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Progress Report No. 3

WHOLESOMENESS OF A GAMMA-IRRADIATED DIET FED TO CHICKENS

Period March 30, 1955, to June 30, 1955

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ABSTRACT

This report covers the progress from March 30 to June 30, 1955, of the three animal feeding and reproduction experiments being conducted at the University of Michigan Fission Products Laboratory. These are (1) the long-term chicken experiment supported by the Office of the Surgeon General of the U.S. Army, (2) the mouse reproduction study, and (3) the long-term rat experiment supported by Michigan Memorial-Phoenix Project No. 41.

The average body weights of the four groups of chickens (control males and females, experimental males and females) have nearly reached their maxima during the past 3 months with the experimental females showing a slightly lower weight relative to the controls, there being no difference between the male groups. Feed efficiency data during this period are difficult to evaluate, but the lower efficiency of feed utilization previously reported for the experimental animals appears not to be the case now. All the control females and all but two of the experimental females have come into egg production with no appreciable difference between the groups in percent egg production nor in average egg weights. Artificial insemination has been adopted as a means of obtaining fertile eggs, but since incubation began on June 14 and no chicks are anticipated until July 5, there is not sufficient egg fertility data to report. The control males showed a superiority in semen production relative to that of the experimental males.

Blood-cell counts for 7 males and 14 females in each of the two groups showed no differences attributable to diet. No pathological incidents have occurred with any of the 82 females. One control and 3 experimental males, however, had to be sacrificed because of severe weight loss or muscular difficulties, while 1 additional control and 2 additional experimental males appear destined to the same fate for similar reasons.

The first litters from the 9 control and 9 experimental parent female mice in the mouse reproduction study have been obtained; the breeding performance was slightly better on the part of the experimental animals. The weanling mice from the experimental females were slightly smaller than those from the control females, but they are showing a slightly greater percent growth rate.

The long-term rat feeding and breeding experiment is now in its eighteenth month and the incidence of tumors, infections, and acute respiratory disease, while increasing, is still about the same among the four groups of animals. A second series of blood-cell counts on a slightly larger number of animals than before revealed no differences between animals on irradiated and control diets. The first crop of third-filial-generation animals has been weaned from the control and experimental females with no differences between them in reproductive performance. Histopathological reports on some of each of the four groups of first-filial-generation rats have no evidence of abnormalities attributable to gamma irratiation of the diet.

OBJECT

The object of the experiments reported on this project is to evaluate the wholesomeness of food and feed receiving a sterilizing dose of gamma radiation.

SECTION I

CHICKEN FEEDING EXPERIMENT

A. INTRODUCTION

The long-term feeding experiment, using chickens as the experimental animal, is in its twenty-eighth week. The period of rapid growth has been completed and the experiment is now entering the reproductive phase. The growth curves for the four groups of animals (males and females on the control and experimental diet) are approaching a maximum. Egg production began on May 10, 1955, and reached a level of about 60% at the end of June, 1955. Data have been accumulated for 7 weeks on egg production, number of pullets laying, and average egg weights. Artificial insemination was started on June 6; incubation of eggs began on June 14; and the first chicks, notwithstanding a famous proverb, are anticipated on July 5. In connection with artificial insemination, it has been possible to score the control and experimental cockerels for semen production. A number of pathological incidents have occurred with male, but not with female, birds and a histopathological report on one is available. During the week of June 20, the second round of blood-cell counts was made. The previous hematologist, being experienced with mammalian rather than avian blood, encountered several difficulties. The second round of counts was made by an experienced avian hematologist, Mrs. C. P. Letts, who received her training and experience under Dr. Alfred Lucas, cytopathologist at the U.S.D.A. Regional Poultry Research Laboratory, East Lansing. Dr. Lucas is one of the foremost authorities on avian blood.

B. MANAGEMENT

1. Changes in the Management of the Cockerels.—The individual cages furnished by Pockman and Sons Company for the housing of laying hens have been proved to be too confining for the males of a medium breed. The dimensions were: 10 inches wide, 18 inches high, and 18 inches long. New cages with corresponding dimensions of 16 x 24 x 22 inches, but similar in other respects to the previous cages, are being installed to give the cockerels sufficient room. The water and feed supply will be the same as before.

It has also been found necessary to trim the combs of the males. The sheer massiveness of the White Leghorn male comb caused facial abnormali-

ties and also presented a hazard to the animals when reaching through the front grill of the cage to obtain food. It is believed that the two birds that suffered neck injuries might have obtained them in this way.

The practice of artificial insemination has been adopted as a means of obtaining fertile eggs because of the greater degree of control over the fertilization process and also because of its greater convenience as compared to penmating. It is ultimately desired to have fertility data on both males and females. The females are to be proven first because of simplicity of this approach, and for this purpose the semen from both the control and experimental males is pooled prior to insemination. The manner of collecting the semen and placing it in the oviduct is standard practice and is becoming more widely adopted as a commercial practice. When fertility records of the females are established, the semen from individual males will be used to establish the fertility of each.

Although semen collections have been made three times weekly since April 27, the quantity of semen obtained has not increased with each collection. Opinions differ as to management practices regarding semen production, one authority claiming that the ration furnished the cockerels should consist largely of whole grain rather than mash and another authority claiming that this is not the case. It is planned in this experiment to offer whole or cracked grain to the cockerels whose semen production is poor to see if it can be increased.

2. Changes in the Diet of the Females.—The Ralston Purina Company, which supplies the commercial mash for this experiment, recommends that when pullets reach 20% egg production, the ration should be switched from the "Growena" to the "Layena." Because of the rapidity with which the pullets came into egg production and the delay in obtaining and irradiating the special antibiotic-free mix of Layena, the rations were not switched at the recommended time. However, by supplying ad libitum commercial oyster shell, which is practically pure calcium carbonate, the calcium level of the Growena was made sufficient. The pullets were switched to the Layena ration during the first week of June when egg production had reached approximately 50%. It has been recommended that a slightly different form of Layena be used for pullets maintained in cages, and use of this form will start during the second week in July.

The composition of the laying mash is presented in Table I which also includes the corresponding composition of the Starteena and Growena mashes. The Layena product does not contain antibiotic supplement. Following irradiation of the diet, a vitamin supplement is added to both the irradiated and control diets as described in Progress Report No. 1.

The mash was originally mixed with an equal weight of water prior to irradiation. This gave a mash whose water content prevented adequate

TABLE I

GUARANTEED ANALYSIS OF THE STARTEENA, GROWENA, AND LAYENA MASHES FURNISHED BY THE RALSTON PURINA COMPANY FOR THE LONG-TERM CHICKEN EXPERIMENT

Ingredient	Type of Mash and When Fed		
	Starteena 0 to 4 wk	Growena 4 wk, 20% Production	Layena* 20% Production
Crude protein, min %	20.0	17.0	18.0
Crude fat, min %	3.0	3.0	2.5
Crude fibre, max %	5.0	7.0	7.0
Nitrogen-free extract, min %	50.0	48.0	42.0
Rock phosphate, %	0.75	1.0	2.0
Calcium carbonate, %	1.5	1.5	3.5

*The ingredients of Layena are: soybean-oil meal, meat scrap, sun-cured alfalfa meal, wheat gray middlings, wheat bran, ground yellow corn, corn gluten feed, ground barley, ground grain sorghums, ground oats, 0.03% vitamin A feeding oil (containing 10,000 U.S.P. units of vitamin A per gram), 0.25% riboflavin supplement, 0.08% D-activated animal sterol (containing 1500 I.C. units vitamin D per gram), 2% low fluorine rock phosphate, 3.5% calcium carbonate (limestone), 0.4% iodized salt, and 0.02% manganese sulfate.

ventilation for the carbon dioxide formation during irradiation and suppressed mold growth when placed in the feeding trough. It was found that lowering the amount of added water so that the mash contains 44% added water, rather than 50%, overcame these difficulties.

3. Facilities for Irradiation of Mash.—A cylindrical mash holder termed the "cheese box," 16-1/2 inches in diameter and 10 inches high was designed, constructed, and placed directly above the source in the cave. The "cheese box" consists of two stainless steel boxes, one inverted inside the other as an alternative to a water-tight lid, for irradiation of wet mash which tends to express some free liquid. Figure 1 is a sketch of the plan and elevation view of the "cheese box." The segment on the north side was removed to avoid interference with the experiments on chemical reactions. The "cheese box" is inverted when 50% of the dose is received so as to provide greater uniformity of dose. A total of 4 days is required to accumulate 3 megarep. The unit holds 60 pounds of wet mash. Figure 1 also shows the elongated No. 10 can (10 inches high) which can be used in the center well position when this position is not being used in other radiation experiments. A

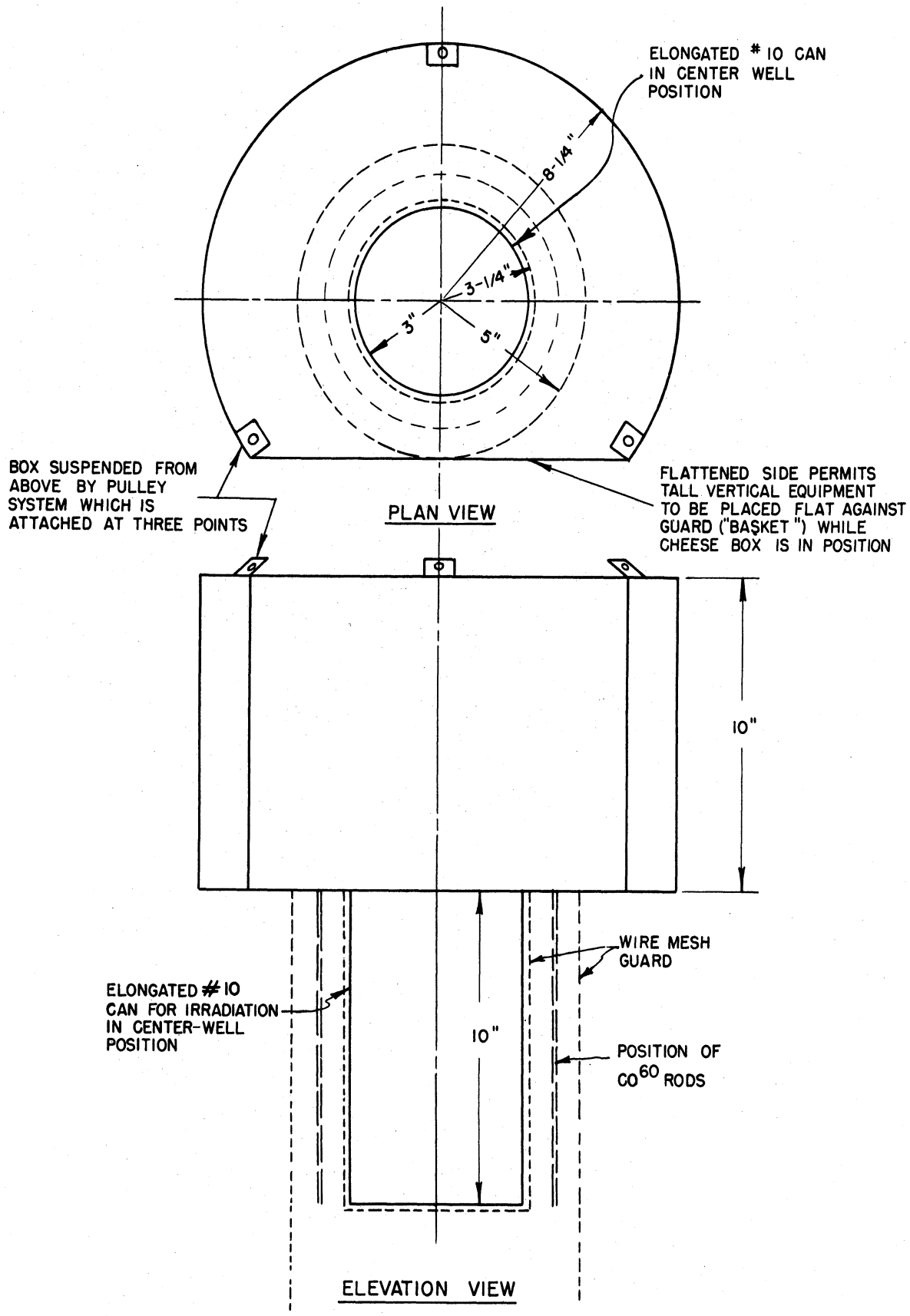

Fig. 1. Plan and elevation showing size and position in radiation cave of "cheese box" and elongated No. 10 can.

15-hour exposure is required in this position to accumulate a 3-megarep dose. This unit holds 9 pounds of wet mash.

In addition to the "cheese box" and the elongated No. 10 can, an annular holder was designed and constructed to utilize the southern half of the radiation field outside the source (see Fig. 2). This holder has an inside radius of 8-1/4 inches and an outside radius of 14-3/4 inches, is 28 inches high, and includes an angle of 170 degrees. (The angle was decreased from 180 degrees so as to clear the elevator rods.) This holder will contain twenty No. 10 tin cans, each holding about 7 pounds of wet mash. These cans are moved from center to upper or lower position when the irradiation is half completed. When the annulus between this holder and the source is empty, a 7-day cycle is required for a 3-megarep dose. When the annulus is filled with diet for the rodent feeding studies an 8-day cycle is required.

Figure 3 shows a photograph of the three containers for the irradiation of wet mash. Note that the "cheese box" has been elevated to permit an operator to insert the elongated No. 10 can in the center well position.

4. Handling and Incubation of Eggs.—Records showing the pullet number, the date laid, and the weight are being maintained for each egg produced since egg production began on May 10. Artificial insemination began on June 6 when egg production reached about 50%. Over a period of 3 weeks all but three birds in each of the two groups were brought into the insemination schedule. Each pullet is inseminated weekly. Two days after each pullet was first inseminated, her eggs were retained for incubation. A single dose of sperm is effective from not less than 2 to not more than 10 days after insemination. Eggs destined for incubation are collected each night and placed directly in a constant-temperature room held at 50°F and approximately 50% humidity, made available through the courtesy of the University Food Service.

Incubation is carried out artificially in a Jamesway Model 2940 Incubator, the standard model for poultry research. Its capacity is 2940 eggs and is carefully designed to achieve the optimum temperature, humidity, and ventilation for chick egg incubation and hatching. Figure 4 shows the machine with a hatching tray above and an incubation tray below, pulled out from each of the compartments. Eggs are incubated in the lower compartment for a period of 18 days and are held in cradles which are rotated every 4 hours. The turning is performed by an automatic turning mechanism; this turning is necessary in order to keep the developing embryo from attaching itself to the inside surface of the egg membrane. Temperature in the incubation compartment is regulated at 99.5 ± 0.25°F. For the remaining 3 days of the 21-day incubation period, the eggs are transferred to individual baskets in the hatching compartment. This enables one to identify every hatched chick as to its dam. The temperature in the hatching compartment is regulated 1 degree higher than that in the incubation compartment. Wet bulb temperature is maintained to about

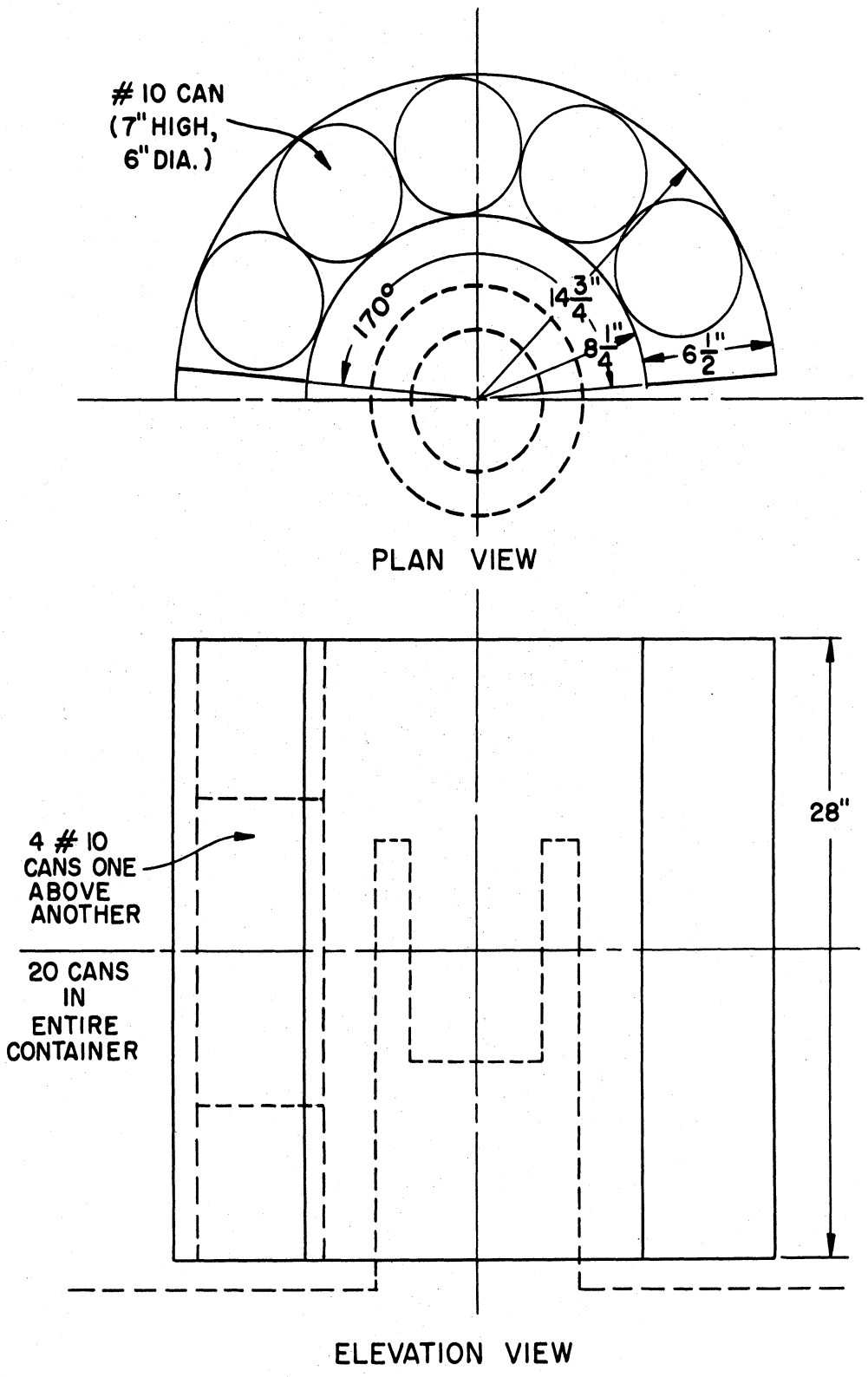

Fig. 2. Annular holder for wet mash.

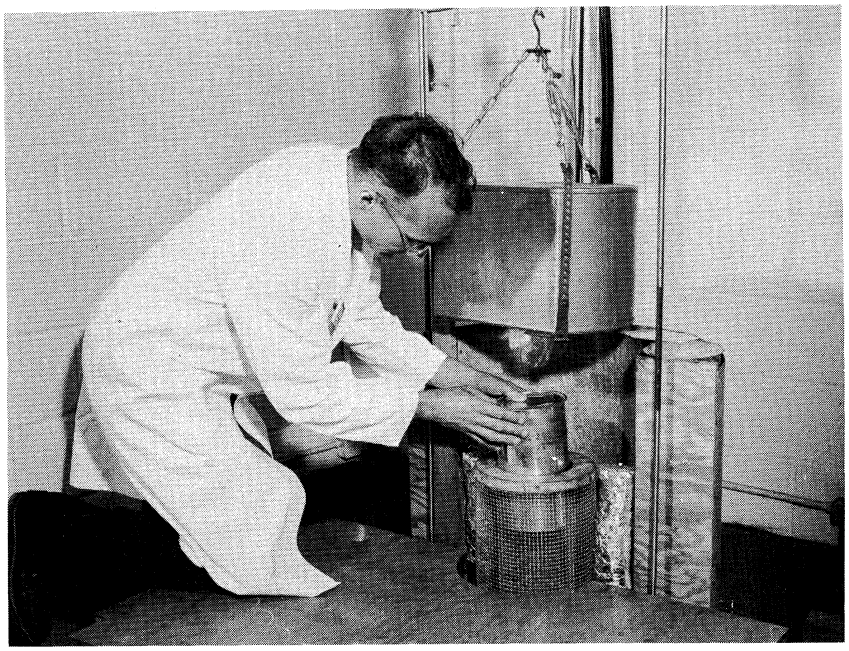

Fig. 3. View of the three containers used for the irradiation of wet mash.

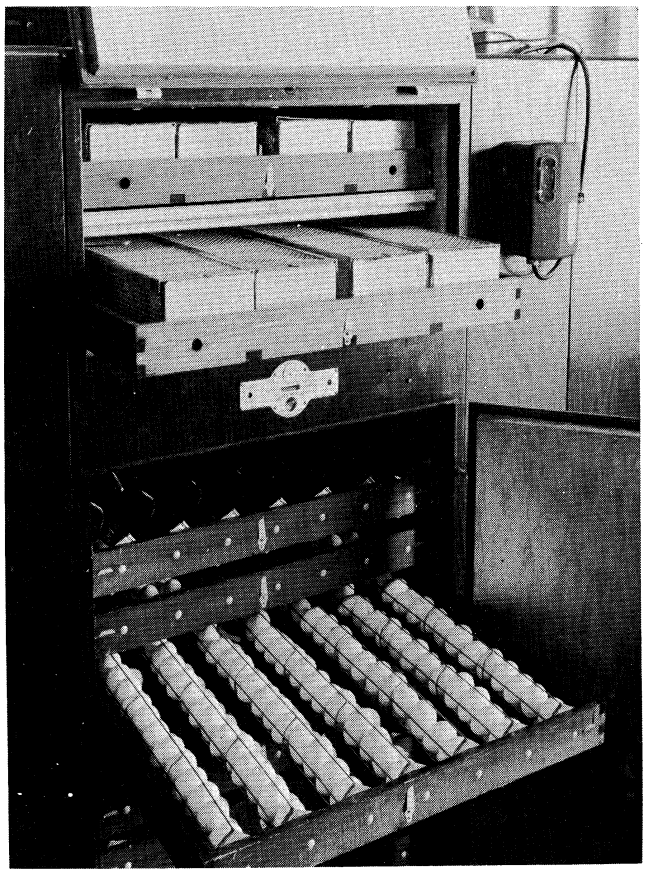

Fig. 4. View of the chick egg incubator showing incubation compartment below and hatching compartment above.

82°F, and adequate ventilation is provided by both automatic and manual controls.

The eggs are candled at the seventh and fourteenth days of incubation. The first candling reveals the infertile eggs as well as those embryos which died before 7 days. Embryos which die before 1 day are difficult to distinguish from infertile eggs, but thereafter it is possible on the basis of size and morphological features to state the day when the embryo died. The second candling would not be necessary were it not for the autolysis which occurs in deceased embryos unless they are removed from the incubation conditions before the end of the incubation period.

The authors wish to acknowledge the assistance given them by Professors Lloyd Champion and Howard Zindell of the Poultry Department, Michigan State University, in the planning and management of the chicken feeding experiment.

C. EXPERIMENTAL RESULTS

1. Growth and Average Body Weight.—Figure 5 is a continuation of Fig. 3 in Progress Report No. 2, in which the average body weights are now shown to the twenty-seventh week of experiment for each of the four groups. The gap between the females did not close even after the period of rapid growth ceased, whereas for the males the difference in the average body weights ceased to exist. This latter observation is noteworthy in that in two other respects, pathology and semen production, the two groups of males differed, but that in no respect other than average body weight have the females shown any differences.

Figures 6, 7, 8, and 9 show representative chickens from each of the four groups. Their ages are 26 weeks and they were chosen on the basis of having a body weight most closely approximating the mean for the group which they represent.

2. Efficiency of Feed Utilization.—Although daily and cumulative records have been kept on the amount of mash supplied the chickens, several factors have entered into the experiment which make it difficult to evaluate the efficiency of feed utilization for the control and experimental birds: (1) the onset of egg production, (2) the slower rate of growth, (3) removal of animals from the experiment, and (4) change in the water content of the mash. For these reasons, the data on which to base feed efficiency calculations are presented in Table II for four consecutive periods from March 21 to June 27. Period I, the first 7 weeks, is the period from the last report to the onset of egg production and shows that the experimental animals used their feed as efficiently as did the control animals. Period II, 2 weeks, is from the beginning of egg production to the time when the water content of the

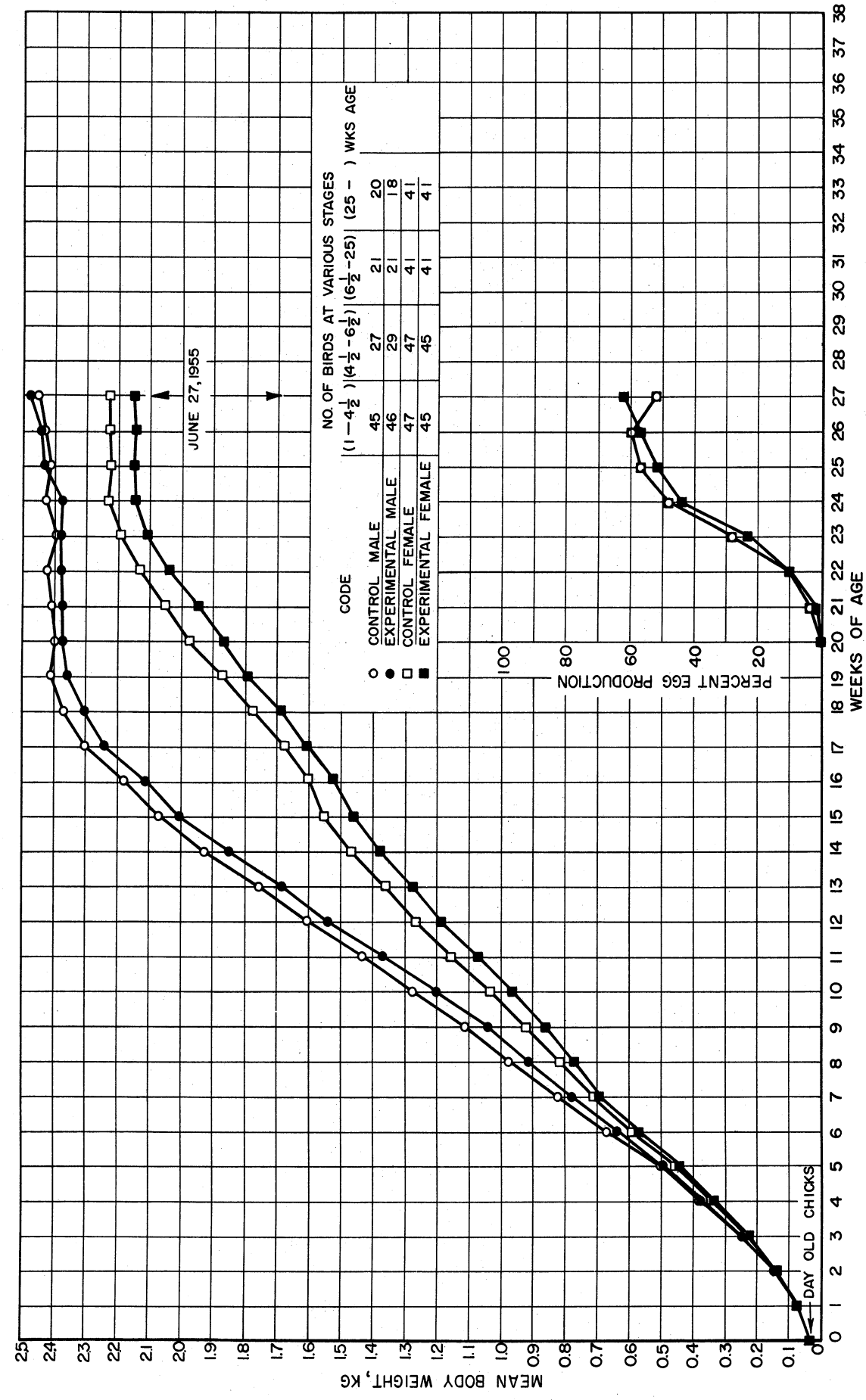

Fig. 5. Growth curves of the four groups of chickens from 1 day to 27 weeks of age; average weekly percent egg production is also shown on the same time scale.

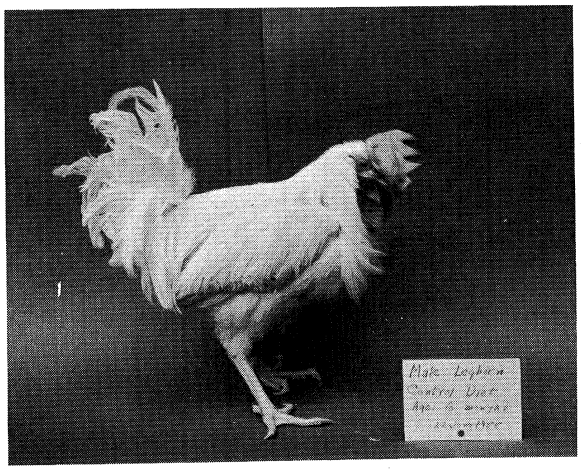

Fig. 6. Typical rooster in control group; age 26 weeks.

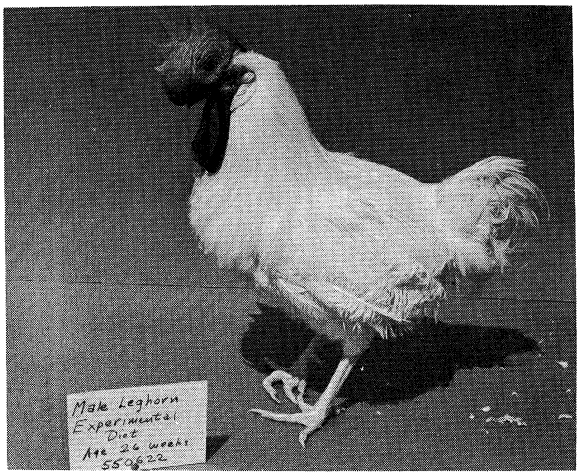

Fig. 7. Typical rooster in experimental group; age 26 weeks.

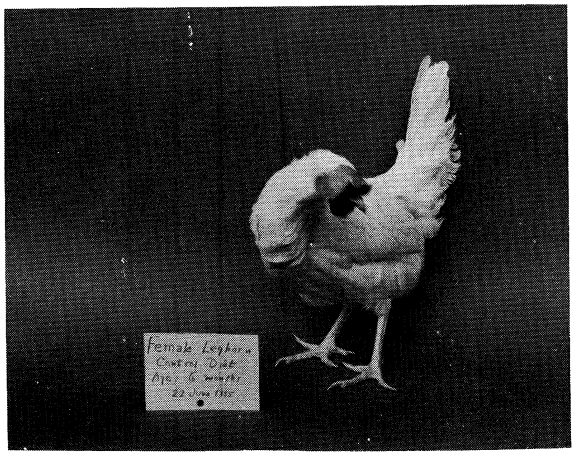

Fig. 8. Typical pullet in control group; age 26 weeks.

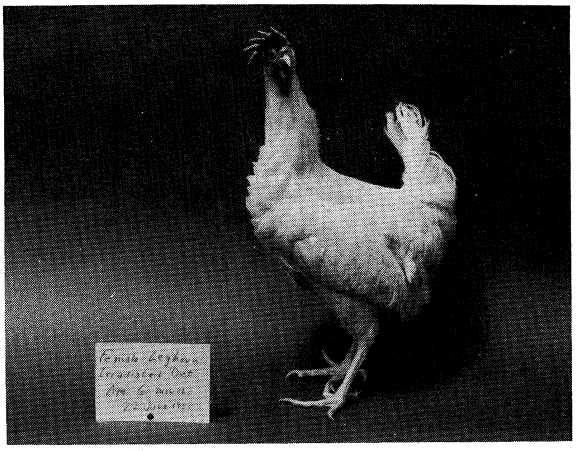

Fig. 9. Typical pullet in experimental group; age 26 weeks.

TABLE II

FEED UTILIZATION IN CHICKEN EXPERIMENT

Group and Period	No. of Weeks	Total Kg Fed to Group	No. of eggs Produced	Total Gain in Weight of Group, kg
Control				
Males and females				
I 21 March-9 May	7	594[1]	---	37.9[4]
II 9 May-23 May	2	168[1]	37	11.0
III 23 May-20 June	4	282[2]	527	4.2
Females only				
IV 20 June-27 June	1	71[2]	163	-0.30[3]
Experimental				
Males and females				
I 21 March-9 May	7	589[1]	---	38.5[5]
II 9 May-23 May	2	163[1]	34	7.4
III 23 May-20 June	4	280[2]	480	5.4
Females only				
IV 20 June-27 June	1	61[2]	175	0.29

[1]Mash consisting of 50% added water.
[2]Mash consisting of 44% added water.
[3]These females lost a total of 0.30 kg.
[4]Efficiency of feed utilization = 0.0638 kg gain/kg fed.
[5]Efficiency of feed utilization = 0.0654 kg gain/kg fed.

mash was reduced and the control animals consumed more, produced more eggs, and showed greater body weight gains. Period III extends to the time at which the food fed to the males in each of the two groups was no longer measured. Feed intake was about the same, but where the controls showed a greater egg production, the experimentals showed a greater body weight increase. Because the males are showing only slight, if any, weight gains and because of the pathological incidents with the males it was felt that feed efficiency data would be meaningless. The last period is only 1 week long at present but will be extended in future reports; it involves the mash consumed by the females only and may be related to egg production and whatever weight gains or losses occur. During this 1 week, the controls consumed more mash but showed a slightly lower egg production and actually lost weight.

It is safe to conclude that there is no longer poorer efficiency of feed utilization which was shown by the experimental groups during the period of rapid growth as reported in the previous report.

3. Male Semen Production.—In preparation for the use of artificial insemination, collections were made three times weekly beginning April 27, before egg production started, for the purpose of preparing the cockerels and to gather data on relative potency of the two groups of birds. For this purpose, an arbitrary score from zero to three was assigned depending on the quantity of semen collected (from none to approximately one fourth of a cc). Table III gives the results of the scores in terms of averages of the 20 control and 19 experimental birds used, and also in terms of the number that had zero scores and those with scores of two to three.

TABLE III

MALE SEMEN PRODUCTION

Date	Average Scores		No. with Zero Score		No. with Score of 2 - 3	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
4 May	1.5	1.0	0	5	7	5
9 May	1.3	0.9	2	4	7	4
11 May	1.6	1.2	1	3	8	5
13 May	1.4	1.0	1	4	6	6
16 May	1.2	0.8	1	9	3	4
18 May	1.6	0.9	2	6	10	4
23 May	1.4	0.8	2	5	7	3
25 May	1.6	1.0	2	5	9	4
27 May	1.6	1.1	2	6	10	6
Average	1.5	1.0	1.5	5.2	7.4	4.6
Standard deviation			±0.7	±1.6	±2.1	±1.0

Data of this sort are subject to a wide variety of influences; much more elaborate experimentation is required to state definitely whether the diet has any effect on semen production. However, from these limited data, it appears that there is a 9:1 probability that the number of birds with zero scores is not due to chance selection of birds, while the corresponding probability for the birds having scores of two to three is about 3:1.

It is also interesting to note that throughout the course of this 3-week period, the semen production of birds did not vary greatly; this is reflected in the constancy of the averages for the two groups of birds. Birds that were poor semen producers at that time still are so, over a month later. As mentioned before, the semen collected to date has come regularly from only 8 control and 5 experimental males.

4. Egg Production, Number of Hens Laying, and Average Egg Weights.— Figure 10 shows the daily percent egg production since it began during the twentieth week of the experiment. Percent egg production is the number of eggs laid each day divided by the total number of birds, including those which have not come into production. The marked daily fluctuation is caused partly by disturbances to pullets which results in delayed laying and partly by not collecting the eggs at exactly the same time at the end of each day. To smooth out the egg production curve, percent egg production was averaged on a weekly basis for each group of pullets and plotted as shown in Fig. 5 along with the growth curve on the same time scale. Here it is apparent that percent egg production by the experimental hens lagged somewhat behind the control pullets during the period of rapid increase in production, but after full production was approached, this difference ceased to exist. The lag in egg production may be related to the slightly lower average body weight of the experimental pullets.

Also shown in Fig. 10 is the number of pullets coming into production each day during the 7-week period shown. The experimental pullets lagged behind the controls slightly as they did in egg production. After 7 weeks, however, all but 4 in each group were laying frequently enough to be put on the insemination schedule, so that with respect to number of pullets laying as well as percent egg production, the slight lag ceased to exist.

Table IV presents egg-weight data in the form of averages for the two groups of pullets for the fifth, tenth, fifteenth, and twentieth eggs laid. It was hoped that by averaging the weights of the eggs of each pullet, regardless of when laid, the fact that the first eggs are usually small would be ruled out as a variable.

TABLE IV

AVERAGE WEIGHT IN GRAMS OF FIFTH, TENTH, FIFTEENTH, AND TWENTIETH EGGS FROM HENS ON THE LONG-TERM CHICKEN EXPERIMENT

Group	5th	10th	15th	20th
Control	43.9 (38)*	45.1 (29)	45.2 (19)	46.9 (19)
Experimental	41.8 (33)	45.6 (27)	46.7 (26)	47.0 (16)

*Numbers in parentheses are the number of eggs averaged.

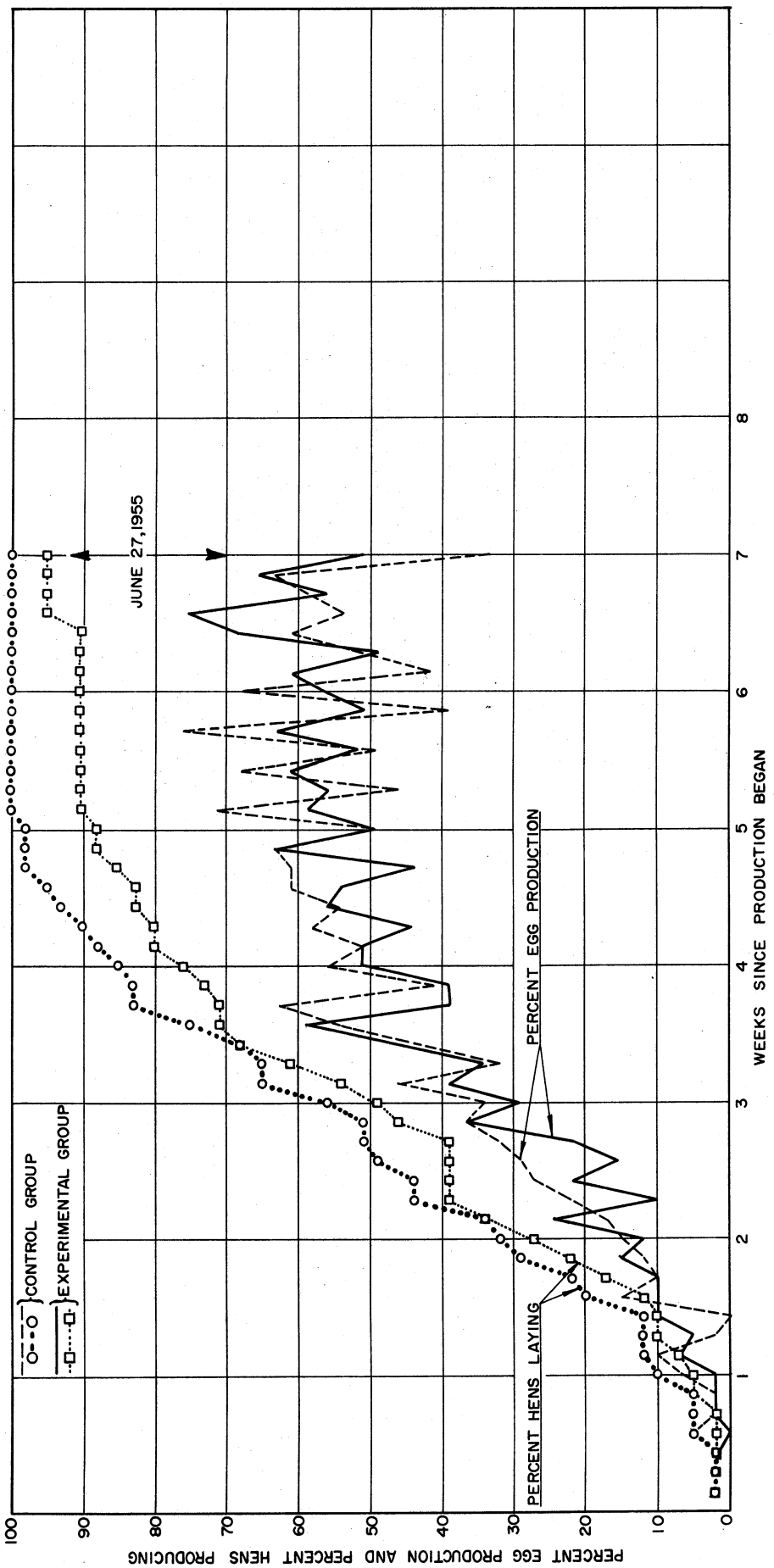

Fig. 10. Curve of percent egg production and percent of pullets in production (having laid at least one egg).

The data show that the differences in egg weight that occur between the two groups correspond to the relative number of eggs averaged, which, in turn, is a reflection of the percent egg production and number of hens laying rather than to any direct effect of the diets fed.

5. Results of Blood-Cell Counts.—Table V shows the results of the blood-cell counts made on 7 male and 14 female birds in each of the two groups. The values are averaged but the ranges shown are the lowest and highest values for the group, not the average of individual ranges. Even though only a third of the total number of birds was used, it is felt that any striking abnormality would be evident and would indicate the desirability of complete blood counts. In addition to the counts made on the blood of the birds during the first series of counts in March, two others, the total red-cell and the thrombocyte counts, were made. The red-cell count, together with the hematocrit and hemoglobin determinations, provides a three-way check on the functioning of the hematopoietic system. The thrombocyte count was of particular interest to investigators who made the count on birds receiving external body irradiation; although feeding an irradiated diet is a much different proposition, there was a possibility that similar effects could be produced in both biological materials.

As in the first series of blood counts, only slight, if any, differences are to be found between the two groups of chickens. The white blood count appears to be slightly elevated although this is not reflected by the range of values. The experimental males, but not the females, show somewhat lower values for polymorphonucleocytes and basophils. Both experimental males and females show lower monocyte and eosinophil counts compared with the corresponding control animals, but they show slightly higher lymphocyte counts. A much larger number of samples would be required before such small differences could be considered real differences. The study of avian hematology is still in its beginning stages and variation in blood-cell counts of the order of those described above cannot as yet be ascribed to specific causes. Large variations have real significance in such diseases as leukemia and anemia, but such small differences as the above, considering the range of values to be found for any determination, cannot be ascribed to effects in the diet.

6. Pathological Observations.—There have been seven pathologic incidents and all these have occurred among the 42 males, none among the 82 females in the experiment. Four males, 3 experimental and 1 control, have been sacrificed and autopsied, and 2 additional experimental and 1 additional control male appear to be afflicted. Table VI-A outlines the gross observations on the 7 birds, while Table VI-B presents the histopathologic report on the 1 bird, Experimental Male No. 167, whose report was available.

The leg weakness is considered to be a symptom of the avian leukosis complex, a nontransmissible group of diseases of genetic origin which seem to attack muscles and nerves. The virus can be transmitted from egg to egg, but

TABLE V

BLOOD-CELL COUNTS OF PARENT-GENERATION CHICKENS ON LONG-TERM EXPERIMENT, JUNE 1955

Group	No. of Animals	Hematocrit, percent	Hemaglobin in Whole Blood, percent	Corpuscular Hemaglobin, percent	Red Blood-Cell Count, millions per cu mm	White Blood-Cell Count, per cu mm	Polymorphonucleocytes	Differential Count % of White Blood-Cell Count			
								Monocytes	Basophils	Eosinophils	Lymphocytes
					Males						
Control	7	37.9 (30.6-41.0)	10.9 (9.8-11.9)	28.7	3.17 (2.74-3.44)	18,784 (9,523-31,818)	20.1 (8-33)	3.8 (0-8)	3.2 (0-6)	1.1 (0-4)	74.8 (59-88)
Experimental	7	36.4* (31.7-45.4)	10.7 (9.1-12.6)	29.4	3.09 (2.59-3.50)	22,439 (9,122-29,058)	15.6 (9-20)	2.9 (1-5)	1.9 (0-4)	0.86 (0-2)	78.6 (73-85)
					Females						
Control	14	29.8 (25.4-71.6)	8.6 (7.6-9.9)	28.8	2.46 (2.07-3.03)	21,644 (11,986-39,878)	27.6 (16-44)	3.3 (0-10)	2.8 (0-8)	1.5 (0-5)	64.4 (45-80)
Experimental	14	28.3 (25.5-31.7)	8.3 (6.8-9.5)	29.3	2.53 (2.05-3.06)	24,800 (10,793-49,492)	25.1 (10-46)	1.9 (0-8)	2.7 (0-16)	0.8 (0-3)	69.2 (51-87)

*Based on 6 animals; one set of samples lost during analysis.

TABLE VI-A

GROSS OBSERVATIONS ON PATHOLOGY
IN THE CHICKEN EXPERIMENT

Group	No.	Gross Observation	Date Noted	Fate
Control	65	Cannot support weight on feet	25 April	Autopsied 9 June
	75	Difficulty in standing, lost 300 grams	30 May	Still living
Experimental	167	Severe leg weakness, loss in weight	4 April	Autopsied 7 April (approx)
	163	Head bent down, tends to squat, not interested in food	7 April	Autopsied 9 June
	157	Severe leg weakness, tends to collapse at ankles	27 April	Autopsied 9 June
	151	Loss over 400 grams, comb shrunken and anemic, no vigor	6 June	Still living
	119	Neck twisted downward	6 June (approx)	Still living

TABLE VI-B

HISTOPATHOLOGICAL REPORT 8069LBG ON EXPERIMENTAL
MALE BIRD NO. 167 SACRIFICED 7 APRIL 1955

Tissue	Observation
Spleen	Congestion. Erythropoietic foci.
Gizzard	Appears normal.
Small intestine	Negative.
Testis	Spermatogenesis reduced.
Heart	Numerous infiltrations of lymphocytes.
Liver	Lymphocyte infiltrations in some of the trinities. No lipoidosis.
Pancreas	Focal infiltrations of lymphocytes.
Lungs	Congestion. Patchy atelectasis. Focal infiltrations of lymphocytes.
Kidney	Focal infiltrations of lymphocytes. No lipoidoisis.

Comment: It is not certain whether the lymphocytic infiltrations are inflammatory or neoplastic. The cells in the vessels are the expected nucleated red blood cells.

so far no cure or more specific etiology is known. The two animals with twisted necks may have obtained them from injuries due to their large combs being caught in the grill through which they reach for food. There is nothing in these results which can be attributed to the diet until further diagnosis is made.

Tissue samples from 3 of the 4 autopsied birds are presently being examined by the histopathologist while the report on the fourth is available; this is shown in Table VI-B. The principal characteristic appears to be lymphocytic infiltration, but it could not be stated whether this was neoplastic or inflammatory.

SECTION II

REPRODUCTION STUDY WITH THE BAGG-STRAIN ALBINO MICE

A. INTRODUCTION

This study, the general plan of which was described in Progress Report No. 2, began in March when the 18 post-weanling Bagg-strain albino female mice were made available through the courtesy of Professor John Allen. These mice were divided into two equal groups and fed the control and experimental diets currently being used in the long-term rat experiment. Ten Bagg-strain albino males from the same colony were placed on commercial laboratory rabbit biscuit, since any effect of either the control or experimental diet on the fertility of the males was to be avoided as much as possible.

In the pilot mouse experiment reviewed in Progress Report No. 1, the reproductive capacity of the first-filial-generation females raised from weanling age on the experimental ration appeared to be affected by the ration. In this experiment, an effort will be made to obtain a greater number of these second-filial-generation females than the 6 used in the pilot experiment. This requires that the original parent stock be bred two or more times to provide a sufficient number of first-filial-generation females. As of the present time, only the first crop of such females has been obtained, but 5-week data on their growth since weaning are available. Also available is a summary of the reproductive performance of the parents in producing the first crop of offspring.

B. MANAGEMENT

1. Housing.—The animals were originally housed in wooden cages with solid bottoms, food being pressed into the wire mesh that formed the front and

top of the cage. Later plastic cages were substituted; these could be kept cleaner, they did not absorb moisture, they provided greater protection to the mice from drafts, and they offered a view of the mice from any point. Figure 11 shows several of the cages as arranged on a rack and Fig. 12 shows an individual cage. The food is placed in an aluminum hopper hung from the wire screen which forms the lid to the cage. A ladder is provided for weanling mice. It has been found that putting the food in hoppers such as these retards the drying out of food and keeps it out of the litter.

2. Diet.—The composition of the diet fed in this experiment is the same as that currently being fed in the long-term rat experiment. It differs from the diet used in the pilot mouse experiment in the essential aspect that the cod-liver oil content has been reduced from 5 to 1%. Because of even this lower level of cod-liver oil, vitamin E cannot be added to the diet but must be given orally. This is done weekly to all mice: the females receive one drop of a solution consisting of 2% alpha-tocopherol acetate in corn oil; the males receive one drop of 0.2% alpha-tocopherol acetate in corn oil. The diet is fed three times daily because even in the aluminum hoppers it dries out quickly and the mice will not consume the dried and caked food.

In order to minimize the possibility that male sterility plays a role in the reproductive capacity of the females, the males are not divided into two groups but are all fed a single diet consisting of a commercial laboratory chow. When the males are at a preweaning age but old enough to eat the maternal diet and when later they are being mated to the females, they consume the same diet as the females, but it is hoped that whatever effect the experimental and control diets have will be minimized.

3. Mating and Management of Litters.—The procedure for breeding the parent female mice is similar to that used in the rat experiment. Each female is mated for 1 week with a male; then all the males are shifted to another female. The females are allowed to be with males until their weekly weighing reveals an increase in weight of about 2 grams, which indicates pregnancy. When pregnant, they are kept in an individual cage. The date of birth and number present in their litters are recorded, and the young are kept with their mothers for 21 days. At weaning, they are divided on the basis of sex, the females being put on the experimental ration and the males on the laboratory chow. Weight records are then maintained on the individual mice each week.

C. EXPERIMENTAL RESULTS

1. Reproduction of Parents.—Table VII is a summary of the reproductive performance of the parent-generation females in producing the first crop of first-filial-generation animals. The number of females bred in each group was 8 rather than the original 9 because 1 female in each group was found to be pregnant when received.

Fig. 11. View of the arrangement of cages in mouse reproduction experiment.

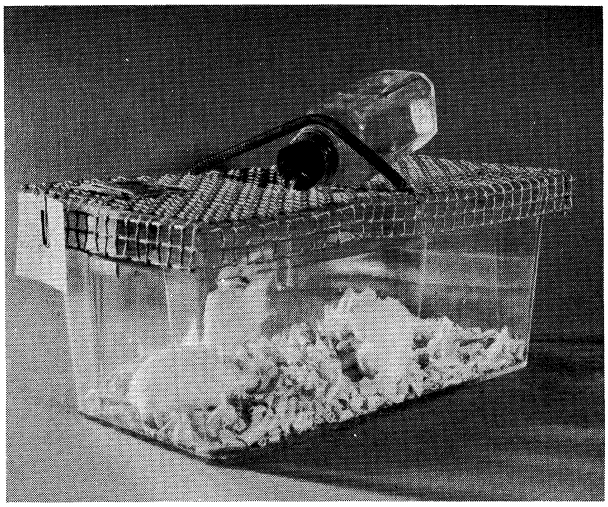

Fig. 12. View of an individual mouse cage showing 1 male with 2 females for breeding.

TABLE VII

DATA ON THE FIRST BREEDING OF THE PARENT-GENERATION MICE

	Control	Experimental
Number of females bred	8	8
Number of males used	5	5
Number of females sterile*	1	0
Number of females conceiving first week	3	4
Number of females conceiving second week	2	2
Number of females conceiving third week	0	1
Number of females conceiving fourth week	1	1
Number of females resorbing fetuses	1	0
Total number of litters born	6	8
Total number of litters born dead**	1	0
Total number of litters born alive not surviving weaning	1	2
Total number of pups born	32	43
Total number of pups born dead	1	3
Percent of pups born dead	3.1	7.0
Number of pups born alive per female bred	3.9	5.0
Total number of pups born alive not surviving weaning	16	18
Average number of pups born alive not surviving weaning	3.2	2.5
Average number of pups born per litter	5.3	5.4
Average number of pups per litter at 5 days	3.0	3.1
Total number of young reaching weaning	15	22
Average number of pups per litter at 21 days	2.5	2.8
Average weaning weight in grams	6.4	6.6
Average number of young weaned per female bred	1.9	2.8
Percent of pups born alive which survived 21 days	48.4	55.0

*Mated four times unsuccessfully, each time to a proven male.

**Only one animal born, and it was a deformed embryo.

Although the difference in the performance of the two groups of mice is probably not significant statistically, the performance seems to have been slightly better on the part of the experimental animals. In the previous pilot experiment, the females were placed on the experimental diets at the time they were mated, whereas in this experiment they were put on the experimental diets about 4 weeks before being bred. If failure to reproduce in the pilot experiment bore some relation to the length of time the females were on the experimental diet, then it is not entirely unreasonable to expect that the diet may have some effect on this first breeding.

2. Growth of First-Filial-Generation Females.—Table VIII shows the average weights of the 9 control and 12 experimental first-filial-generation female mice. The weaning weight is the average weight of the animals in each litter; the other weights are actually interpolated weights, inasmuch as the animals were not of the same age, i.e., the weights shown for the seventh, fourteenth, etc., days are determined by straight-line interpolation between the weights which are always determined on the same day of the week rather than on the seventh, fourteenth, etc., days of age for each mouse. This procedure is used in the rat experiment and has been checked to find that it gives a close approximation of the actual weight for the day for which the weight was interpolated.

TABLE VIII

AVERAGE (INTERPOLATED) WEIGHT IN GRAMS OF FIRST-FILIAL-GENERATION MICE

Group	No. of Mice	Days Since Weaning				
		0	7	14	21	28
Control	9	7.1	9.0	11.7	13.0	14.2
Experimental	12	6.2	8.1	10.9	12.4	13.8

The slight lag in the average weights of the experimental mice appears to decrease percentagewise with time, suggesting that the lag is due primarily to the fact that the experimental females were smaller as weanlings.

SECTION III

THE LONG-TERM RAT FEEDING AND BREEDING EXPERIMENT

The long-term rat feeding and breeding experiment, whose progress is reported below, is supported entirely by Michigan Memorial-Phoenix Project No. 41. Because of its possible interest to the Office of the Surgeon General and to others participating in the study of the irradiation of foods, its inclusion in this report is considered warranted.

A. INTRODUCTION

The long-term rat experiment is now in its eighteenth month and the first crop of fourth-generation or third-filial-generation animals is being weaned from the parents. A report complete in all except the histopathological examination of the autopsied members was reported for the first-filial-generation animals in Progress Report No. 2. In this report the histopathologic data are presented. A similar report on the second-filial-generation animals will not be available until the next report when the second crop of third-filial-generation animals will have been weaned. Except for the data on the second filial generation, the present section of this report will be devoted to the status of the original parent-generation animals, which are nearly 18 months old.

B. PATHOLOGICAL REPORT OF THE FIRST-FILIAL-GENERATION ANIMALS

After the animals of the first filial generation had given birth to their second crop of young and these had been weaned, the parents were all sacrificed and autopsied. Sections of the vital organs of each of the 40 females and 24 males were preserved. The tissues from 3 males and 6 females in each of the two groups were sent to the histopathologist for examination. Because the animals were only about 6 (?) months old when autopsied and there had been no evidence during reproduction, by gross examination, or from blood-cell determinations that the experimental animals suffered from any abnormality, only this fraction of the total animals was examined histopathologically. The reports turned out to be brief and repetitious and were summarized as shown in Table IX. The 18 animals are listed by experimental group, and the pathologist's comments on each of the tissues listed across the top is given in the boxes.

Careful examination of the chart shows that none of the positive pathological indications predominates in any one group. Many of the observations made are directly attributable to the high fat content of the diet, viz., abundant quantities of lipids in the adrenal cortex diffuse fatty infiltration in the liver, although no lipoidosis was apparent. Congestion was noted in about half the samples of spleens from each of the four groups. The samples of kidney tissue appeared almost completely devoid of lipoidosis in all four groups. Abundant mucin was noted in about half the samples of small intestine from each of the four groups. Most of the reports on the uterus were negative, while the observations on the testes were obscured by post-mortem change. Nearly all the lung tissue samples, regardless of origin, showed patchy atelectasis, emphysema, and varying degrees of dilatation of the bronchi.

These animals were young when sacrificed (males, about 21 weeks and

TABLE IX

HISTOPATHOLOGICAL REPORT ON THE VITAL ORGANS OF A PORTION OF THE FIRST-FILIAL-GENERATION RATS FROM THE LONG-TERM RAT FEEDING EXPERIMENT

The histopathological report on 3 of the original 12 animals in each of the two male groups and on 6 of the original 20 animals in each of the two female groups is shown. The tissues of the unexamined animals are available for histopathological study.

Key to Abbreviations

c.l.	cortical lipids	d.f.i.	degenerative fatty infiltration
e.	emphysema	p.m.c.	post-mortem change
l.h.	lymphoid hyperplasia	atel.	atelectasis
d.b.	dilation of bronchi	sper.	spermatogenesis

Group	Rat No.	TISSUE							
		Adrenal	Heart	Liver	Spleen	Kidney	Small Intestine	Uterus or Testis	Lung
					MALES				
Experimental	D♂ 6	c.l. moderate amount	No lipoidosis	No lipoidosis	l.h.	No lipoidosis	Abundant lymphoid tissue	p.m.c. active sper.	Patchy e. and atel., bronchiectasic, no fat emboli
	D 26	c.l. abundant amount	No lipoidosis	Well-marked d.f.i.	Congestion	No lipoidosis	Negative	No report	Slight patchy atel., occasional perivascular lymphocytic foci
	D 54	c.l. abundant amount	No lipoidosis	Patchy d.f.i.	Negative	Moderate congestion	Abundant mucin	p.m.c. active sper.	Patchy atel. and patchy lymphocytic foci
Control	A♂ 13	c.l. moderate amount	No lipoidosis	Well-marked d.f.i.	Congestion	No lipoidosis, congestion	p.m.c.	p.m.c.	Patchy atel. and e., no fat emboli
	A 15	c.l. abundant amount	No lipoidosis	Abundant c.l.	Congestion	No lipoidosis	Negative	p.m.c. active sper.	Patchy e. and atel., dilated bronchi, lymphatic foci about bronchi
	A 47	c.l. scant amount	No lipoidosis	No lipoidosis	Negative	No lipoidosis	Abundant mucin	p.m.c.	Patchy atel., moderate dilation of bronchi
					FEMALES				
Experimental	D♀ 18	c.l. abundant amount	No lipoidosis	No lipoidosis	Congestion, numerous pigment-containing cells	No lipoidosis	Negative	No report	Patchy atel. and e., dilation of bronchi
	D 28	c.l. moderate amount	No lipoidosis	No significant lipoidosis	Negative	Negative	Abundant mucin	Negative	Patchy atel. and e., moderate dilation of bronchi
	D 36	Negative	No lipoidosis	No lipoidosis	Congestion	No lipoidosis	Abundant lymphoid tissue	Negative	Patchy atel., d.f.i.
	D 48	c.l. abundant amount	No lipoidosis	No significant lipoidosis	Negative	No lipoidosis	Abundant mucin	Focal leukocytic infiltration in the endometrium	Patchy atel.
	D 58	c.l. abundant	No lipoidosis	Slight d.f.i.	Congestion	No lipoidosis	Negative	Ovary and fallopian tube negative	No fat emboli, patchy e. and atel.
	D 62	c.l. abundant	No lipoidosis	Slight d.f.i.	Congestion	Negative	Negative	Ovary and fallopian tube negative	Marked congestion
Control	A♀ 11	c.l. normal amount	Negative	No lipoidosis	Congestion	No lipoidosis	p.m.c.	Negative	Patchy e. and atel.
	A* 25	No report	No lipoidosis	Well-marked d.f.i.	Congestion numerous pigment-containing phagocytes	Well-marked d.f.i.	Negative	No report	Patchy e. and atel., bone marrow, giant cell emboli
	A 27	c.l. abundant	Patchy d.f.i.	Slight d.f.i.	Negative	Moderate congestion	Abundant mucin	Normal	Patchy e. and atel., no fat emboli
	A 29	c.l. abundant	No lipoidosis	Well-marked d.f.i.	Numerous pigment containing phagocytes l.h.	No lipoidosis	Abundant mucin	No report	Patchy e. and atel.
	A 33	c.l. normal amount	No lipoidosis	No lipoidosis	Negative	No report	Abundant mucin	Negative	Patchy atel., focal lymphocytic infiltration, moderate d.b.
	A** 51	c.l. abundant amount	No lipoidosis	Minimal d.f.i.	Negative	No lipoidosis	Abundant mucin	No report	Patchy atel.

*Esophagus, moderate desquamation of keratohyalin.

**Esophagus, abundant desquamated keratohyalin.

females, 25 weeks of age) and there had been no gross pathological observations. The histopathological examination summarized above showed no other effect attributable to gamma irradiation of the diet consumed.

C. PATHOLOGICAL STATUS OF THE PARENT-GENERATION RATS

1. Average Body Weights.—The average body weights of the parent animals have continued to be determined weekly and have increased slowly since the last growth curve was presented. Because 14 animals have either died or been sacrificed since then, and because of the variable effect of varying degrees of respiratory infection on average weights, insufficient evidence is attached to the average weights to warrant presentation of growth curves. In Table X, however, is presented a brief summary of average body weights; they are presented for the forty-ninth week of the experiment, where the growth curve shown in Progress Report No. 1 terminated; for the sixty-first week, as reported in Progress Report No. 2; and for the seventy-fifth week.

TABLE X

AVERAGE BODY WEIGHTS IN GRAMS OF THE PARENT-GENERATION ANIMALS

Group	Week of Experiment		
	49th	61st	75th
Control male	*(30) 612	(28) 656	(26) 703
Experimental male	(29) 587	(27) 633	(27) 656
Control female	(31) 340	(29) 373	(27) 413
Experimental female	(31) 335	(28) 362	(27) 404

*Number of animals are in parentheses.

It is evident that, in spite of animals lost from the experiment (which were 4 in number from each group except control males from which 5 were lost) and in spite of the presence of at least mild disease in half the animals, the relative position of the four groups of animals with respect to average body weights has not changed.

2. Blood-Cell Counts on Animals from the Parent Generation.—In March, 1955, a second series of the complete blood-cell counts was made on representatives of the parent generation of animals. The first series of counts was made in July, 1954, on 27 individual animals and was reported in Progress Report No. 1. No differences were found among the arithmetic averages of each of the four groups for percent hemoglobin, percent hematocrit, percent reticulo-

cytes, the white blood count, the eosinophil count, or the percent lymphocytes and polymorphonuclear leukicytes in the differential count. The second series was extended to 30 animals (7 from each of the two male groups, 8 from each of the female groups) and, in addition to the above determinations, the differential count was extended to include basophils, monocytes, and the segmented form of polymorphonucleocytes. The counts were performed by Mrs. J. A. S. Wilson, an experienced mammalian hematologist, who was employed as a hematologist at Simpson Memorial Institute for Blood Diseases at the University of Michigan. The authors are grateful to Dr. F. H. Bethell for use of the hematologic facilities at the A.E.C. Laboratory for Biologic Effects of Radiation.

The results in terms of arithmetic averages for each of the above tests for each of the four groups of animals are given in Table XI. The range of values for each average value is the lowest and highest values for the group as a whole, rather than the average values for the individual ranges. The table reveals only trivial differences among the groups for every type of blood cell counted. The values for percent hemoglobin and percent hematocrit are the same as those shown in Progress Report No. 1; the white counts reported here are slightly lower as are also the eosinophils and lymphocytes. The values for the polymorphonucleocytes, however, are higher than those found in July, 1954.

3. Current Gross Pathological Observations.—Because of the increasing occurrence of various degrees of respiratory infection, tumors, and other diseases among the parent-generation animals during the past few months, a systematic check is being made weekly of each of these animals. Every animal is examined individually for any gross external abnormalities, and an effort is made to detect internal tumors. The degree of respiratory infection, if present, is rated mild or slight if it is confined to sniffling and a rattly throat, moderate if the rattling in the chest can be felt, and severe if there is visible heaving of sides and loss of weight.

With these data, a record is kept which is shown in graphical form in Fig. 13. The four groups of parent rats are shown in separate graphs. Each bar in each graph represents the fate of the original 31 animals in each group as it stands on the particular week examined. The data were presented in this way because all pathological occurrences except deaths and malignant tumors varied in incidence and because this form of presentation permitted a rapid means of accounting for all the animals originally on the experiment.

Among the males the number of pathological incidents occurs among over half of the animals, mostly consisting of mild respiratory infection. There appears to be little difference between the control and irradiated males as to distribution of diseases except in the case of tumors; they have the same amount of respiratory infection and unclassified diseases (infections, abscesses, weight losses), although 4 males on the experimental diet have had tumors to 1 male on the control diet. Among the females, those on the control

TABLE XI

BLOOD-CELL COUNTS ON REPRESENTATIVE PARENT RATS ON LONG-TERM EXPERIMENT, MARCH 1955

Group	No. of Animals	Hemoglobin, percent	Hematocrit, percent	Reticulocytes, percent of red cell count	White Blood Cells, per cu mm	Eosinophils, per cu mm	Differential Count % of White Blood-Cell Count					
							Lymphocytes	Polymorphonucleocytes	Monocytes	Eosinophils	Stabs***	Basophils
Males												
Control	7	15.2* (14.3-16.4)	.494 (.484-.506)	3.9 (2.0-4.8)	8,470 (6,600-10,500)	123.7 (44.4-210.9)	72 (63-80)	24 (18-33)	1 (0-3)	3 (2-6)	.4 (0-1)	.1 (0-1)
Experimental	7	15.1 (13.6-17.1)	.488 (.458-.511)	3.8 (1.4-5.4)	9,220 (7,550-14,200)	100.8 (33.3-233.1)	65 (57-74)	30 (24-35)	.7 (0-3)	3 (1-6)	1 (0-3)	.7 (0-4)
Females												
Control	8	15.1 (14.2-16.1)	.472 (.441-.519)	3.8 (2.6-5.6)	5,910 (3,750-9,300)	65.2 (11.1-99.9)	63 (54-72)	30 (22-39)	2 (0-8)	3 (1-6)	2 (0-7)	.2 (0-1)
Experimental	8	14.6 (13.6-15.4)	.476 (.429-.524)	4.5 (2.7-6.4)	6,050 (3,350-7,850)	61.8** (33.3-122.1)	66 (59-73)	28 (19-35)	1 (0-3)	3 (1-5)	1 (0-4)	0 (0-0)

*Single number is average value for the animals in the group; the numbers in parentheses are the lowest and the highest values among the animals in the group.

**Data for 7 rather than 8 animals.

***Nonsegmented form of polymorphonucleocytes.

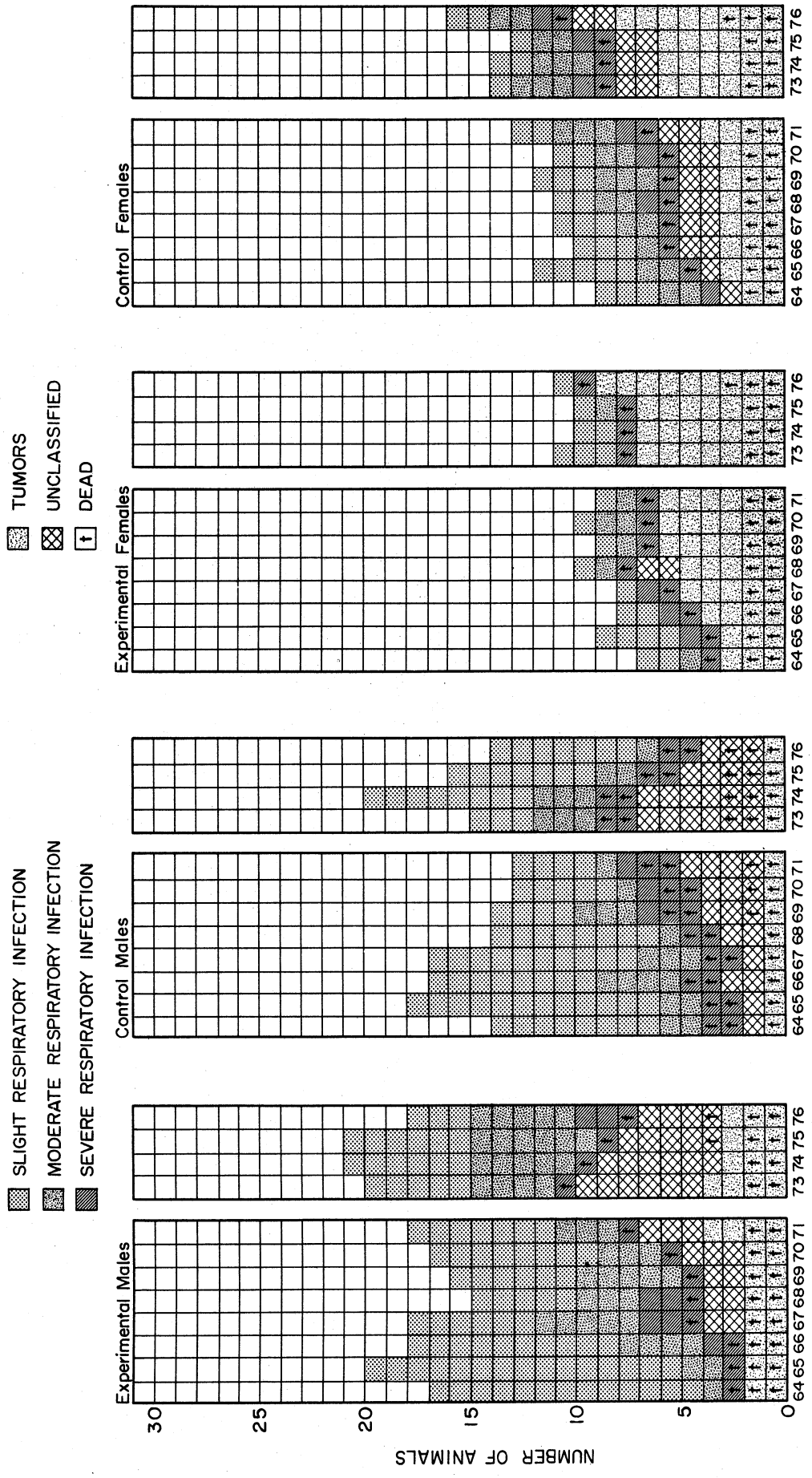

Fig. 13. Pathologic status each week of parent-generation rats on the long-term feeding experiment.

diet show a greater number of pathological incidents than those on the irradiated diet, this increase being in the greater number of cases of respiratory infection and unclassified disease. Compared to the males, the females as a group show a greater number of tumors but a much fewer number of cases of respiratory infection and unclassified disease.

In regard to the incidence of tumors, it was of interest to plot their occurrence among each of the four groups over the entire duration of the experiment to date. Figure 14 shows such a graph, in which tumors occurring among the different groups are indicated by different symbols. Beginning at about 14 months, the incidence of tumors among the females begins and increases rather abruptly, their being eight cases among the experimental females and seven among the control females. The nature of this sudden increase would indicate that tumor formation may be related to a fundamental change which occurs normally in rats of this age. Most of the tumors have been mammary tumors, and two very recent cases have been tumors of the uterus.

Four experimental male rats and 1 control male rat have produced tumors, but these appear to occur randomly. This 4:1 occurrence cannot be considered significant at the present time, but careful attention will be given this feature.

4. Summary of the Pathology of the Parent Animals Lost from the Experiment Since Its Beginning.—Since the last report, the histopathological reports of 9 parent-generation rats which died or were sacrificed have become available. This brings to 17 the total of parent rats lost from the experiment. In order to evaluate the relative standing of the four groups of rats, these nine reports are shown in Table XII together with references to the 8 other animals which have died or been sacrificed.

Of the 4 males whose pathology report is shown, 3 were sacrificed because of acute respiratory infection; the evidence of bronchitis and pneumonia in the lung confirms this. The fourth one, a control male, died suddenly; autopsy revealed the lung tissue to be very congested with blood.

Of the 5 females whose pathology report is shown, 3 were sacrificed because of tumors. The pathologist's report stated that each of these was an adenofibroma of the mammary gland, while one in addition was adenocarcinoma. Two of these cases occurred among experimental females, while the other was that of a control female. One control female had an abscess on the neck which may have started as an infection of the middle ear. A third control female died suddenly without apparent cause. The post-mortem changes were extensive and obscured the pathological diagnosis.

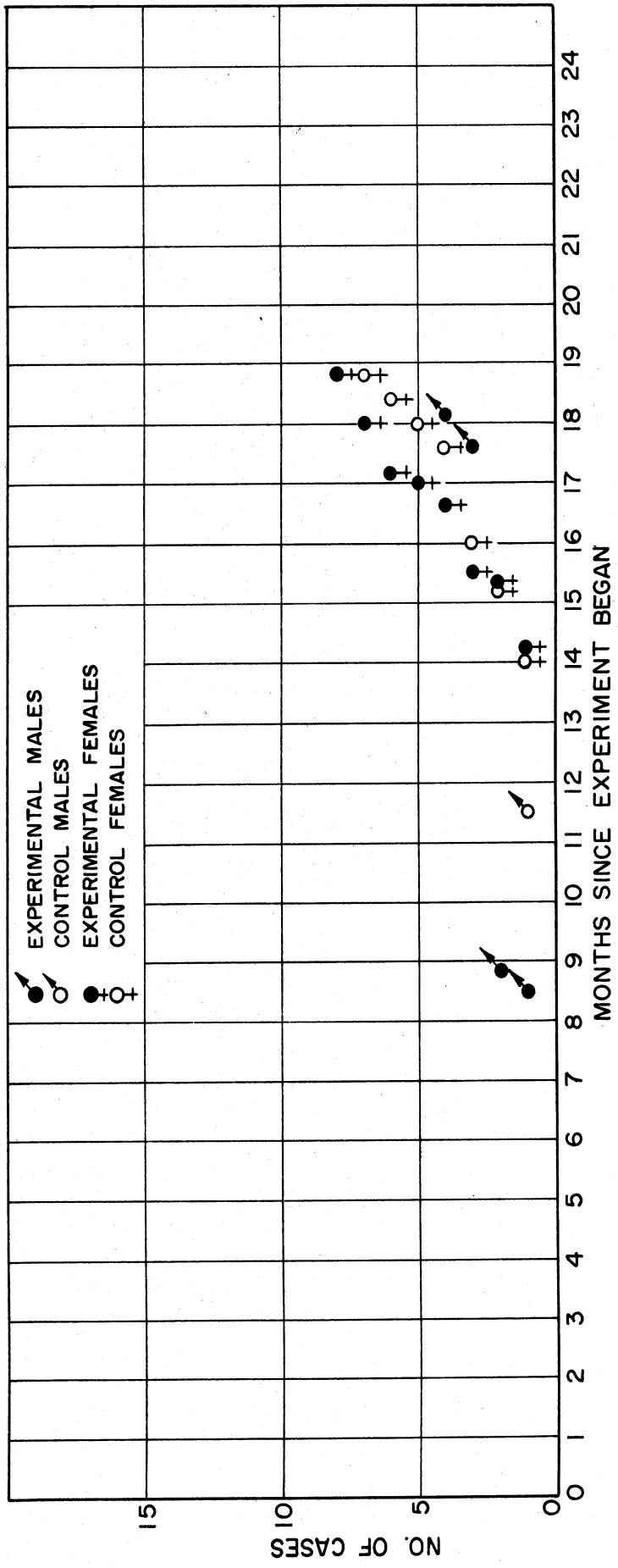

Fig. 14. Graph showing the incidence of tumors in the parent-generation rats from the beginning of the experiment.

TABLE XII

PATHOLOGY RECORD OF 17 PARENT-GENERATION RATS
TAKEN FROM EXPERIMENT SINCE ITS BEGINNING JANUARY 13, 1954

Group	Animal and Report No.	Observation and Date Noted	Histopathological Report	
			Tissue	Observation
			MALES	
Experimental	$2R_3$	Lateral tumor 16 Aug 54	(See Progress Report No. 1, p. 37)	
	$4R_1$	Scrotal tumor 23 Aug 54	(See Progress Report No. 1, p. 39)	
	$6R_3$ 8066LBG	Acute respiratory infection 23 Feb 55	Heart	Patchy degenerative fatty infiltration.
			Kidney	Congestion. Hyaline casts in a few tubules. Well-marked degenerative fatty infiltration of tubular epithelium.
			Lung	Patchy emphysema and atelectasis. Acute purulent bronchitis and lobular pneumonia. Bronchiectasis. Metaplasia of bronchial mucosa to squamous cell type. No fat emboli. Severe pulmonary infection.
			Testis	Advanced post-mortem change. Spermatogenesis active.
			Spleen	Congestion. Numerous pigment-containing cells.
			Small intestine	Post-mortem change.
			Liver	Congestion. Well marked degenerative fatty infiltration.
			Adrenals	Cortical lipids in normal amount.
			Comment:	The degenerative fatty infiltration of liver, kidneys and heart may well be due to toxin as a result of the pneumonia. The metaplasia of the bronchial epithelium may have as a possible cause avitaminosis.
	$2R_2$	Severe weight loss followed by death 15 June 55	(Pathologist's report not yet available)	
Control	7_0	Lateral tumor 15 Nov 54	(See Progress Report No. 2, p. 21)	
	$3L_1$ 8071LBG	Acute respiratory infection 22 Feb 55	Heart	No lipoidosis.
			Kidneys	Post-mortem change. No lipoidosis.
			Lung	Acute purulent bronchitis and lobular pneumonia. Patchy emphysema and atelectasis.
			Testis	Post-mortem change. Active spermatogenesis.
			Spleen	Congestion. In addition to the brown pigment in phagocytes, there is coal black pigment scattered throughout.
			Small intestine	Post-mortem change.
			Liver	Well-marked degenerative fatty infiltration.
			Stomach	Post-mortem change.
			Comment:	The pneumonia is severe enough to be the cause of death. The degenerative fatty infiltration of the liver is probably the effect of toxin from the pneumonia.
	$1R_2L_1$ 8073LBG	Acute respiratory infection 22 Feb 55	Heart	Patchy degenerative fatty infiltration.
			Kidney	Intense acute passive congestion. Well-marked degenerative fatty infiltration. Numerous hyaline casts.
			Lung	Bronchiectasis. Bronchiectatic abscesses. Acute purulent bronchitis and severe purulent lobular pneumonia. No fat emboli.
			Testes	Active spermatogenesis.
			Spleen	Congestion. Numerous pigment-containing cells.
			Liver	Well-marked degenerative fatty infiltration. Congestion.
			Adrenal	Negative.
			Aorta	Negative.
	$5R_2$ 8072LBG	Died suddenly 30 April 55	Heart	No lipoidosis.
			Kidney	Marked congestion. No lipoidosis.
			Lung	Intense acute passive congestion. Patchy emphysema and atelectasis. No fat emboli.
			Spleen	Marked congestion. Numerous pigment-containing cells.
			Small intestine	Advanced post-mortem change.
			Liver	Congestion. No lipoidosis.
			Adrenal	Cortical lipids abundant.
	$1R_1L_3$	Severe loss of weight 9 June 55	(Pathologist's report not yet available)	

TABLE XII (concluded)

Group	Animal and Report No.	Observation and Date Noted	Histopathological Report	
			Tissue	Observation
		FEMALES		
Experimental	$6R_1L_1$	Respiratory infection 19 Jan 55	(See Progress Report No. 2, p. 23)	
	$4R_2L_3$ 8068LBG	Series of at least 3 mammary tumors 9 Feb 55	Heart	No lipoidosis.
			Kidney	Congestion. No lipoidosis.
			Lung	Patchy emphysema and atelectasis.
			Uterus	Negative.
			Spleen	Congestion. Foci of erythropoiesis.
			Small intestine	Post-mortem change.
			Liver	Congestion. No lipoidosis.
			Adrenal	Foci of lymphocytes.
			Mammary tumor	A large tumor mass consisting in part of an adenofibroma. There is also extensive infiltration of a poorly differentiated adenocarcinoma. Extensive necrosis of the carcinoma.
			Bone	After decalcification: bone marrow about 50% cellular. Spinal ganglia are included with this specimen.
	$4R_1L_3$ 8067LBG	Lateral tumor 23 March 55	Heart	No lipoidosis.
			Kidney	Congestion. No lipoidosis.
			Lung	Patchy atelectasis and emphysema. Acute purulent bronchitis and confluent lobular pneumonia. Severe process. Marked degree of bronchiectasis. No fat emboli.
			Uterus	Negative.
			Liver	Congestion. Well-marked degenerative fatty infiltration.
			Adrenal	Lipid in moderate amount.
			Mammary gland	Adenofibroma.
			Esophagus	Abundant desquamating keratohyalin.
			Comment: The pneumonia is severe enough to cause death. The hepatic lipoidosis may be the result of the toxin from the pneumonia.	
	$2R_2$	Large uteral tumor 28 June 55	(Pathologist's report not yet available)	
Control	$3R_1L_1$ 8070LBG	Mammary tumor 2 Feb 55	Heart	No lipoidosis.
			Kidney	A few hyaline casts. Congestion. A few small infiltrations of lymphocytes.
			Lung	Patchy emphysema and atelectasis.
			Uterus	Negative.
			Spleen	Intense acute passive congestion. Numerous pigment-laden cells.
			Small intestine	Post-mortem change.
			Liver	Congestion. Minimal degenerative fatty infiltration.
			Adrenals	Small cortical adenoma. Moderate cortical lipids.
			Tumor	The tumor is a large lobulated mass of adenofibroma of the mammary gland. No malignancy.
			Bone	After decalcification: bone marrow about 50% cellular.
	$1R_1$ 8065LBG	Sudden, severe weight loss followed by death 9 April 55	Heart	Post-mortem change. No lipoidosis.
			Kidney	Advanced post-mortem change. Well-marked degenerative fatty infiltration.
			Lung	Post-mortem change. Patchy emphysema and atelectasis. Some increase in leukocytes in the blood stream. Bone marrow giant cell emboli. No fat emboli.
			Liver	Advanced post-mortem change. Widespread infiltrations of neoplastic cells in relation to the hepatic trinities. Reticulum cell sarcoma. Slight fatty and well-marked degenerative fatty infiltration.
	$1L_2$ 8074LBG	Boil on neck 10 March 55	Heart	Slight patchy degenerative fatty infiltration.
			Kidneys	Negative.
			Lung	Patchy emphysema and atelectasis.
			Uterus	Negative.
			Spleen	Enormous numbers of cells filled with brown pigment. Scattered coal black pigment.
			Small intestine	Negative.
			Liver	Congestion. Slight degenerative fatty infiltration. The stellate cells of Kupffer contain abundant lipid.
			Adrenal	Abundant cortical lipids.
			Comment: This is the first rat in this group to show lipid, as a foreign material, in the Kupffer cells.	
	1L	Large uteral tumor 26 June 55	(Pathologist's report not yet available)	

SECTION IV

FUTURE PROGRAM

A. LONG-TERM CHICKEN EXPERIMENT

Facilities are available at the Fission Products Laboratory for incubating all the eggs from the pullets now on the experiment. The pullets will be inseminated with semen pooled from both control and experimental males in order to establish female fertility. This will make it possible to determine male fertility by inseminating proven females with semen from individual cockerels.

Facilities are available for raising 50 chicks per week to 4 weeks of age, or approximately 1 out of 5 chicks hatched assuming 60% egg production and an 80% hatch. This will provide some data on survival and carry-over effects from the maternal diet. It is of interest to raise a first filial generation, especially with respect to males. It is important to repeat the experiment with respect to semen production, and this will be done with first-filial-generation males. As they become larger and demand additional space, the parent males whose semen production was poor can be sacrificed.

With respect to females, there is no effect of irradiation of the diet which requires repeating other than the difference in growth rate and average body weight even after the period of rapid growth has been passed. To repeat this would require space which can be obtained only by replacing the present hen batteries by triple-deck units or by sacrificing some of the present pullets. The fact that nearly all the pullets are good layers makes the latter alternative undesirable. By virtue of this good performance, an excellent basis is available for determining the effect of continued feeding of the gamma-irradiated diet on egg production and its seasonal variations.

B. THE MOUSE REPRODUCTION STUDY

In the previous pilot experiment, it was observed that first-filial-generation females born from parents put on the experimental diet at the time of breeding were not able to reproduce. If the effect was due to infertility of the first-filial-generation females, then this could have been caused by having been raised on the experimental diet or from being the offspring of parents which were fed the experimental diet at least for a short period. These two effects will be tested separately by starting to breed the first-filial-generation females while breeding of the parents is continued. If the effect on reproduction is real and bears some relation to the length of time

the animals, regardless of which generation they belong to, are fed the experimental diet, the parent mice as well as the first-filial-generation females should show reproduction failure.

There is reason to believe that the experimental diet may cause sterility in male Bagg-strain albino mice. When a sufficient number of first-filial-generation male mice become available, they will be bred to females of proven fertility. The males will have been raised from weanlings on a laboratory diet known to be nutritionally adequate for mice; one third of the mice will be continued on this ration while being mated to females; another third will be given the control diet (unirradiated rat diet), while the other third will be fed the experimental diet (irradiated rat diet). The reason for two control groups rather than one is the suspicion that the unirradiated rat diet may affect the fertility of the male mice.

C. LONG-TERM RAT EXPERIMENT

The long-term rat experiment represents an investment at the present time of considerable magnitude, and every effort will be made to make the most of it. Because of the just sufficient numbers of animals for proper statistical evaluation, animals have not been sacrificed when the first signs of a disability arise and when it might best be possible for a pathologist to determine the primary effect, if any, of irradiation of the diet. However, should this experiment be terminated at the end of this calendar year, when support from the Michigan Memorial-Phoenix fund will be terminated, all the animals will be subjected to extensive histopathological and other examinations.

However, these animals, because they are under a degree of nutritional stress as well as being old and therefore more vulnerable to dietary defects, are becoming increasingly valuable for the purpose of the experiment. Despite the fact that 14% of the original number of animals have died or been sacrificed and that the tumor incidence among females has shown a steady increase over the past 2 or 3 months, it is expected that the parents will survive the 2-year period. Longevity data, or the effect of diet on survival, were one of the original purposes of the experiment and this will not be available if support for the experiment is not continued after the end of the year. Although the number of generations required to detect dietary effects on reproduction is not established, it should be desirable to continue the reproduction phase of the experiment beyond the fourth filial generation which is expected by the end of the year.

There appears to be need for a long-term rat feeding experiment in which the wholesomeness of irradiated whole wheat grain is to be determined. Previous experiments have shown that insect infestations of wheat grain, which cause losses of hundreds of millions of dollars annually in this country, can

be halted by gamma irradiation of only 10,000 rep. Not only is this process feasible from an engineering and hence economic standpoint, but there is every reason to believe that, because of the low moisture content of wheat (10 to 12%), the effect of irradiation on wholesomeness should be at a minimum. However, the oil of the wheat germ may be particularly vulnerable, and for this reason it is desirable to test by animal feeding experiments whether such effects are unwholesome.