3	0	0	2	2	2	2	
No. females conceiving 1st wk	8	3	6	5	5	4	10	12	
No. females conceiving 2nd wk	1	3	6	6	3	4	3	4	
No. females conceiving 3rd wk	3	4	3	7	4	3	3	0	
No. females conceiving 4th wk	0	0	0	0	3	2	1	0	
No. females conceiving after 4th wk	4	5	3	1	1	1	0	2	
No. females resorbing fetuses	2	0	0	0	2	0	0	0	
Tot. No. litters born	16	15	18	19	16	14	17	18	
Tot. No. litters born dead	1	1	2	0	1	0	1	1	
Tot. No. litters born alive not surviving weaning	1	1	0	1	1	3	1	0	(5)
Tot. No. pups born	157	119	184	195	145	115	173	188	
Tot. No. pups born dead	5	3	22	9	17	11	17	13	
% pups born dead	3.18	2.52	11.95	4.62	11.72	9.56	9.83	6.91	
No. pups born alive per female bred	7.6	5.8	8.1	8.3	6.4	5.2	7.8	8.8	
Tot. No. pups born alive not surviving weaning	25	21	17	24	30	23	21	14	(5)
Ave. No. pups born per litter	9.8	7.9	10.22	10.26	9.1	8.2	10.18	10.44	
Ave. No. pups per litter at 5 days	8	6.6	8.4	9.3	6.1	5.6	8.5	9.2	
Ave. No. pups per litter at 21 days	8	6.6	8.0	8.6	6.1	5.6	7.6	8.7	(5)
Tot. No. young disposed of (4)	0	0	0	0	0	0	5	7	
Ave. wt. of young at 21 days, grams	48.6	45.5	45.5	47.7	57.8	52.7	52.9	49.9	(5)
Tot. No. young reaching weaning	127	95	145	162	98	81	135	161	(5)
Ave. No. young weaned per female bred	6.43	4.8	7.2	8.1	4.9	4.1	6.5	8.1	(5)
% pups born alive which survived 21 days	83.5	81.8	89.4	87.1	76.6	77.9	86.5	92.0	

(1) Mated six times unsuccessfully.
(2) Mated unsuccessfully with at least one female which later became pregnant by another male.
(3) Mated only with pregnant or sterile females.
(4) For purposes of reducing litters to ten after birth.
(5) Data do not include those of pups from females conceiving after 4th week.

groups did not survive to weaning age, whereas only three litters from the control groups did not. The percentage of pups born dead was greater among the control groups in all four comparisons. As to the average number of pups per litter at 21 days (time of weaning), the ratio of those in the experimental groups to those in the control groups steadily increased throughout the course of the four successive breeding periods from .83 to 1.15; the average weight of the pups in the experimental groups, however, was slightly less.

The third line from the bottom on Table IV shows the total overall performance, namely, the number of pups weaned per female used for the breeding. In this respect the control animals of the parent generation did somewhat better, but in the case of the first filial generation, the experimental animals did better. This overall performance is the result of two things, reproduction and lactation. The former is given by the number of pups born alive per female bred; in this respect, there was a steady increase in the performance ratio of experimental to control animals from 0.76 to 1.13 throughout the course of the four breeding periods. Lactation is a complicated process which cannot be precisely evaluated with the data in the table, but the expression which most closely reflects it is the percentage of pups born alive which survive to weaning. The experimental and control animals are found to be practically identical in their lactation performance. The data show that lactation for both the parent and first filial generations was improved when each was bred the second time, but this is the only point of difference supported by the data.

D. SELECTION OF THE SECOND-FILIAL-GENERATION RATS

After the second litters from the first filial generation of rats had been weaned, the first filial generation was sacrificed. The number of rats in the second filial generation was reduced to twenty females and twelve males in each of the two groups. Each of the fifteen litters in the control group and seventeen in the experimental group are represented. If the litter contained three or more males, one male was chosen whose weight was closest to the litter average, and if the litter contained two or more females, one female was chosen. This method of selection is a departure from the completely random method used in selecting members to constitute the first filial generation.

E. RED-BLOOD-CELL DATA FROM FIRST-FILIAL-GENERATION RATS

At the time the first-filial-generation rats were sacrificed, blood samples were taken by tail nicking, and the percent hematocrit, percent hemoglobin, and percent corpuscular hemoglobin were determined. The methods are the same as those described in section I.C.4 of this report for the chick. The results are presented in Table V. No differences are found in any of the comparisons between control and experimental animals. The values for the

TABLE V. RED BLOOD-CELL DATA ON FIRST FILIAL GENERATION

Group	No. of Animals	Average Weight, Time of Sacrifice (grams)	Hematocrit, %		Whole Blood Hemoglobin, %		Corpuscular Hemoglobin, %		White-Cell Count** cells per cu mm	
			Average	Range	Average	Range	Average	Range	Average	Range
Control males	11	528	49.9	46.7-54.4	16.8*	16.2-18.1	34.2*	29.8-38.7	10,950	10,000-11,650
Experimental males	12	508	48.2	39.2-50.8	15.6*	12.7-17.0	32.6*	30.4-34.6	11,710	9,400-17,250
Control females	20	317	50.9	29.9-57.2	17.2	9.2-19.8	33.7	31.6-36.6		
Experimental females	18	304	51.4	48.4-55.6	17.3	15.4-18.9	33.6	30.2-36.9		

*Excluding 5 animals whose hemoglobin values were determined by another method.
**Only 5 male rats in each of the two groups.

females are practically identical; those for the males may not be significantly different owing to smaller numbers of animals. From these data it may be concluded that the hemopoietic functions of second generation rats are not affected by irradiation of the diet of both the second generation rats and their parents.

F. PRESENT STATUS OF THE PARENT GENERATION OF RATS

The growth curves for the parent generation rats appear to be levelling off. As of the 23rd of March, 1955, the 61st week of the experiment, the average weight and number of animals in each group is shown in Table VI.

TABLE VI. AVERAGE WEIGHT OF PARENT RATS, 61 WEEKS

Group	No. in Group	Average Weight
Control males	28	656 grams
Experimental males	27	633 "
Control females	29	373 "
Experimental females	28	362 "

These weights are 9-10% greater than the last weights (50th week) shown in Progress Report No. 1, for this project, and the relative difference between the different groups has not changed.

There were thirty-one animals in each group or 124 altogether at the beginning of the experiment 61 weeks ago; there are still 112 in good health. The remaining twelve animals were sacrificed or isolated from the experiment due to the occurrence of acute respiratory infection or to the incidence of carcinoma. Table VII presents the pertinent data with respect to these 12 animals.

The incidence of tumors to date is three among control animals (one male, two females) and four among the experimental animals (two males, two females). The incidence of respiratory infection is two among the control animals (both males) and three among the experimental animals (two males, one female). As of the present writing, a control female became inflicted with a severe inflammation in the left eye.

Of the five animals which died or have been sacrificed, the pathology reports of two, Experimental males $2R_3$ and $4R_1$ are given in Progress Report No. 1 (Engineering Research Institute Project 2307). The pathology report of one, Control male $3L_1$, is not yet available. The pathology reports

TABLE VII. CURRENT PATHOLOGICAL RECORD OF THE LONG-TERM RAT EXPERIMENT

Group	Animal Number	Observation	Date Noted	Fate	Location of Figures	Location of Pathologist's Report
Control male	A♂7_0	Lateral tumor	15 Nov 54	Sacrificed 16 Dec	Prog Rpt No. 1, Fig. 14	This report, p. 21
" "	A♂$3L_1$	Acute respiratory infection	23 Feb 55	Died 8 Mar		Pathologist's report not yet available
" "	A♂$1R_2L_1$	Acute respiratory infection (lost 78 gm in 2 wks)	20 Mar 55	Still living		
Experimental male	D♂$2R_3$	Lateral tumor	16 Aug 54	Sacrificed 13 Nov 54	Prog Rpt No. 1, Fig. 13	Prog Rpt No. 1, p. 37
" "	D♂$4R_1$	Scrotal tumor	23 Aug 54	Sacrificed 10 Sep 54	Prog Rpt No. 1, Fig. 15	Prog Rpt No. 1, p. 39
" "	D♂$6R_3$	Acute respiratory infection	23 Feb 55	Still living		
" "	D♂$4R_1L_3$	Acute respiratory infection	23 Mar 55	Still living		
Control female	A♀$3R_1L_1$	Lateral (mammary?) tumor	2 Feb 55	Still living	This report, Fig. 13	
" "	A♀$1L_2$	Tumor on neck	10 Mar 55	Still living	This report, Fig. 14	
" "	A♀$3L_3$	Left eye protruding due to infection and swelling	30 Mar 55	Still living		
Experimental female	D♀$6R_1L_1$	Respiratory infection	19 Jan 55	Sacrificed 9 Feb 55		This report, p. 23
" "	D♀$4R_2L_3$	Series of at least 3 mammary tumors	9 Feb 55	Still living	This report, Fig. 15	
" "	D♀$4R_1L_3$	Lateral tumor	23 Mar 55	Still living	This report, Fig. 16	

for the other two, Control male 7_0 and Experimental female $6R_1L_1$ are presented in Tables VIII and IX, respectively, in this report. Pictures of the seven animals bearing tumors to date are available either in this report or in Progress Report No. 1, as indicated in Table VII.

There is no evidence in these data to support an unfavorable conclusion with regard to the irradiation of the diet. Most of the illness has occurred since the first year of experiment, and during the second year of experiment, which is "middle age" for a rat, the occurrence of tumors and degenerative diseases can be expected to increase. Degenerative diseases take many forms and may obscure less pronounced effects due to the irradiation of the diet. On the other hand, if a particular pattern of degenerative diseases occurs among animals on the irradiated diet, this will be of particular significance. Careful records of daily observations are being maintained toward this end.

TABLE VIII. HISTOPATHOLOGICAL REPORT A♂7_0, 4325-LBG
ON CONTROL MALE RAT WITH LATERAL TUMOR

The tumor is a malignant supporting-tissue neoplasm, made up of large atypical polygonal cells arranged in sheets and in syncytial fashion. There are no adequate grounds for specific classification of this sarcoma. This has been compared with the scrotal tumor of 1832-LBG. They do not appear to be the same type of neoplasm, although both are derived from supporting tissue.

Tissue	Observation
Heart	Negative.
Lung	Patchy emphysema. Fat stain negative.
Spleen	Negative.
Small intestine	Negative.
Liver	Recent, probably terminal, peri-vascular hemorrhages in portal areas. Diffuse heavy lipidosis. Some of the lipid is present as large droplets in hepatic cells, but much of it is in fine droplets in lining cells of the sinuses.
Kidney	Negative.
Bone	No disturbance of growth. The marrow is about 90% cellular and is composed of the usual cell types.
Testis	The seminiferous tubules contain many spermatozoa but all are necrotic. Many of the cells have pyknotic nuclei. Very few division figures are present.

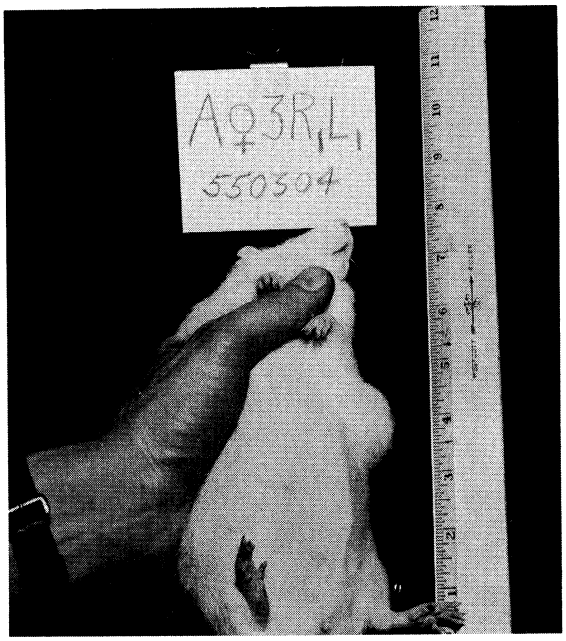

Fig. 13. Female rat of the parent generation on the control diet with a lateral tumor.

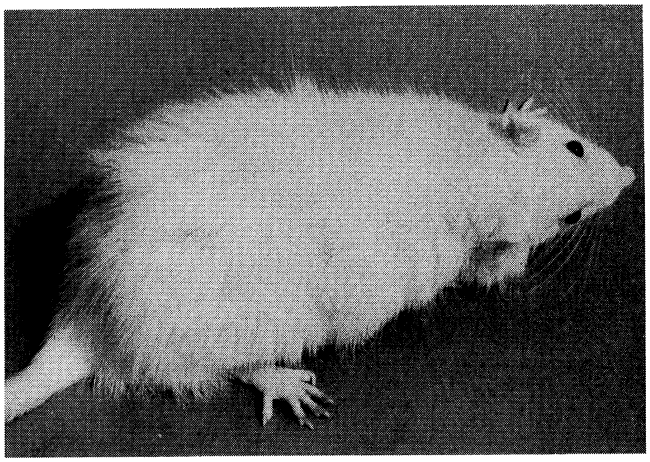

Fig. 14. Female rat of the parent generation on the control diet with tumor on neck. The rat usually holds head in the twisted position shown.

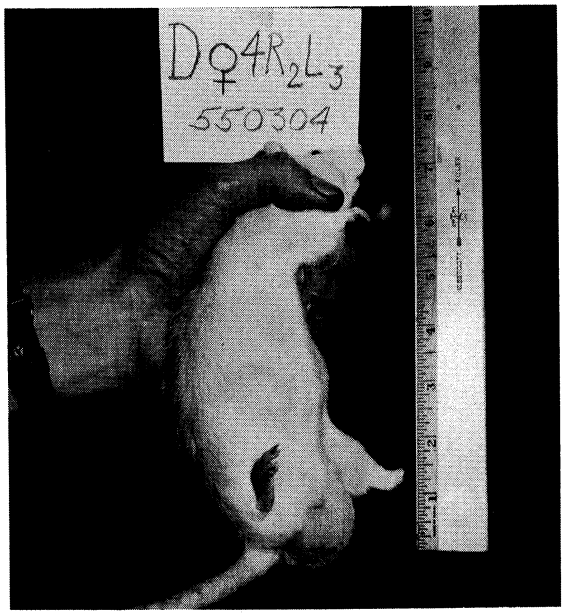

Fig. 15. Female rat of the parent generation on the irradiated diet, with at least three mammary tumors. The large tumor extending from the rear of the animal grew very rapidly and became necrotic in about seven weeks.

Fig. 16. Female rat of the parent generation on experimental diet with a lateral tumor.

TABLE IX. HISTOPATHOLOGICAL REPORT D♀6R_1L_1, 6024-LBG ON EXPERIMENTAL FEMALE RAT WITH ACUTE RESPIRATORY INFECTION

Tissue	Observation
Liver	Cloudy swelling. Slight degenerative fatty infiltration. Lipid in stellate cells of Kupffer.
Esophagus	Cornification of the epithelium.
Stomach	Slight increase in mucin.
Colon	Negative.
Ovaries	Corpora lutea. Normal ovaries.
Fallopian tubes	Negative.
Spleen	Congestion. Foci of erythropoiesis.
Lungs	Dilated bronchi filled with pus. Bronchiectasis. Lipophages in the bronchial submucosa. Purulent lobular pneumonia with acute abscesses. Fat stain shows no fat emboli, but numerous lipophages about the bronchi.
Adrenal	Cortical lipids abundant.
Kidneys	Slight patchy degenerative fatty infiltration.
Heart	Negative.

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