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WHOLESOMENESS OF A GAMMA-IRRADIATED DIET FED TO CHICKENS

Period July 1, 1955 to September 30, 1955

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ABSTRACT

This report covers the progress from July 1 to September 30, 1955, of the four experiments on animal feeding and reproduction being conducted at The University of Michigan Fission Products Laboratory. These are (1) the long-term chicken experiment and (2) the mouse reproduction study, both supported by the Office of the Surgeon General of the U.S. Army; (3) the long-term rat experiment and (4) the wheat-feeding rat experiment, both supported by Michigan Memorial-Phoenix Project No. 41.

The average body weights of the male birds in the chicken experiment show slight increases but no significant difference between control and experimental groups. While both groups of pullets show average body-weight increases of 9%, the control pullets still have a 3% greater weight than the experimental pullets. The amount of mash consumed, the weight gain, and the number of eggs produced reveal no difference between the control and experimental pullets with respect to efficiency of feed utilization. The results of thrombocyte counts made on the blood of 7 males and 14 females on each diet were negative with respect to the effect of gamma-ray sterilization of the diet. About the same number of control as of experimental roosters have died or have been sacrificed. No control and only one experimental pullet has been found to be infertile. No male from either group which has been used in insemination has been proven infertile. Egg production by experimental pullets has been slightly greater than that by control pullets, but hatchability of the eggs from the experimental pullets has been lower. These differences have ceased to occur during the last month of the period reported. One hundred and forty-five first-filial-generation chicks were raised on the control diet and one hundred and forty-four on the irradiated diet with no significant differences in growth rate.

In the mouse reproduction study, a second but smaller crop of first-filial-generation mice has been obtained from the parents. Too few animals were involved, however, to provide reliable data on breeding performance of the parents and on growth rate of the young.

In the long-term rat experiment, the increased mortality in both groups of parents and the decidedly poor breeding performance of the second-filial-generation animals have suggested that the low level of dietary manganese which was previously thought adequate is too low. The level of dietary manganese has been increased, together with the level of vitamin B_{12} and vitamin E. Little significance, however, can be attached to the recent data from this experiment with respect to the effect of gamma irradiation of the diet.

An experiment in which rats have been fed a diet containing 70% of wheat which received 10,000 rep of gamma radiation (dose required to sterilize adult insects and insect eggs in infested wheat) was started. The growth rate of the parent males and of the parent females on the two diets were identical. The first breeding of the animals has just begun.

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.

I. STUDIES SUPPORTED BY ERI PROJECT 2307

A. Chicken Feeding Experiment

1. INTRODUCTION

The long-term chicken feeding and breeding experiment is now in its thirty-ninth week. The parent generation of chickens has come to maturity with little further change in body weight. Egg production has increased from about 60% to its apparent maximum of about 65% since the last report was written. Fertility, embryo mortality, and hatchability have been computed on the basis of data from about 3600 eggs. Two hundred and eighty-nine first-filial-generation chicks have been raised to four weeks of age to provide a further means of checking the wholesomeness of a radiation-sterilized wet mash. Thirty-six males from this group of chicks will be continued on the experiment to provide replacements for some of the parent males. Besides providing a first filial generation to carry to maturity, these replacements will provide an opportunity to check the effect of irradiation of the diet on semen production by the males. A similar replacement program for females is contemplated. There has still been no serious pathological case among the females, but three more control and four more experimental males have died or been sacrificed since the last report. A program of artificial insemination has been in progress for over nine weeks, the goal of which is to mate each pullet with all the males whose semen is available. The data on the thrombocyte count of the blood of representative pullets and roosters are now available.

2. MANAGEMENT

a. Changes in Diet and Housing of Roosters.—The enlarged rooster cages mentioned in Progress Report No. 3 have been installed, and a view of the new arrangement is shown in Fig. 1. Injuries to the males have been lessened, and the additional head room is also making it possible to reduce the size of the combs of the roosters by clipping without continued injury to the wound while it is healing. Five males in each of the two groups have had their combs clipped or "dubbed" to date, and this has been accomplished without any effect on the weight or semen production of these birds. The remaining roosters with the exception of those to be replaced will be dubbed in the near future.

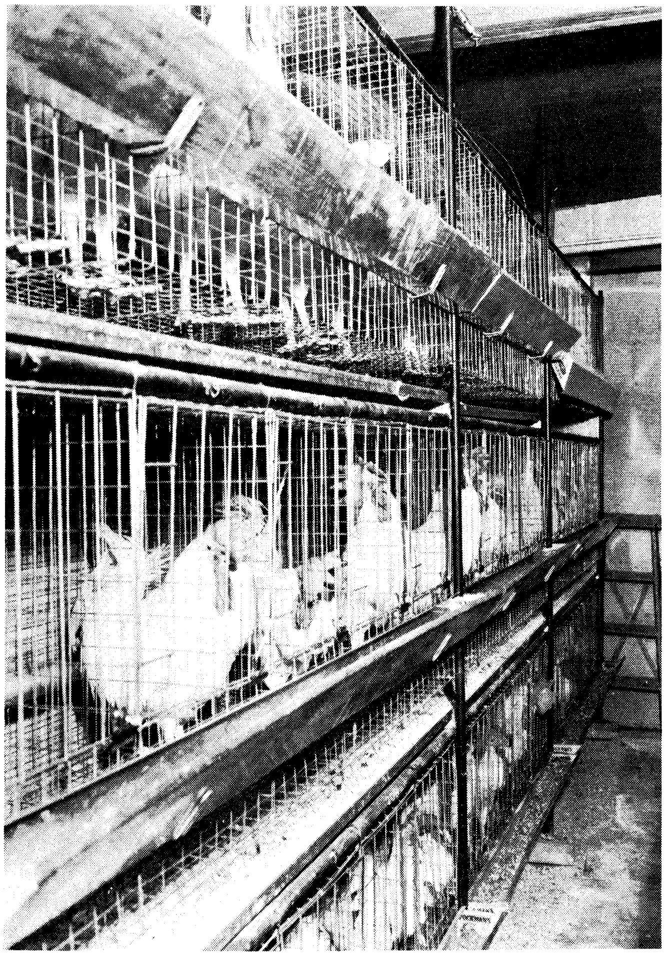

Fig. 1. View of the battery of enlarged rooster cages. There are 42 cages arranged in three decks of 14 cages each. Water is supplied continuously from a 2-inch aluminum pipe running through the rear of the cages. The ration is supplied from the conventional hopper.

As mentioned in the previous report, there are various opinions as to the effect of the diet on semen production. A mixture of whole or cracked grains has been recommended for roosters if high semen production is desired. A supplement of "Rob Roy Scratch Grain" has been fed *ad libitum*, together with the Growena mash. The grain is soaked in water and given a dose of 3 megarep of gamma radiation. The composition of the "Rob Roy Scratch Grain" as well as the Growena is given in Table I. An effort is made to keep both grain and mash before the roosters in approximately equal amounts.

b. *Changes in the Diet of the Pullets*.—The selection of diets to be used in this experiment is based on the recommendation of Mr. Charles Guider, the local representative of the Ralston Purina Company which manufactures the diets being used in these experiments. Since the pullets reached 20% production, they were first fed Flock Layena, then Cage Lanena, and are about to be fed a mixture of Cage Layena and Lay Chow. The guaranteed analysis and a list of the ingredients of each of these mashes is given in Table I. It would have been preferable to have fed the birds the

TABLE I

GUARANTEED ANALYSIS OF THE RATIONS FURNISHED BY THE RALSTON PURINA COMPANY AND THE USE OF THESE RATIONS IN THE LONG-TERM CHICKEN FEEDING EXPERIMENT

Name of Ration	Birds Receiving Each Ration	Guaranteed Analysis of Each Ration					
		Crude Protein, min %	Crude Fat, min %	Crude Fibre, max %	N.F.E.(1) min %	Rock Phosphate, %	Calcium Carbonate, %
Starteena	Starting chicks for the first 4 weeks.	20.0	3.0	5.0	50.0	0.75	1.5
Growena	To pullets from 4 weeks until they reach 20% egg production; to cockerels until they are used for breeding; thereafter mixed with Scratch Grain.	17.0	3.0	7.0	48.0	1.0	1.5
Rob Roy Scratch Grain(2)	To roosters when they are used for breeding; it is mixed 50-50 with Growena.	10.0	2.5	5.0	--	--	--
Flock Layena	To pullets after they reach 20% production and when maintained in pens or on range.	18.0	2.5	7.0	42.0	2.0	3.5
Cage Layena(3)	To pullets after they reach 20% production and when maintained in individual cages.	16.0	3.0	5.5	50.0	1.5	4.0
Lay Chow	To pullets fed Cage Layena and for breeding; it is mixed 50-50 with Cage Layena.	20.0	2.5	6.6	46.0	2.5	1.5

(1) Nitrogen-free extract, the equivalent of the carbohydrate portion of the diet.

(2) Consists of wheat, cracked corn, barley, oats, buckwheat grain, and small, broken, and imperfect grains of wheat.

(3) Consists of ground yellow corn, soybean oil meal, ground oats, wheat standard middlings, ground barley, ground grain sorghums, dehydrated alfalfa meal, corn gluten feed, meat scrap, fish meal, animal fat (preserved with butylated hydroxyanisole), vitamin B_{12} feed supplement, vitamin A feeding oil, riboflavin supplement, niacin, D-activated animal sterol, 1.5% low fluorine rock phosphate, 4% calcium carbonate, .25% iodized salt, and .01% manganese sulfate.

breeding ration, which is the Cage Layena—Lay Chow mixture, since the time they reached 20% production. The ingredients are nearly the same in all these diets, however, and there are only small differences in the protein, calcium, and phosphorus contents, the three constituents of principal importance in devising a breeding ration.[1]

c. Insemination Program.—Artificial insemination provides a greater measure of control over conception than does natural mating.[2] It is planned that every pullet, on the control and irradiated diet alike, be inseminated by all the males that are producing sufficient semen to be included in the program. Each female is inseminated once a week and two consecutive times with each male. Following this, a week is skipped to minimize the possibility of the semen of two males being confused. With the 21 males presently being used, 1722 matings are possible of which 253 have so far been achieved. Other details on the progress of the insemination program are given in Table II. The majority of the pullets have received the semen of three or four roosters. The larger number of control rather than experimental roosters being used reflects the difference in semen production reported earlier.[3]

TABLE II

STATUS OF INSEMINATION PROGRAM
AS OF SEPTEMBER 23, 1955

		Control	Experimental	Total
No. of pullets in program		41	41	82
No. of males in experiment		17	16	33
No. of males used to date		12	9	21
No. of times pullets in each group were inseminated*		126	127	253
No. of pullets receiving semen of designated number of roosters:	1	0	1	1
	2	6	7	13
	3	27	20	47
	4	7	13	20
	5	1	0	1
No. of times roosters from each group were used in insemination**		149	104	253

*Twice in succession with semen of an individual rooster (control or experimental).

**Twice in succession for an individual pullet (control or experimental).

d. <u>Handling</u> <u>and</u> <u>Incubation</u> <u>of</u> <u>Eggs</u>.—Individual egg weights have been recorded up to August 25. It is believed that it would be sufficient to collect data on egg weights for biweekly periods from time to time in the future, when it is desired to check possible differences between eggs from control and experimental pullets.

Until September 8, the eggs were collected each afternoon in plastic boxes. This may not have afforded the necessary circulation of air to cool the eggs quickly from body to room temperature so as to avoid injury to the germinal cell.[1] Henceforth, they are being collected in rubberized wire baskets and the time of collection has been changed to the following morning. Since August 10, the eggs have been stored in an air-conditioned room at about 76°F prior to incubation; henceforth, they will be stored at 50°F at the Food Service Building as originally planned.

The present schedule of incubation began on July 11; the eggs of half the pullets on the control diet and half of those on the experimental diet are placed in the same incubation tray and started on Mondays; eggs from the other halves are started on Thursday. Thus, fluctuation in temperature and humidity in the incubator need not affect one group of eggs more than the other. Pedigreed chicks can be obtained by placing the eggs in individual compartments during the last three days of incubation. Such an arrangement is shown in Fig. 2, showing a picture taken at the time of hatching of a group of pedigreed chicks.

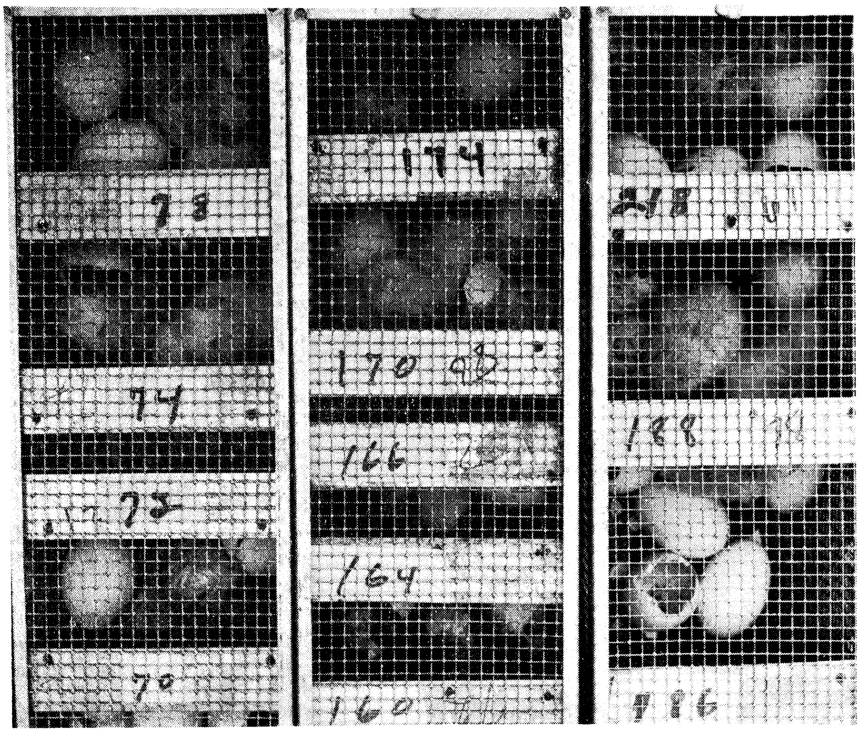

Fig. 2. View of the individual pedigree baskets which contain the fertile eggs of an individual pullet for one week. The picture was taken during the last (or twenty-first) day of incubation.

e. Raising of First-Filial-Generation Chicks.—All chicks prior to the ninth hatch (incubation period ending August 8) were disposed of after the data for the hatch had been collected. Chicks from the ninth through thirteenth hatches were hatched from pedigree baskets, wing-banded and identified as to dam and sire, and placed in the Jamesway Chick Battery shown in Fig. 3. This battery has a capacity of about 500 day-old chicks and about 150 four-week-old chicks. It is equipped with a "contact-heating" arrangement to simulate natural conditions, and it is the preferred battery by poultry research workers. The chicks are being raised on irradiated and control Starteena (see Table I) to four weeks of age. Half the chicks on each diet were from pullets on the irradiated mash and the other half from pullets on the control mash. Feed and water were supplied *ad libitum*, and the chicks were weighed twice weekly.

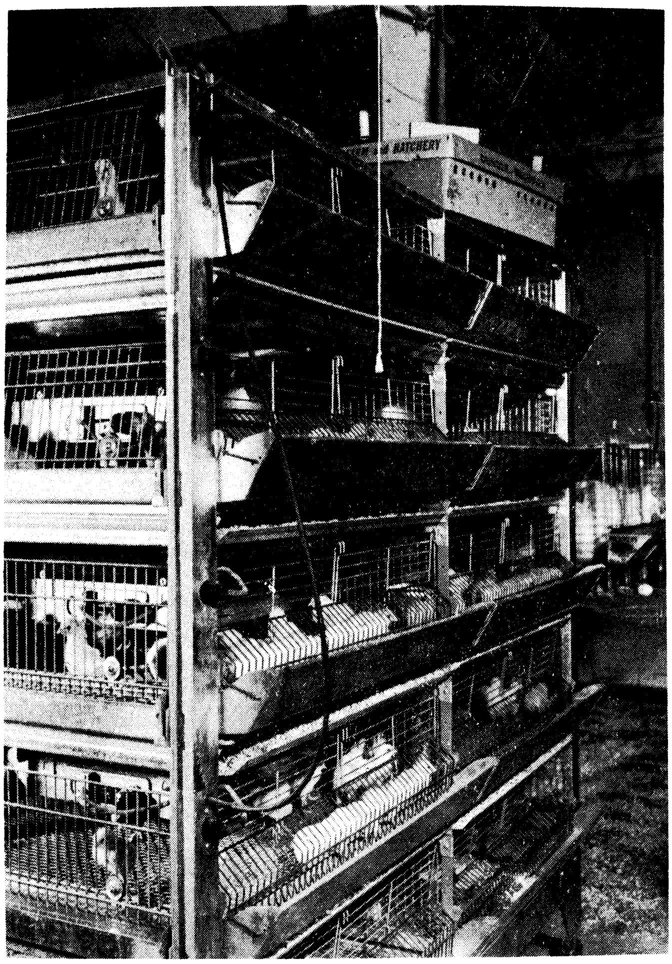

Fig. 3. View of the chick battery showing chicks from hatch numbers 9 through 13 from the top to the bottom deck, respectively. The age of each hatch is half a week older than that below it.

The chicks from hatch numbers 9, 10, and 11 have reached four weeks of age. These chicks can be classified into four groups as follows:

Group 1: Chicks from control pullets which are raised on the control diet

Group 2: Chicks from control pullets which are raised on the experimental diet

Group 3: Chicks from experimental pullets which are raised on the control diet

Group 4: Chicks from experimental pullets which are raised on the experimental diet.

Nine males from each of these classifications will be selected to furnish replacements of the parent-generation roosters. These males are now being fed Growena wet mash. The selections were made on the basis of the closeness of the fourth-week weight to the average weight for the group.

3. RESULTS

a. Average Body Weight of Parents.—Table III presents data on the average body weight for the four groups of chickens for the period since the last report. There has been no overall change in the average body weight for the males. Possibly as a reaction to being moved to their new cages, the average weight for the males fell 0.12 to 0.14 kg for a period of two or three weeks and then returned. The control females have gained an average of 0.17 kg and the experimental females gained an average of 0.16 kg during this period, thus continuing to show no significant differences in body weight traceable to diet.

TABLE III

AVERAGE BODY WEIGHTS OF CHICKENS IN LONG-TERM FEEDING EXPERIMENTS FROM TWENTY-SEVENTH TO THIRTY-NINTH WEEK OF EXPERIMENT

Date	Week of Experiment	Average Weight of Group, kg			
		Males		Females	
		Cont.	Exp.	Cont.	Exp.
27 June 1955	27	2.45	2.47	2.22	2.15
4 July	28	2.45	2.47	2.25	2.15
11 July	29	2.45	2.48	2.23	2.10
18 July	30	2.48	2.48	2.28	2.16
25 July	31	2.48	2.50	2.28	2.17
1 August	32	2.42	2.47	2.30	2.18
8 August	33	2.33	2.35	2.26	2.17
15 August	34	2.36	2.33	2.28	2.19
22 August	35	2.45	2.42	2.37	2.27
29 August	36	2.46	2.43	2.39	2.31
5 September	37	2.49	2.43	2.42	2.33
12 September	38	2.51	2.39	2.42	2.33
19 September	39	2.55	2.49	2.44	2.36

b. Efficiency of Feed Utilization by Pullets.—Table IV gives the amount of mash supplied, the average weight gain, and the number of eggs produced per pullet per week for the 11-week period since June 27. It is possible that the experiment pullets tended to convert a slightly greater portion of the feed into eggs rather than body weight, but the differences are too slight to be of significance.

TABLE IV

EFFICIENCY OF FEED UTILIZATION BY PULLETS
FOR THE 11-WEEK PERIOD FROM
JUNE 27 TO SEPTEMBER 12, 1955

	Group	
	Control	Experimental
Average amount of feed consumed per bird per week, in grams of dry mash	743	736
Average gain in weight per bird per week, in grams	17.7	16.0
Average number of eggs produced per bird per week	4.5	4.7

c. Thrombocyte Counts of Blood of Parent Chickens.—In Progress Report No. 3 it was reported that thrombocyte counts were to be included with the count of other blood cells made at that time. Inasmuch as this required special equipment which was in the hands of Dr. Alfred Lucas in East Lansing, Michigan, these counts had to be made there and reported later. The average values are given in Table V. Given the wide range of values within each group, the average values are remarkably close and show further negative evidence of radiation effects on the diet.

TABLE V

AVERAGE THROMBOCYTE COUNT IN BLOOD OF REPRESENTATIVE
CHICKENS ON CONTROL AND IRRADIATED WET MASH

Group	No. of Birds	Average Thrombocyte Count Cells/cu mm	Range
Males			
Control	7	31,430	14,628-57,120
Experimental	7	31,840	12,190-50,002
Females			
Control	14	25,244	9,744-40,986
Experimental	14	27,113	11,700-50,500

d. Pathological Observations on Chickens.—Table VI summarizes the pathological observations made on the four control and five experimental roosters that died or were sacrificed since the experiment began. One control and one experimental rooster are included that were reported in Table VI-A of Progress Report No. 3 but which since recovered. There has still been no mortality among the pullets, but one pullet on the control diet is listed as having lost 620 grams in three weeks and has difficulty standing. At the present time the pullet appears to be recovering.

The evidence is that one of the control roosters died of respiratory infection and two others with what appeared to be an accumulation of bile. There does not appear to be any previous avian pathology in which this observation is reported.[4] Among the experimental roosters, one died of bronchitis. Another died of what may have been the Leukosis complex which sometimes attacks the nerves leading to the legs. Two others died with the enlarged gall bladders and large quantities of bile present in the digestive tract.

e. Egg Production.—Figure 4 shows graphically the percent egg production by both groups of pullets since egg production began. At the beginning, production by the control pullets increased faster than did that by the experimental pullets, but thereafter egg production by the experimental pullets was greater. For the 10-week period from July 11 through September 15, the 41 control pullets produced 1746 eggs, for an overall percent production of 61.0, whereas the 41 experimental pullets (of which two were not laying) produced 1857 eggs for an overall percent production of 64.5. No argument is being presented either way at this time for the effect of diet irradiation on the egg production record of the pullets.

f. Fertility of Pullets and Roosters.—All the 41 control pullets and all but one of the 41 experimental pullets have produced eggs and are therefore fertile. One of these experimental pullets produced only one egg. Twelve of the 17 roosters on the control diet have been used in insemination and fertile eggs were produced by the pullets following the insemination. Nine of the 16 roosters on the experimental diet have been used in insemination with the same result. An attempt will be made to obtain semen from the remaining roosters for artificial insemination, and data on these animals will be presented in the next report.

When the semen of an individual rooster has been used successfully on all the pullets (control and experimental alike), it may be removed from the experiment and replaced by a first-filial-generation cockerel. About half the parent roosters will be kept in order to obtain longevity data even after the fertility data are complete for that male.

When the fertility data are complete, i.e., when all the roosters from which semen was obtainable have each been used to inseminate all the pullets, the data will be examined for possible differences in potency, duration of viability of semen after insemination, motility of semen (as

TABLE VI

CURRENT RECORD OF PATHOLOGY AMONG PARENT CHICKENS IN THE LONG-TERM CHICKEN FEEDING EXPERIMENT

Group	Bird No.	Gross Observation	Date Noted	Pathologist's Report
Control Males	65	Cannot support own weight.	25 April	Heart: Occasional perivascular infiltration of lymphocytes. No lipoidosis. Liver: No lipid in the liver cells. Fat droplets in some of the stellate cells of Kuppfer. Spleen: Negative. Pancreas: Negative. Gizzard: Negative. Kidneys: Negative. Small Intestine: Negative. Testis: Spermatogenesis active. Lungs: Catarrhal bronchitis. Bronchiectasis. No fat emboli.
	75	Difficulty in standing; lost 300 grams.	30 May	Recovered.
	204	Sudden weight loss followed by death.	18 July (approx.)	Gall Bladder: Dilated. Gizzard: Negative. Heart: Negative. Liver: No lipoidosis. Kidneys: Slight patchy degenerative fatty infiltration. Testis: Aspermatogenesis. Spleen: Negative. Small Intestine: Negative. Pancreas: Negative. Comment: Dilatation of gall bladder.
	69	Bile in feces, 650-gram weight loss.	18 Aug. (approx.)	Autopsy showed greatly enlarged gall bladder and bile present in all parts of the intestine.
	208	Sudden weight loss followed by death.	14 Sept.	Pathologist's report not yet available.
Control Female	86	620-gram weight loss in 3 weeks; cannot stand.	5-19 Sept.	Still living.
Experimental Males	167	Severe leg weakness, loss in weight.	4 April	See Progress Report No. 3, p. 17.
	163	Head bent down; tends to squat; not interested in food.	7 April	Heart: Negative. Liver: Negative. Lungs: Chronic catarrhal bronchitis. Gizzard: Negative. Small Intestine: Negative. Kidneys: Negative. Spleen: Negative. Testis: Spermatogenesis active.
	157	Severe leg weakness; tends to collapse at ankles.	27 April	Liver: Slight degenerative fatty infiltration. Heart: Negative. Kidney: No significant lipoidosis. Spleen: Vessels are thick-walled. Numerous eosinophils. Lungs: Marked atelectasis. Post-mortem change. Gizzard: Negative. Testis: Post-mortem change. Spermatogenesis active. Small Intestine: Negative.
	151	400-gram weight loss; comb shrunken and anemic.	6 June	Recovered.
	119	Neck twisted downward.	6 June (approx.)	Autopsy similar to that of Control Male No. 69.
	159	Died without prior weight loss; autopsy revealed green discoloration on its right side, visible through skin, and also extending into muscle.	1 Aug.	Kidneys: Congestion. No lipoidosis. Liver: Congestion. Heart: Negative. Spleen: Negative. Testis: Active spermatogenesis. Gizzard: Negative. Adrenal and Lung: No sections found either in fat stain or H and E.

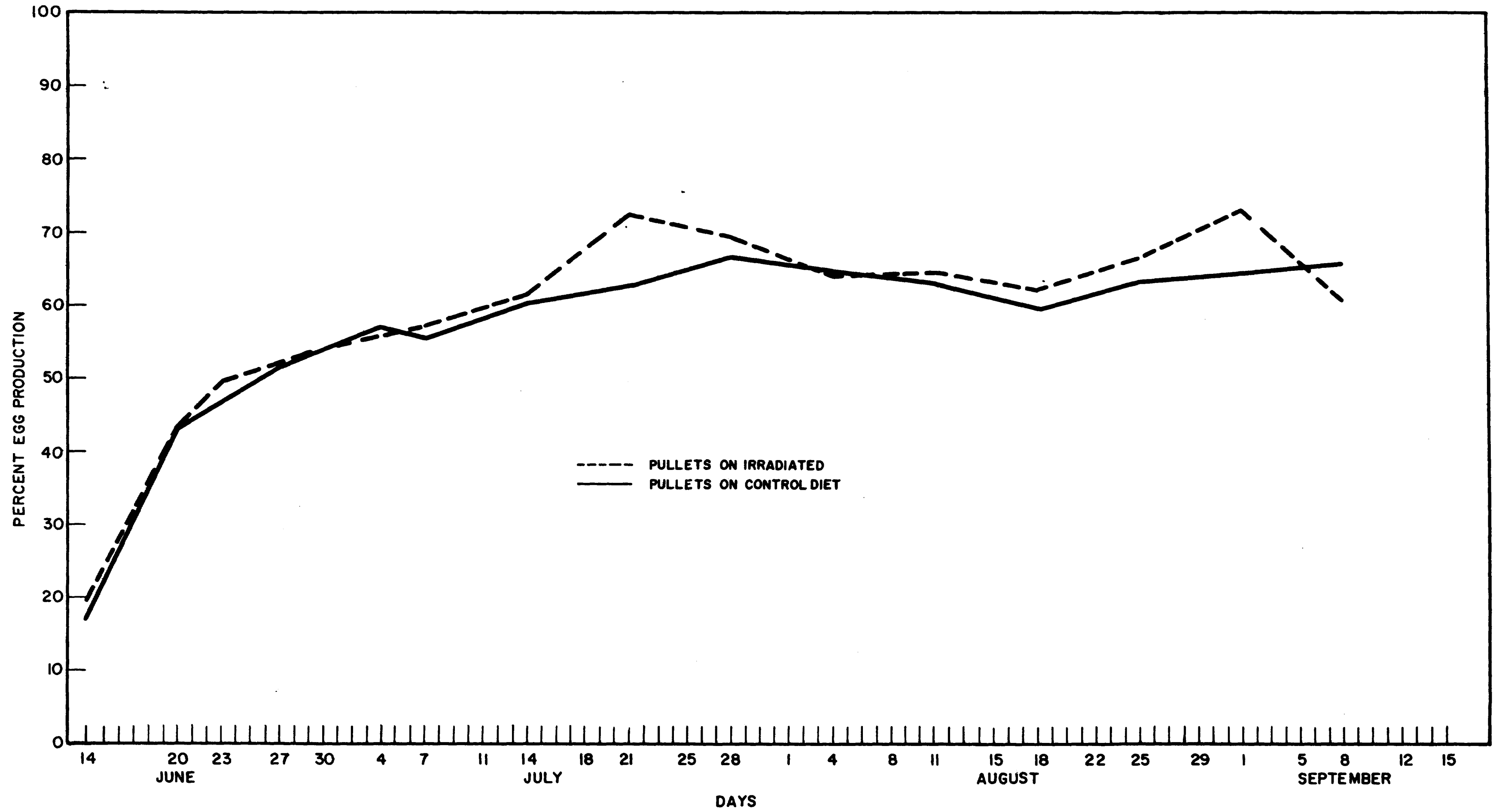

Fig. 4. Average weekly percent egg production of control and experimental pullets. Percent egg production is the number of eggs laid per day per total number of pullets in the group times 100.

examined microscopically), etc. It will be difficult to obtain unequivocal data on these points because an infertile egg may be the result of failure to conduct the insemination properly. There is no way of being certain on this point; when there is doubt as to whether the insemination was done properly, this is recorded.

g. Embryo Mortality and Hatchability of Eggs.—Table VII presents the data on embryo mortality of eggs incubated since July 7, at which date the present schedule of setting both control and experimental eggs together in the same incubation tray was begun. The eggs are examined by candling (allowing light to shine through the egg) after seven and after fourteen days of incubation. On the twenty-first day, the number of chicks, the number of eggs with pipped shells, and the number of eggs whose shells are intact are counted. Gross examination of the embryos which die during incubation will be presented in the next report.

The number of fertile eggs set and the number which died during the first week of incubation are practically the same for both groups for the period considered. The great majority of the embryos which are found to have died during the first week actually died during the first two days; this may be partly caused by improper egg handling. Embroyos which die after the first two days of incubation may be considered to be inherently defective. In Table VI, considerably greater embryonic mortality after the first week of incubation occurred among eggs from pullets on the irradiated diet than from those on the control diet. Embryonic mortality, however, is high in both groups. The usual incidence of mortality ranges between 20 and 30% (or in terms of hatchability, 80 and 70%) of fertile eggs set.[2] With eggs from control pullets in this experiment, the incidence is 36% (64% hatchability) on the average, ranging from a low of 31 for the third hatch to a high of 81% (69 and 19% hatchability, respectively). Whether a difference of 7.5% in embryonic mortality is significant in an experiment where it is 36% for the untreated group will be explored by a statistician and reported in Progress Report No. 5. It is worth noting, however, that this 7.5% difference arises during the second and third week of the incubation period, where the embryonic mortality for the control group is only 17.1%.

Figure 5 presents the percent hatchability data on an average weekly basis for the eggs from the control and experimental pullets. This graph, of course, reflects the same observation commented on in the previous paragraph, but shows that nearly all the difference in percent hatchability occurred during the earlier weeks of the experiment. During the last month, there has been no observable difference in hatchability.

h. Growth Rate of First-Filial-Generation Chicks.—Figure 6 shows the growth rate of the chicks on the control and irradiated Starteena diet for each of the five hatches. No distinction is shown between males and females and none between chicks from control and those from experimental pullets. When the data for the twelfth and thirteenth hatches are complete, the body weights will be grouped and averaged according to the sex, origin, and diet of the chicks.

TABLE VII

EMBRYO MORTALITY DURING INCUBATION OF EGGS FROM PULLETS ON IRRADIATED AND CONTROL DIETS SET SINCE JULY 7, 1955*

Diet of Pullets	Fertile Eggs Set	Died During First Week of Incubation	Died During Second Week of Incubation	Died During Third Week of Incubation	Pipped**	Hatched
			In Number of Eggs			
Control	609	116	21	53	30	389
Experimental	619	118	36	68	48	349
			In Percent of Fertile Eggs Set			
Control	(100)	19.1	3.5	8.7	4.9	63.8
Experimental	(100)	19.1	5.8	11.0	7.8	56.3

*Date at which present schedule of setting eggs was begun.

**Chick succeeds in breaking shell but is unable to emerge.

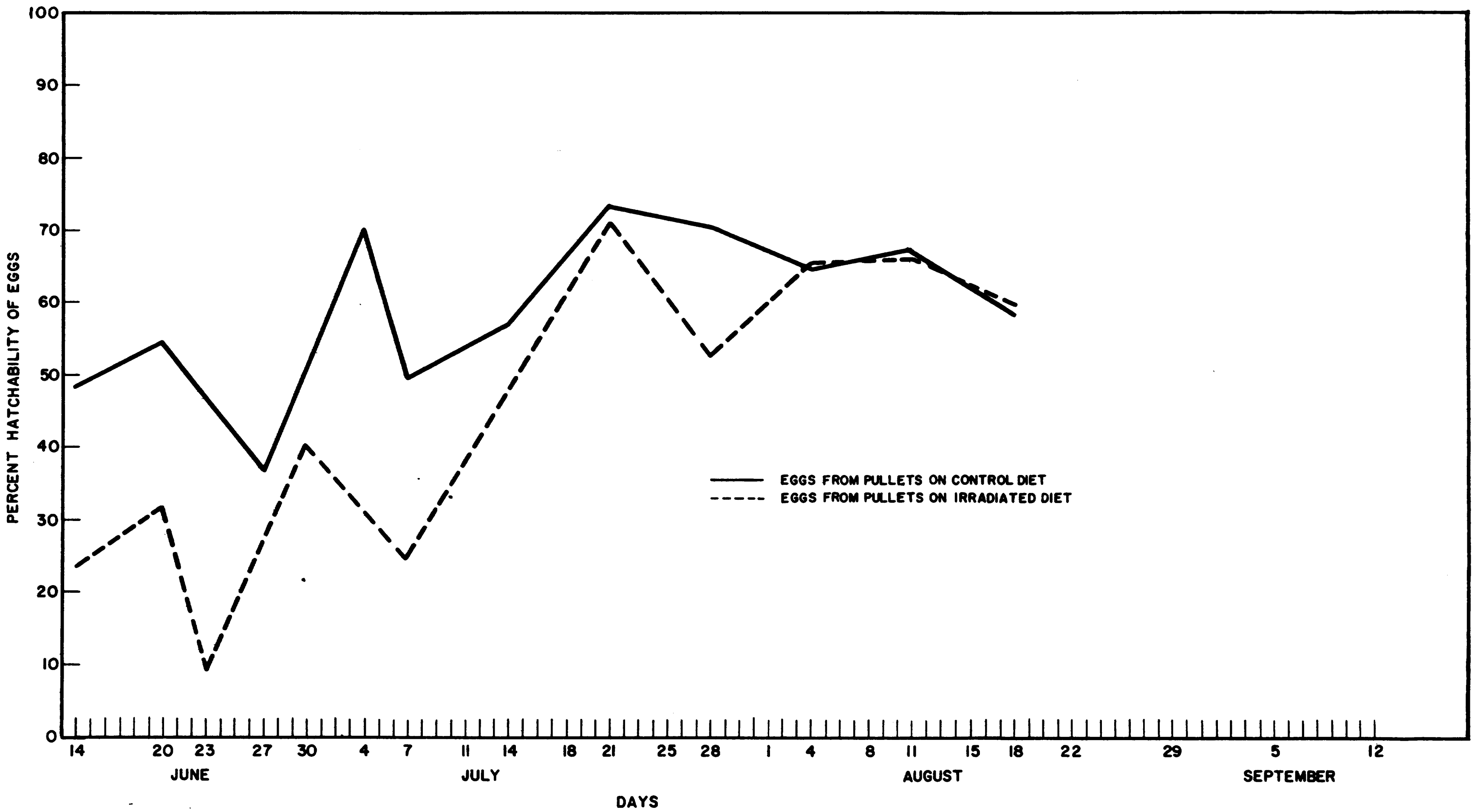

Fig. 5. Weekly percent hatchability of eggs from control and experimental pullets. Percent hatchability is the percent of chicks hatching of the number of fertile eggs incubated.

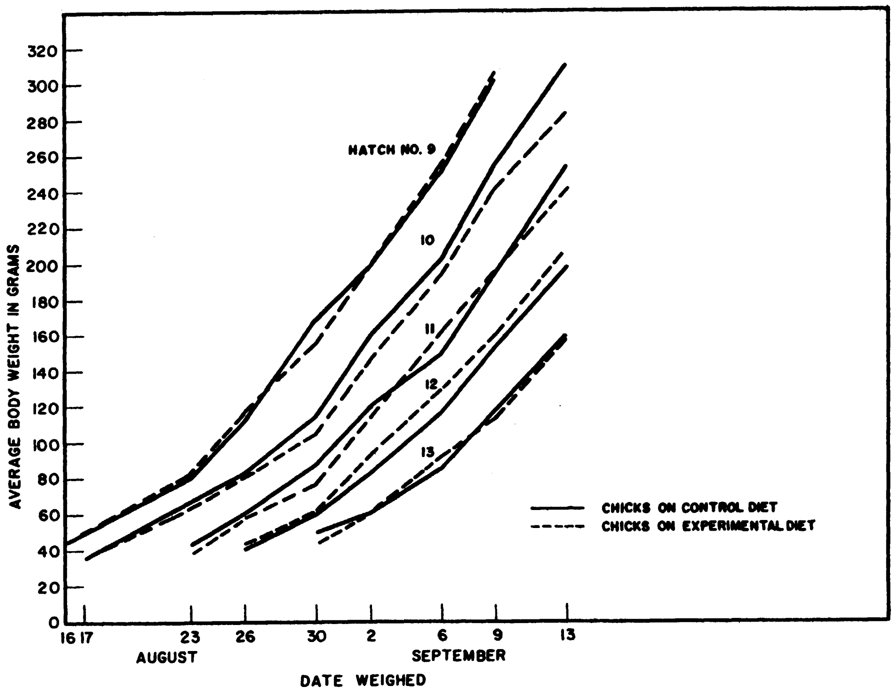

Fig. 6. Growth curves of first-filial-generation chicks. The hatches were started half a week apart. Only the chicks in the first two hatches reached four weeks of age when this report was written.

The data in its present form, however, reveal no net differences in the performance of the chicks on the two diets. The small differences may be due to different proportions of males and females in each group.

B. Reproduction Study with Bagg-Strain Albino Mice

1. INTRODUCTION

As outlined in previous reports, the objective of this experiment is the determination of the breeding performance of a significant number of first-filial-generation, female, Bagg-strain albino mice raised on the control and irradiated diets. Since only nine parent female mice were available for each of the groups when the experiment began, it was necessary to breed the parent generation two or three times. At the present time the available data consist of the performance of the parent mice during the two breeding periods and of the average growth rates of the two groups of litters of the first-filial-generation females on each diet.

2. MANAGEMENT

The first litters of first-filial-generation females were obtained with reasonable success. The record breeding occurred during the extreme hot weather prior to housing the animals in air-conditioned quarters. The results of the second breeding were inferior in both groups of animals with respect to number of pups born alive. Several measures were taken to improve the diet and the environment of the animals so as to increase the number of animals raised. The vitamin E supplementation was increased fivefold. (The females now receive one drop of a solution consisting of 10% alpha-tocopherol-acetate in corn oil; the males still receive the same amount of a 0.1% solution.) The mice have also been moved into air-conditioned quarters where they are also more secluded than before. The loss of pre-weanling mice that occurred prior to these changes has now ceased altogether. However, much time was lost as a result of the difficulties in the second breeding, and at the present time only 13 experimental and 9 control first-filial-generation females have been obtained. In spite of the small number, they were mated with the parent-generation males and some of the litters have been obtained.

However, in spite of the improvements in the management, none of the young from over eight litters from both groups of females survived. The manner in which the young were lost is very characteristic of a manganese deficiency in rats.[5] The mice are fed the same diet fed to the rats on the long-term experiment (see Part A, Section II), and the rats have also been suffering from difficulties explanable at least partly by too low an intake of manganese. On September 27, the rat and mouse diet was supplemented with 14.8 mg-% of manganous sulfate monohydrate, enough to supply the daily requirement of 0.8 mg per day for the rat and presumably a corresponding amount for the mouse.

Both the parent females and the first-filial-generation females will be bred again when it is established that the manganese supplement restores the full nutritional value of the diet.

3. RESULTS

a. Breeding Performance of the Parent Mice.—Table VIII gives the performance of the parent mice during their first and second breeding. The chief difference between the first and second breeding for each group is in the number of pups born dead or dying very shortly after birth. Death at birth is a symptom of manganese deficiency as mentioned previously.

There are too few pups from the second breeding to allow conclusions to be drawn other than that these animals have proven more difficult to raise than the albino rats.

The small number of pups born and the low manganese level probably invalidate conclusions from these data. While the control females produced more young and their weaning weights were greater, the experimental females were able to wean twice as many young. There were no differences of this kind observed during the first breeding.

TABLE VIII

DATA ON THE BREEDING OF THE PARENT-GENERATION MICE

	First Breeding		Second Breeding	
	Control	Experimental	Control	Experimental
No. of females bred	8	8	7	6
No. of females used	5	5	5	5
No. of females sterile	1*	0*	1/	0
No. of females conceiving first week	3	4	2	3
No. of females conceiving second week	2	2	3	1
No. of females conceiving third week	0	1	0	0
No. of females conceiving fourth week	1	1	0	0//
No. of females resorbing fetuses	1	0	1	1
Total no. of litters born	6	8	5	5
Total no. of litters born dead	1**	0**	0	0
Total no. of litters born alive, not surviving weaning	1	2	1	1
Total no. of pups born	32	43	30	22
Total no. of pups born dead	1	3	8	4
% of pups born dead	3.1	7.0	26.7	18.2
No. of pups born alive per female bred	3.9	5.0	3.1	3.0
Total no. of pups born alive, not surviving weaning	16	18	15	6
Average no. of pups born alive, not surviving weaning	3.2	2.5	2.1	1.0
Average no. of pups born per litter	5.3	5.4	4.3	3.7
Average no. of pups per litter at 5 days	3.0	3.1	1.7	2.7
Total no. of young reaching weaning	15	22	7	12
Average no. of pups per litter at 21 days	2.5	2.8	2.3	4.0
Average weaning weight in grams	6.4	6.6	9.2	6.4
Average no. of young weaned per female bred	1.9	2.8	1.0	2.0
% of pups born alive which survived 21 days	48.4	55.0	31.8	66.7

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

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

/Previously proven fertile, but in breeding for second litter, mated five times with proven males unsuccessfully. Female was below normal breeding weight at time of second breeding.

//One female in group conceived after eight weeks of continued mating.

b. Growth Rates of First-Filial-Generation Females.—Figure 7 shows the growth of the two groups of female offspring from the two breeding periods of the parents. The first crop of females appeared to show identical rates of growth. The sudden increases in weight indicate periods of pregnancy. The beginning of the growth curves for the second crop reflect the fact that the weaning weights of the control females were one and one-half times that of the experimental female weanlings, but this difference ceased to exist after the first few weeks.

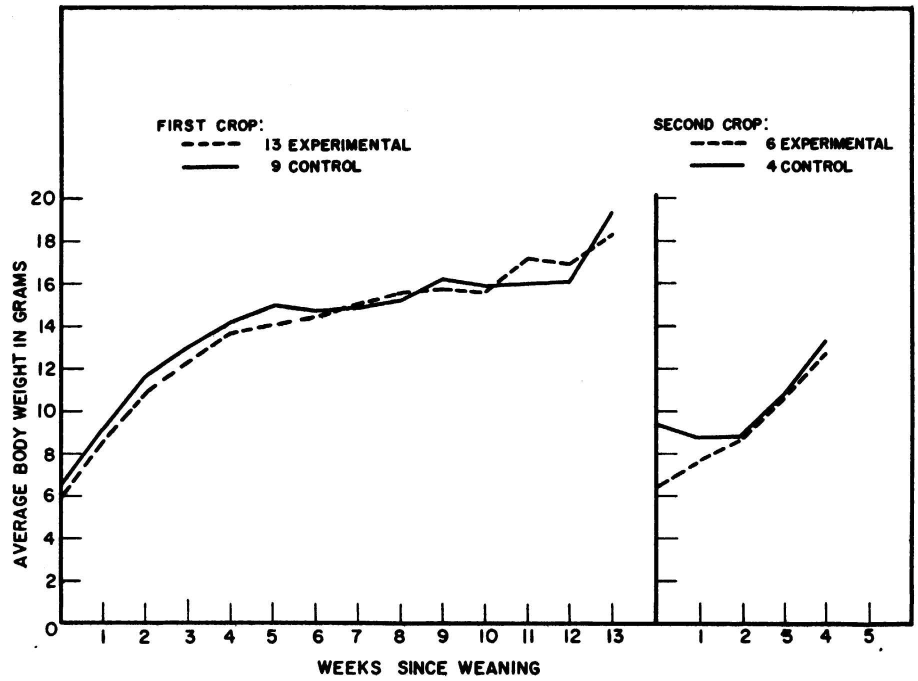

Fig. 7. Growth curves of first-filial-generation female mice from each of the two breedings of the parent mice. Comments on the irregularities in these growth curves are found in the text.

II. STUDIES SUPPORTED BY MICHIGAN MEMORIAL-PHOENIX PROJECT NO. 41

A. The Long-Term Rat Feeding and Breeding Experiment

The progress of the long-term rat feeding and breeding experiment and the initiation of the wheat-feeding experiment are reported in the following sections and are both supported entirely by Michigan Memorial-Phoenix Project No. 41. Because of possible interest of these experiments to the Office of the Surgeon General and to others participating in the study of the irradiation of foods, the inclusion of this material in this report is considered warranted.

1. INTRODUCTION

The long-term rat experiment is now in its twentieth month and the weaning of the second crop of the third and last filial-generation-animals will be completed shortly. Data on growth rate of second-filial-generation animals, on pathology of parent rats lost from the experiment, and on reproduction of second-filial-generation females are not available. The performance of control and experimental animals alike during the past three months has not been as satisfactory as anticipated. The following section presents a discussion of the difficulties with the objective of determining the cause and the necessary corrective measures to be taken.

2. RECENT CHANGES IN PERFORMANCE OF ANIMALS

a. Comparative Growth Rates of Three Generations of Male Rats.—Growth rates of three generations of male rats are compared in Fig. 8. The growth rates of both groups of parent- and first-filial-generation males are almost identical, but those of the second filial generation are lower. There has been no change in the diet fed these animals during this period and no other change in the management of the experiment. Apparently either a toxic factor has inadvertently been introduced or a dietary deficiency is becoming evident.

b. Reproduction During the First Breeding of the Second-Filial-Generation Rats.—Table IX compares the breeding performance of the parent-,

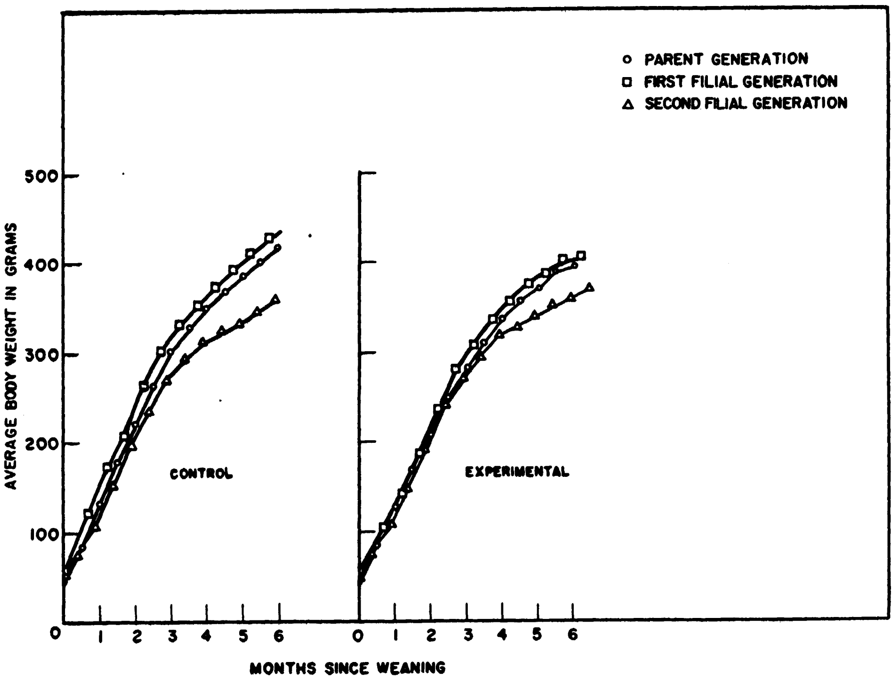

Fig. 8. Comparative growth curves of three generations of male rats in the long-term rat feeding and breeding experiment.

first-filial-, and second-filial-generation rats on both diets during the first time each generation was bred. The performance of the second-generation females was actually superior to that of the two earlier generations with regard to the following: sterility of males and females, time of conception, total number of litters born alive and born dead, total number of pups born alive and born dead, and therefore, the number of pups per litter and the number born per female bred.

However, the percentage of young surviving to weaning and the average weight at weaning are lower for the second-filial-generation rats on both diets than for the previous two generations. At least 80% of the pups born alive from the first two generations survived weaning, whereas not more than 67% of those from the second filial generation did so. The average weight of the pups from the first two generations was greater than 45 grams, whereas that of the pups born from the last generation was less than 27 grams. The ability of females to have normal litters but their complete inability to raise them is characteristic of only a limited number of dietary abnormalities.

c. Average Body Weight of Parent Rats.—Table X presents the average body weights of the four groups of parent-generation rats for several intervals since the forty-ninth week of the experiment. Figure 11 in Progress Report No. 1[6] shows the growth curves for these four groups up to the forty-ninth week. The data in Table X in this report consist of the average body weights only of those animals which were alive on the ninetieth

TABLE IX

DATA ON THE FIRST BREEDING OF PARENT-, FIRST-FILIAL-, AND SECOND-FILIAL-GENERATION RATS

	1st Breeding of Parent Gener.		1st Breeding of First Filial Gener.		1st Breeding of Second Filial Gener.	
	Cont. Group	Exp. Group	Cont. Group	Exp. Group	Cont. Group	Exp. Group
No. females bred	20	20	20	20	20	20
No. males used	20	20	12	12	10	10
No. females sterile (1)	2	5	2	1	1	0
No. males sterile (2)	8	9	4	3	0	2
No. males not proven (3)	2	3	0	0	0	0
No. females conceiving first week	8	3	6	5	12	12
No. females conceiving second week	1	3	6	6	6	4
No. females conceiving third week	3	4	3	7	1	2
No. females conceiving fourth week	0	0	0	0	0	1
No. females conceiving after fourth week	4	5	3	1	0	1
No. females resorbing fetuses	2	0	0	0	0	0
Total no. litters born	16	15	18	19	19	20
Total no. litters born dead	1	1	2	0	0	0
Total no. litters born alive, not surviving weaning	1	1	0	1	3	3
Total no. pups born	157	119	184	195	195	204
Total no. pups born dead	5	3	22	9	3	0
% pups born dead	3.18	2.52	11.95	4.62	1.5	0
No. pups born alive per female bred	7.6	5.8	8.1	8.3	9.6	10.2
Total no. pups born alive, not surviving weaning	25	21	17	24	64	76
Average no. pups born per litter	9.8	7.9	10.22	10.26	10.3	10.2
Average no. pups per litter at 5 days	8	6.6	8.4	9.3	8.7	8.4
Average no. pups per litter at 21 days	8	6.6	8.0	8.6	6.7	6.4
Total no. young disposed of (4)	0	0	0	0	3	7
Average weight of young at 21 days, grams	48.6	45.5	45.5	47.7	27.1	27.0
Total no. young reaching weaning (5)	127	95	145	162	128	128
Average no. young weaned per female bred	6.43	4.8	7.2	8.1	6.4	6.4
% pups born alive which survived 21 days	83.5	81.8	89.4	87.1	66.7	62.7

(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) Does not allow for animals that were disposed that might have survived to weaning age.

TABLE X

AVERAGE BODY WEIGHTS IN GRAMS OF PARENT-GENERATION RATS ALIVE ON THE NINETIETH WEEK AT INTERVALS DURING THE SECOND YEAR OF THE EXPERIMENT

Date	Week of Experiment	Group			
		Males		Females	
		18 Cont. Animals	21 Exp. Animals	21 Cont. Animals	21 Exp. Animals
22 Dec. 54	49*	605	586	346	332
12 Jan. 55	52	626	602	354	339
9 March 55	60	650	626	372	360
18 May 55	70	681	650	395	377
26 July 55	80	697	649	427	408
31 Aug. 55	85	646	638	430	412
28 Sept. 55	90	665	625	444	423

*Growth curves for these animals up to and including the forty-ninth week are presented in Fig. 11, p. 27, of Progress Report No. 1.

week of the experiment, as it would be misleading to compare averages which include weights of animals which died prior to the ninetieth week.

Both groups of males showed average body-weight gains up to about the eightieth week of the experiment, but showed declines in average body weight after that point. The females did not show weight declines, but their rate of increase nearly halted during the period following the eightieth week. It was during this period that the second-filial-generation animals were producing their first litter of young. The breeding performance of these animals is given in Table IX, and their failure to raise their young is apparent.

d. Occurrence of Hairballs in Stomachs of Rats.—Since July 15, 17 parent rats from all four groups and two second-filial-generation rats have died with hairballs in their stomachs. In some of the cases the hairball completely filled the stomach and obstructed the passage of food. Figure 9 shows a hairball from an experimental female rat; the hairball had the shape of the stomach which it nearly filled. This rat also bore a tumor, a photograph of which is included in the figure. Figure 10 shows the same hairball sectioned so as to show that it was a nearly solid mass of hair. Of the 17 hairballs occuring among parent rats, six were from control males, three from experimental males, three from control females, and five from experimental females. The two hairballs found upon autopsy of the second-filial-generation females were both from experimental females. The histopathological reports for many of these animals cite evidence of inanition.

The hair of the parent male rats is loose enough to be easily pulled from the skin, although that of the females is not quite so loose.

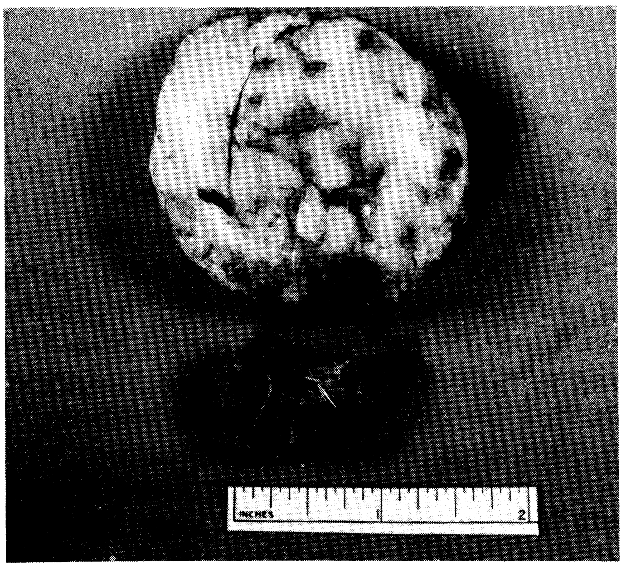

Fig. 9. View of tumor and hairball taken from an experimental female parent rat ($8R_1L_1$) on September 6, 1955. The hairball filled and took the shape of the stomach and is believed to be the immediate cause of death.

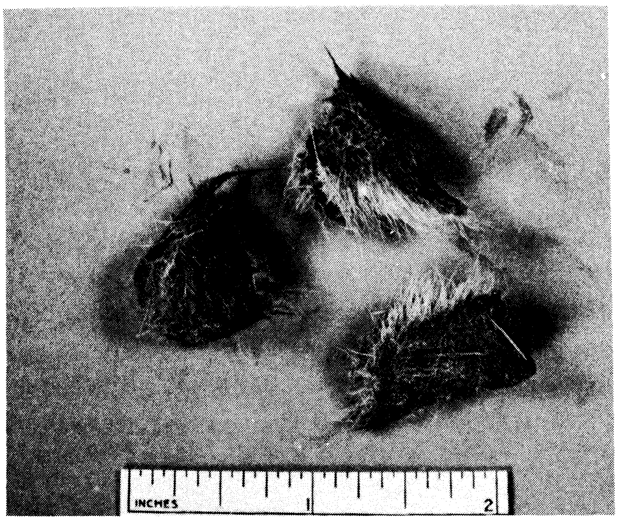

Fig. 10. The same hairball as shown in Fig. 9 has been sectioned to show that it was a solid mass of hair.

Alopecia (loss of hair) was also observed to be frequent among the third-filial-generation weanlings, and among some of the mice in the mouse reproduction study. These observations suggest that the occurrence of hairballs is secondary to some abnormity resulting in loss of hair. The hair probably accumulates in the stomach as a result of the normal licking habits of the animals.

e. Growth, Mortality, and General Behavior of Second- and Third-Filial-Generation Animals.—For the first time in this experiment, deaths have occurred among second- and third-filial-generation animals after they have been weaned to both the control and irradiated diets. A total of seven second-filial-generation rats died (one control male, two control and four experimental females). Hairballs were found in the stomachs of two, and possibly three of these animals, as mentioned previously. Two third-filial-generation rats died (both control males) out of a total of 32 selected from the first group. The females in this group have gained an average of less than 2 grams during the past two weeks, and the males have shown no change in weight during this period (normal gain would be of the order of 20-30 grams). Many of these animals show alopecia, distended and hard abdomins, diarrhea, hyperirritability, and listlessness.

f. Analysis of the Cause of the Experimental Difficulties.—As the difficulties occurred with animals in both the experimental and control groups, the irradiation of the diet is eliminated as a cause. As the management of the colony has not been changed in the past few months, it may be ruled out as a variable. The evidence, therefore, indicates that the difficulties have been caused by some factor present in both the control and irradiated diets.

The evaluation should be made with respect to the control diet which, because it is prepared fresh and stored under refrigeration for not more than a day, is not likely to have become defective as a result of mixing ingredients and storage thereafter. When the recommended requirements of the rat are compared with the composition of the diet, it is apparent that the dietary level of manganese that has been used for the past several months is less than a fifth of the amount that previous experimental work has shown to be necessary. The principal source of this dietary essential is the manganous sulfate in the Hubbel-Mendel-Wakeman salt mixture[7] which at a 4% level supplies 0.5 mg-% manganese to the final diet. Allowing another 0.5 mg-% from the canned meat[8] (this is believed to be a generous estimate), which is the only other possible source, produces a total in the diet of only 1 mg-%.

There is considerable difference of opinion in the literature regarding the manganese requirement by the rat. This uncertainty cannot be dispensed with simply by adding a liberal allowance because of the reported growth retardation effect from an excess of manganese. Orent and McCollum[9] report that 0.005% (5.0 mg-%) is the minimum requirement for manganese; females on their "manganese-free" diet are able to "grow to maturity in a normal manner, go through normal estrual cycles and when mated with potent males produce the normal number of young. However they fail to suckle their young." Daniels and Everson[10] report that 4 to 6 mg-% in a milk diet allowed females to wean their young successfully. Becker and McCollum [11] fed from 5.0 to 100 mg of manganese daily to rats with "excellent reproduction" and found no evidence of toxicity from even the highest level of manganese, although it did cause a slight retardation in growth. Skinner[12] found that diets supplying 10 mg per day per rat resulted in completely successful rearing of young.

On the other hand, McCarrison[13] reported that only 0.015 mg of manganese (as manganese chloride) per day was required for rapid growth and that a supplement of 0.56 mg manganese (as manganic oxide) had a retarding effect on growth. McCarrison's use of two different forms of manganese, the lack of attention paid to reproduction, and the indication that the diet was not manganese-free to begin with serve to invalidate his work. The work of Perla, Perla, and Sandburg[14], however, is more pertinent. They found that supplementing an adequate normal diet with either 400 micrograms of thiamin daily or with 2 mg manganese resulted in females exhibiting cannibalism, loss of maternal instinct, and interference with lactation. Supplementation with both thiamin and manganese, however, did not result in this disturbance. Thus, added manganese reduced the undesirable reaction to excess thiamin and vice versa. This is of particular interest in the rat experiment because the thiamin level is probably excessive. This would suggest that more benefit would be obtained from a higher-than-normal level of manganese rather than from a lower level.

Further evidence that the diet was deficient in manganese is the fact that in both the rat and mouse experiment the females were able to have normal litters but were unable to suckle the young, which is characteristic of manganese deficiency.

It is now believed that the manganese level in the present diet should have been held at from 5 to 10 mg-% rather than at the level of 1.0 mg-%. When the diet was originally devised, a supplement of manganese was added to furnish 4.8 mg-% (equivalent to about 1 mg per day). When this diet was modified for use in the present program, the supplement was omitted because of the evidence at that time of the undesirable effect of too much manganese.

In an attempt to correct these difficulties the original procedure of using a manganese supplement has been restored and also three other changes were made. The supplement of dibasic potassium phosphate used in the original diet, but later omitted, was also restored to assure adequate potassium. The amount of vitamin B_{12} furnished by the canned meat is not known. To eliminate a possible deficiency the requirement by the rat for this new vitamin is being met by a supplement which provides about 10 micrograms daily. The third change is the use of the stable alpha-tocopherol acetates as a source of vitamin E rather than the nonacetylated vitamin which is not stable.

After feeding the modified diet for only five days, the reaction of the animals was dramatic and indicates that the right corrective measures were taken. The routine observations that will continue to be made will allow one to evaluate the wisdom of this change.

3. PATHOLOGICAL OBSERVATIONS ON PARENT RATS

Since the last report the number of parent-generation rats lost from the experiment has increased from 17 to 41. Table XI presents the

TABLE XI

PATHOLOGY RECORD OF 33 PARENT-GENERATION RATS
TAKEN FROM EXPERIMENT SINCE ITS BEGINNING JANUARY 13, 1954

Group	Case No.	Animal and Report No.	Observation and Date Noted	Histopathological Report: Tissue	Histopathological Report: Observation
Experimental Males	1	$2R_3$	Lateral tumor 16 Aug 54	(See Progress Report No. 1, p. 37)	
	2	$4R_1$	Scrotal tumor 23 Aug 54	(See Progress Report No. 1, p. 39)	
	3	$6R_3$	Acute respiratory infection 23 Feb 55	(See Progress Report No. 3, p. 31)	
	4	$2R_2$ 345LBH	Severe weight loss followed by death 15 June 55	Heart	Well-marked degenerative fatty infiltration.
				Lungs	Congestion. Emphysema. No fat emboli. Bone marrow giant cell emboli.
				Kidney	Marked acute passive congestion. Scattered hyaline casts. Well-marked degenerative fatty infiltration. Serous atrophy of perirenal fat.
				Adrenal	Marked congestion. Cortical lipids in normal amount.
				Adipose Tissue	Some loss of fat.
				Liver	Intense acute passive congestion. Well-marked degenerative fatty infiltration confirmed by fat stain.
				"Pus Sac"	A sac lined by cornifying squamous epithelium and filled with desquamated keratohyalin and pus. Inflammatory infiltrations in the subepithelial tissue. This appears to be an epithelial inclusion cyst with secondary infection.
				Testes	Post-mortem change. Spermatogenesis active.
				Spleen	Numerous hemophages.
				Comment:	There is no evidence of pneumonia. The marked degenerative fatty infiltration of heart, liver, and kidney may be due to the toxin from the pyogenic infection of the feet.
	5	$2R_1L_3$ 1118LBH	Animal moribund; 3/4-inch ventral abscess filled with green pus. Sacrificed 29 July 55	Heart	Patchy degenerative fatty infiltration.
				Small Intestine	Negative.
				Stomach	Negative.
				Lungs	Patchy emphysema.
				Spleen	Negative.
				Testis	Post-mortem change. Spermatogenesis active.
				Liver	Well-marked degenerative fatty infiltration.
				Kidneys	Congestion. Well-marked degenerative fatty infiltration.
	6	$2L_3$	Died suddenly and violently with bleeding from nose or mouth. Three hairballs, approx. 3/8 inch in diam, in stomach. 17 Aug 55	(Pathologist's report not yet available)	
	7	$4L_3$	Animal listless for 3 weeks before death; 3 hairballs in stomach.	(Pathologist's report not yet available)	
Control Males	8	7_0	Lateral tumor 15 Nov 54	(See Progress Report No. 2, p. 21)	
	9	$3L_1$	Acute respiratory infection 22 Feb 55	(See Progress Report No. 3, p. 31)	
	10	$1R_2L_1$	Acute respiratory infection 22 Feb 55	(See Progress Report No. 3, p. 31)	
	11	$5R_2$	Died suddenly 30 Apr 55	(See Progress Report No. 3, p. 31)	
	12	$1R_1L_3$ 344LBH	Severe loss of weight 9 June 55	Kidneys	Congestion. A few hyaline casts. Well-marked degenerative fatty infiltration.
				Testis	Post-mortem change. Active spermatogenesis.
				Lungs	Patchy emphysema. Peribronchial lymphocytic infiltration. Occasional fat emboli.
				Liver	Well-marked degenerative fatty infiltration confirmed by fat stain.
				Heart	Sclerosis of small arteries.
				Spleen	Congestion.
				Adrenal	Cortical lipids abundant. Serous atrophy of periadrenal fat.
				Comment:	The tissue changes do not explain the cause of death. The serous atrophy of the adipose tissue indicates that the animal had used up the stored fat (panniculus) and was in a state of malnutrition.
	13	$3R_2$ 1108LBH	Sickly 2 days prior to death preceded by vomiting. Four hairballs, approx. 1/2 inch in diam, in stomach; spleen smaller than normal.	Small Intestine	Post-mortem change.
				Lungs	Congestion. Patchy atelectasis. Dilatation of bronchi. No fat emboli.
				Liver	Congestion. Well-marked degenerative fatty infiltration.
				Kidneys	Congestion. Well-marked degenerative fatty infiltration.
				Heart	Slight degenerative fatty infiltration.
				Spleen	Erythropoietic foci.
				Adrenals	Serous atrophy of periadrenal fat.
				Testes	Post-mortem change. Active spermatogenesis.
				Comment:	Cachexia. Inanition.
	14	$1R_1L_2$ 1115LBH	Developed a hard growth off right side of jaw. It dislocated his jaw and evidently caused pain. Slight bleeding from eye and nose. Sacrificed 26 July 55	Liver	Well-marked degenerative fatty infiltration.
				Heart	Patchy degenerative fatty infiltration.
				Kidneys	Well-marked degenerative fatty infiltration. Serous atrophy of perirenal fat.
				Adrenals	Cortical lipids abundant.
				Spleen	Negative.
				Stomach	Negative.
				Pancreas	Negative.
				Lungs	Congestion. Slight patchy atelectasis.
				Testis	Post-mortem change. Spermatogenesis present.
				Small Intestine	Post-mortem change.
				Parotid Gland	Marked degenerative changes; old hemorrhage, chronic inflammation, epithelial proliferation, degenerative changes in the epithelium. Adjacent to the parotid is a highly cellular spindle-celled neoplasm, a spindle cell sarcoma. We cannot determine with certainty that this neoplasm has origin in the parotid gland.

TABLE XI (continued)

Group	Case No.	Animal and Report No.	Observation and Date Noted	Histopathological Report: Tissue	Histopathological Report: Observation
Control Males (cont.)	15	$1L_1$ 1119LBH	Listless but showed no weight loss before death. Large hairball completely filled stomach. 31 July 55	Adrenals Heart Liver Kidneys Lungs Adipose Tissue Small Intestine Spleen Testis	Cortical lipids in moderate amount. Negative. Marked degenerative fatty infiltration. Slight degenerative fatty infiltration. Congestion. Edema. Serous atrophy. Advanced post-mortem change. Post-mortem change. Post-mortem change. Apermatogenesis active.
				Comment:	This group as a whole (1106LBH through1119LBH) shows evidence of cachexia (or inanition), indicating that the animals had used up their stored fat.
	16	$5L_3$ 1113LBH	Found dead with a discharge from mouth and ulcerated rear feet. Enlarged liver, inflamed lungs, 3 hairballs in stomach, and stomach filled with gas. 8 Aug 55	Kidney Testes Liver Heart Adrenals Spleen Lungs "Tumors"	Marked congestion. Well-marked degenerative fatty infiltration. Post-mortem change. Spermatogenesis active. Well-marked degenerative fatty infiltration. Marked congestion. Patchy degenerative fatty infiltration. Congestion. Hemorrhage. Abundant cortical lipids. Serous atrophy of peri-adrenal fat. Congestion. Congestion. Edema. Emphysema and patchy atelectasis. The so-called "growths" are cystic epididymi and not tumors.
				Comments: Cachexia.	
	17	$3R_1$	Died suddenly with 2 large hairballs in region and food in cardiac region of stomach. 25 Aug 55	(Pathologist's report not yet available)	
Experimental Females	18	$6R_1L_1$	Respiratory infection 19 Jan 55	(See Progress Report No. 2, p. 23)	
	19	$4R_2L_3$	Series of at least 3 mammary tumors 9 Feb 55	(See Progress Report No. 3, p. 32)	
	20	$4R_1L_3$	Lateral tumor 23 Mar 55	(See Progress Report No. 3, p. 32)	
	21	$2R_2$ 347LBH	Large uteral tumor 28 June 55	Heart Liver Lungs Kidney Spleen Colon Voluntary Muscle Esophagus Bone Uterine Tumor	Negative. Minimal degenerative fatty infiltration. Numerous lipophages. Dilatation of bronchi. Acute passive congestion. No lipoidosis. Large lymph follicles. Fecal material in the lumen. Abundant mucin. Negative. Abundant keratohyalin on the surface. After decalcification: With bone marrow about 60% cellular. A deciduoma. Found also in 341LBH, female control rat 1L.
	22	$4R_1L_1$ 1112LBH	Posterior, ventral tumor above endoderm. Slight-to-moderate respiratory infection for 4 months preceding death. Two hairballs, approx. 3/8 inch in diam, in stomach. 7 Aug 55	Heart Liver Ovary Kidneys Small Intestine Lungs Spleen Tumor	Negative. No lipoidosis. Negative. Advanced post-mortem change. Serous atrophy of fat. Post-mortem change. Congestion. Patchy emphysema and atelectasis. Foci of erythopoiesis. Adenofibroma of mammary gland.
				Comment: Cachexia.	
	23	$2R_1L_2$ 1111LBH	Animal emaciated and eyes inflamed before death. Slightly enlarged spleen and kidneys; 2 hairballs, approx. 1/4 inch in diam, in stomach. 9 Aug 55	Heart Kidneys Liver Adrenals Lungs Spleen Omentum Small Intestine Ovary Fallopian Tube Uterus	Patchy degenerative fatty infiltration. Patchy degenerative fatty infiltration. Congestion. Well-marked degenerative fatty infiltration. Congestion. Cortical lipids abundant. Intense acute passive congestion. Hemorrhages. Bronchietasis. Congestion. Marked degree of serous atrophy of fat. Post-mortem change. Negative. Negative. Negative.
				Comment: Marked degree of cachexia.	
	24	$6R_1$	Listless, eyes light in color. Abnormal kidneys, heart, intestinal tract, and ovary; also 2 hairballs about 5/8 inch in diam in stomach. 26 Aug 55	(Pathologist's report not yet available)	
	25	$4L_2$	Three hairballs in stomach. 30 Aug 55	(Pathologist's report not yet available)	
	26	$8R_1L_1$	Large anterior ventral tumor and history of respiratory infection. Hairball approx. 1/4 x 1 inch found in stomach. 6 Sept 55	(Pathologist's report not yet available)	

TABLE XI (concluded)

Group	Case No.	Animal and Report No.	Observation and Date Noted	Histopathological Report: Tissue	Histopathological Report: Observation
Control Females	27	$3R_1L_1$	Mammary tumor 2 Feb 55	(See Progress Report No. 3, p. 32)	
	28	$1R_1$	Sudden, severe weight loss followed by death. 9 Apr 55	(See Progress Report No. 3, p. 32)	
	29	$1L_2$	Boil on neck 10 Mar 55	(See Progress Report No. 3, p. 32)	
	30	$1L_1$ 341LBH	Large uteral tumor 26 June 55	Heart	Dilatation of vessels. Congestion. No lipoidosis.
				Lungs	Atelectasis. Patchy emphysema. Increase in leukocytes in the blood stream. Bland emboli in arteries. No fat emboli.
				Esophagus	Abundant keratinization of epithelium.
				Stomach	Post-mortem change.
				Spleen	Hyperplasia of lymph follicles. Numerous megakaryocytes.
				Kidneys	Congestion. Post-mortem change. No lipoidosis.
				Liver	Well-marked degenerative fatty infiltration.
				Tumor (Uterine)	This is a deciduoma. Deciduoma has been produced in experimental animals by the injection of substances such as progesterone.
				Fallopian Tube	Negative.
	31	$1R_3$ 1109LBH	Died suddenly 20 July 55	Liver	Well-marked degenerative fatty infiltration.
				Heart	Negative.
				Kidneys	Advanced post-mortem change.
				Lungs	Congestion. Edema. Patchy atelectasis. No fat emboli. Dilatation of bronchi.
				Ovary	Normal.
				Small Intestine	Post-mortem change.
				Adrenals	Negative.
				Spleen	Foci of erythropoiesis.
				Adipose Tissue	Serous atrophy.
				Comment: Cachexia. Inanition.	
	32	$3R_1$ 1106LBH	Mammary tumor first noted 23 May. 25 July 55	Kidneys	Congestion. A few cystic tubules. Marked degenerative fatty infiltration.
				Spleen	Foci of erythropoiesis. Post-mortem change.
				Stomach	Post-mortem change.
				Pancreas	Some islets are of large size.
				Small Intestine	Post-mortem change.
				Mammary Gland	Adenofibroma. No malignancy.
				Adrenals	Post-mortem change. Serous atrophy at periadrenal fat.
				Heart	No lipoidosis.
				Liver	Acute passive congestion. Marked degenerative fatty infiltration. Black granular pigment in the stellate cells of Kupffer.
				Lungs	Congestion. Edema. Hemorrhage. Patchy atelectasis. Bronchiectasis. Bronchiectatic abscesses. Chronic fibroid pneumonia. Bone marrow giant cell emboli. No fat emboli.
				Large Bronchi	Dilatation. Chronic mucopurulent bronchitis.
				Bronchial Lymphnodes	Congestion. Serous atrophy of fat.
				Comment: Bronchiectatic abscesses. Cachexia. Marked degenerative fatty infiltration of liver and kidneys.	
	33	$1R_2L_2$ 1107LBH	Increasingly severe respiratory infection in 4 months preceding death; slow weight loss, abnormal lungs, and small nodules on intestines. 5 Aug 55	Heart	Slight degenerative fatty infiltration.
				Ovary	Developing ova. Corpora lutea.
				Lung	Emphysema. Patchy atelectasis. Severe active chronic purulent pneumonitis with bronchiectasis and bronchiectatic abscesses.
				Liver	Well-marked degenerative fatty infiltration
				Spleen	Congestion. Foci of erythropoiesis. Hyperplasia of lymph follicles.
				Kidneys	Post-mortem change. Scattered dilated tubules. Slight patchy degenerative fatty infiltration.
				Comment: Severe chronic purulent pneumonitis with bronchiectatic abscesses. Cachexia.	

pathology data on all but eight of these animals. It is a continuation of Table XII in Progress Report No. 3, and the histopathological reports of animals lost previous to the last report are presented there. The eight animals not listed in Table XI in this report are those which died since the table was prepared.

Out of the 33 animals listed in Table XI, 17 (ten males and seven females) were control and 16 (seven males and nine females) were experimental animals. Among the 24 animals lost since the last report, 17 animals (nine control and eight experimental) died with hairballs. There is little in these data to indicate that irradiation of the diet has played a part in these pathological occurrences.

The following comments apply to animals listed in Table XI whose histopathological reports became available since the time of writing the last progress report.

Among five control males, hairballs were found in three upon death.

One was sacrificed with an inflammation of the parotid gland and a spindle cell sarcoma adjacent to the parotid gland. The fifth was sacrificed because of severe weight loss; the pathologist's report was summarized as malnutrition.

Of two experimental males, one appeared to have died of a pyogenic infection and the other was sacrificed because of a ventral abscess.

Of four control females, two bore tumors and were sacrificed; one of these females also had a severe respiratory infection. Of the other two, one died of inanition and the other of bronchiectasis.

Of three experimental females, two bore tumors. One of these also had a large hairball in its stomach and severe respiratory infection. The third animal had hairballs in its stomach.

Figure 11 is a continuation of Fig. 13 of Progress Report No. 3, which is a graphical presentation of the overall pathological status of the long-term rat experiment. Very few males in either group are now free of any affliction. The incidence of respiratory infection has increased among the control males and observations of unclassified diseases (infections, listlessness, etc) have also increased. The increase in pathological occurrences among the experimental males appears chiefly in the category of unclassified diseases. Both groups of females appear particularly afflicted with tumors, but few respiratory infections or unclassified diseases have appeared in either group.

Figures 12 and 13 show a control and an experimental female rat, respectively, each of which bears an unusually large mammary tumor. These animals will be sacrificed shortly, and the pathological observations will appear in the next report.

B. The Wheat-Feeding Experiment

1. INTRODUCTION

Insect infestation of stored grain, flour, cereal meals, and other cereal products results in a great annual loss of these staple food items. An extensive series of tests was reported by Baker, Taboada, and Wiant[15,16] in "The Lethal Effect of Electrons on Insects Which Infest Wheat, Flour, and Beans." As a result of these studies, the following conclusion, among others, was reached: "An electron dose of 10,000 rep will sterilize flour beetle and granary weevil eggs, and this same dose will prevent the adults from reproducing." Goldblith[17] made the observation that a given ionizing radiation dosage produces similar biological effects whether from electrons or from gamma radiation. It is concluded that a gamma radiation dose of 10,000 rep might be sufficient for control of insect infestation of screened flour.

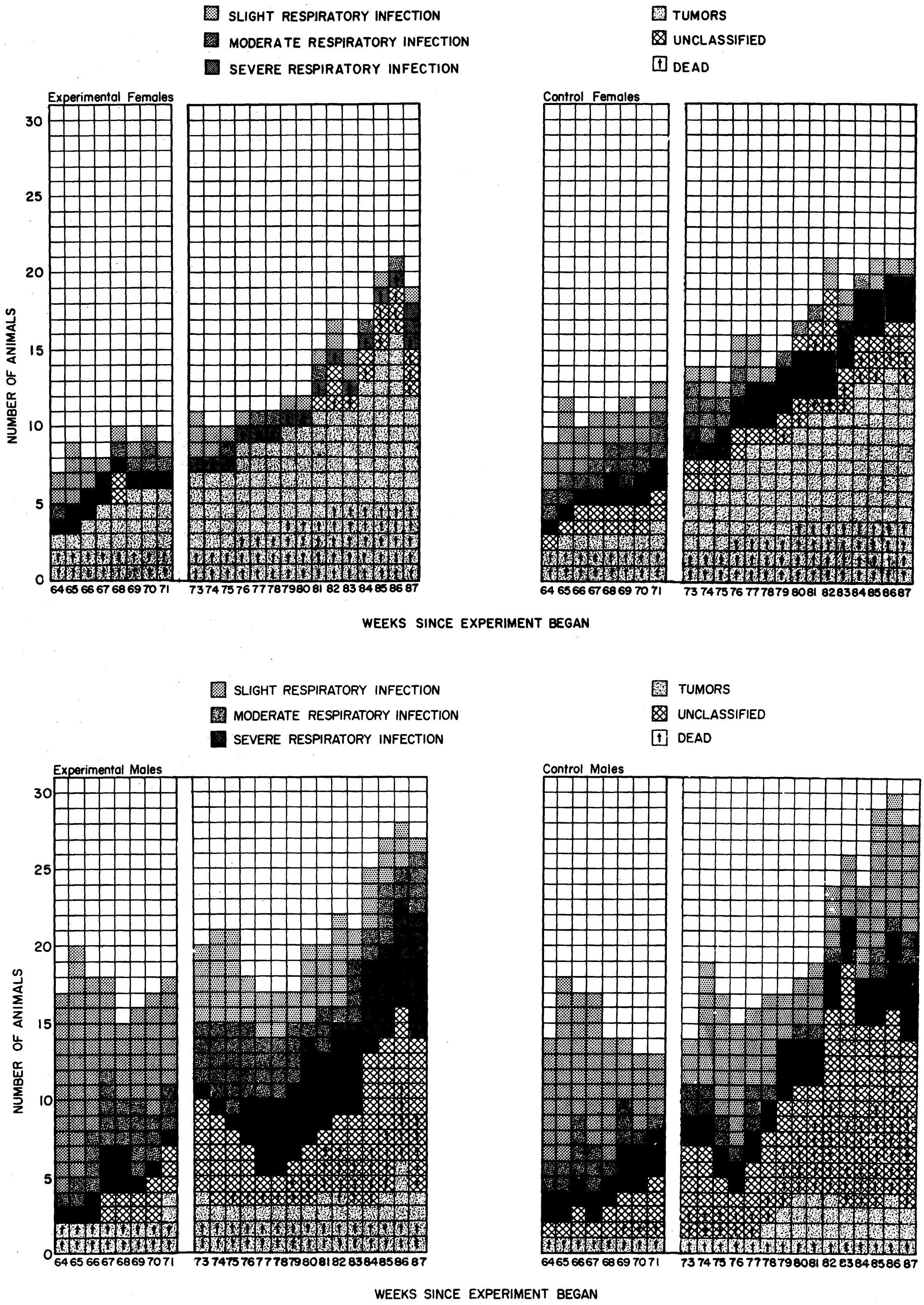

Fig. 11. Summary of weekly pathologic status of parent-generation rats on the long-term feeding experiment.

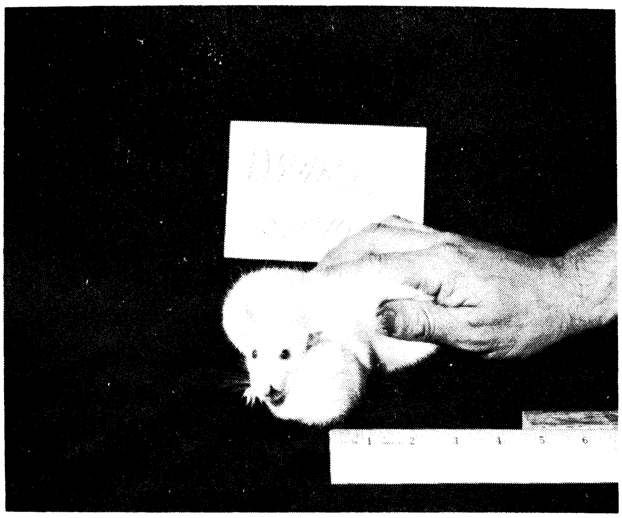

Fig. 12. Experimental female parent rat with two large mammary tumors prior to sacrifice and autopsy on September 7, 1955.

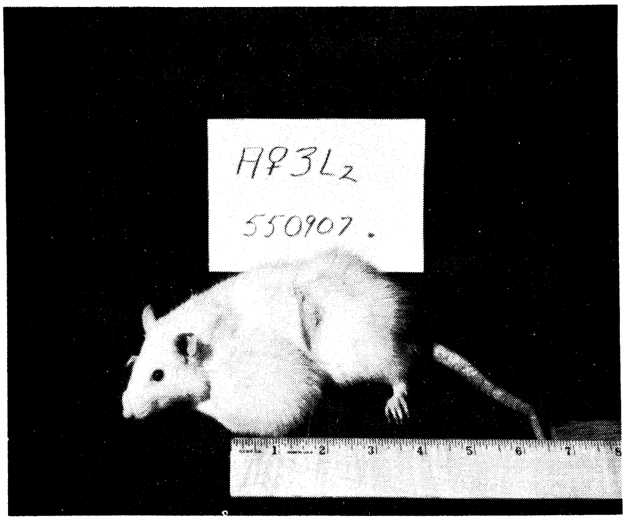

Fig. 13. Control female parent rat with large mammary tumor prior to sacrifice and autopsy on September 7, 1955.

Brownell, et al.,[18] have designed a gamma irradiation facility for this purpose and have shown its economic feasibility. The question of wholesomeness of wheat receiving this low dose must also be demonstrated to provide assurance that the wheat or flour receiving a dose of 10,000 rep is free of toxicity. The observations to date indicate that animals fed a diet receiving four million rep of gamma radiation have normal growth, reproduction, longevity, etc. However, a food receiving only 1/400th of this dose and intended for consumption by humans should be tested directly. For this reason an experiment was recently initiated at the Fission Products Laboratory with support by Michigan Memorial-Phoenix Project No. 41 to determine the wholesomeness of 10,000 rep of gamma irradiated wheat using albino rats as the experimental animals. The plan of the experiment and a description of its initial phases follow.

2. EXPERIMENTAL DESIGN

a. Objectives.—Previous work has shown that thiamin, riboflavin, pantothenate, pyridoxine, folic acid, and thiotic acid in aqueous solution are altered structurally by less than 1 megarep of gamma radiation, while a dose of 2 megarep was required to produce changes in niacin.[19] This effect was not brought about when the vitamins were irradiated in the dry state, indicating that the free radicals produced in water by ionizing radiation were responsible for the inactivation of the vitamins. Because water is present in nearly all food materials, it is now anticipated that some vitamin destruction might occur in foods exposed to gamma radiation. For this reason the diets which receive 3 to 4 megarep of radiation in the wholesomeness studies receive a supplement of all vitamins at their recommended allowances.

However, the low dose given to wheat provides an opportunity to determine whether or not vitamins may remain unaffected at a dose of gamma radiation which, while low, is expected to have promising uses. The study of wheat in this respect is also opportune inasmuch as what is believed to be a highly radiosensitive vitamin, vitamin E, is abundantly present in the oil of the wheat germ. Thiamin, riboflavin, pyridoxine, folic acid, and biotin are also radiosensitive, but since the moisture content of the whole-grain wheat is low (approximately 12%), it is believed that these vitamins would be less vulnerable to radiation than vitamin E. Vitamin E is the vitamin most directly involved in reproductive processes. A dietary deficiency of this vitamin makes male animals irreversibly sterile and female animals unable to produce young. Therefore, a reproductive study with rats using irradiated wheat is an excellent criterion for determining, in addition to freedom from toxicity, whether or not the nutritional values in wheat remain unchanged when the whole wheat grain is treated with 10,000 rep of gamma radiation.

Thus, the primary objective in the plan of this experiment is to devise a diet which consists of as large a proportion of wheat as possible and which does not provide more than the recommended allowance of any of the individual nutrients. The second objective is to design the experiment so that it will yield as much data on reproduction as possible. A third objective is to minimize the time and expense involved in managing the experiment.

b. Diet.—Whole-grain wheat does not by itself constitute an adequate diet for the rat. It has too low a protein content (approximately 12%), and the protein is deficient in the essential amino acid lysine. Calcium, sodium chloride, iron, vitamin A, riboflavin, and possibly some of the other B vitamins are also not supplied in adequate amounts by a diet consisting solely of whole wheat. Milk protein is frequently used to supplement the proteins of cereals, because it supplies adequate amounts of lysine and tryptophane. Skim milk powder, besides increasing the protein level, would also supply some of the minerals. In addition, it would raise the level of those B vitamins, especially ribovlavin, not adequately supplied by the wheat. Table XII lists the recommended dietary requirements of the rat, the composition of whole wheat and of dried skim milk, and the composition of the experimental diet consisting of 70% whole wheat, 15% skim milk powder, 14% casein, and a supplement of calcium, salt, and vitamin A. The level of skim milk powder does not supply an excess of B vitamins.

The wheat is irradiated in the form of the whole, intact grain inasmuch as this is the form which would be irradiated commercially. To prepare the dietary mixture, advantage was taken of the adhesive property of the casein in milk and the glutin in wheat to form a cake or biscuit which would be easy to feed. This required that the wheat be ground coarsely after irradiation, in which form it was believed to be more acceptable to rats. A laboratory-scale power-driven burr mill manufactured by the Laboratory Construction Corporation was purchased for this purpose.

A batch (5.4 kg) of wheat diet is made by mixing the following in a Hobart mixer:

Coarsely ground wheat	2800 grams
Casein	560
Skim milk powder	600
Calcium carbonate	30
Sodium chloride	10
Water	1400
	5400 grams

Mixed in this fashion the diet is a thick paste which is spread out in a layer about 3/4-inch deep. This is warmed gently under infrared lamps until the cake is hard and dry. It is then broken up into small pieces and stored in the refrigerator until fed.

c. Housing and Manner of Feeding.—Figure 14 is a top view of two animals on the wheat experiment in a standard rat cage. A hopper made of 2 x 2 light-gauge hardware cloth has been fastened inside the front screen. The pieces of wheat diet are placed inside this wire cloth, and the rats are able to reach and chew the diet through the cloth openings without difficulty. This arrangement keeps the wheat diet off the cage bottom, minimizes wastage by allowing the rat access only to a small bit at a time, and makes it very simple to replenish the supply of diet. Recently a manufactured type of hopper, which permits solid bottoms to be inserted into the cages for breeding purposes, was substituted.

d. Selection of the Animals.—The animals consisted of the first crop of third-filial-generation rats from the long-term rat feeding and breeding experiment. Twenty females and 12 males were used on the irradiated wheat diet and an equal number on the control wheat diet. The animals in each group were drawn from both control and experimental litters in the long-term rat experiment. For a period of about two weeks following their weaning they were fed a regular laboratory biscuit prior to being fed the wheat diet.

TABLE XII

COMPOSITION OF THE WHEAT DIET

Nutrients Required by the Rat	Req't by Rat per 100 g of Diet (3)	Content in 100 g of Whole Wheat	Content in 100 g of Skim Milk Powder	Diet Composition; Contribution to 100-g Diet by			
				70 g of Whole Wheat	15 g of Milk Powder	Supple-ment	Total
Protein, g	30 (4)	12	37	8.4	5.5	14.0 (12)	27.9
Fat, g		1.7	1.0	1.2	.15	---	1.35
Carbohydrate, g		75.5	51 (9)	53	7.5	---	61
Calories		360	225	252	34	63 (12)	349
Calcium, g	0.5	.057	1.33	0.04	.20	0.3 (13)	0.54
Potassium, g	0.5	.47	1.6	0.33	.24	---	0.57
Sodium, g	0.25	.06	0.6	0.04	.09	0.1 (14)	0.23
Magnesium, g	.04	.170	0.133	0.12	.02	---	0.14
Phosphorus, g	0.4	.37	1.0	0.26	.15	---	0.41
Manganese, mg	4	4.6	(10)	3.2	Negl	---	3.2
Iron, mg	25	5.7		4.0	Negl	20 (15)	24
Copper, mg	0.5	0.8		0.6	Negl	---	0.6
Vitamin A, I.U. (1)	100	20-25	(11)	14-17	Negl	200 (16)	200
Vitamin E, mg	25	(8)		(8)	Negl	---	(8)
Vitamin D, I.U.	(5)	---		---	Negl	---	---
Thiamin, mg	0.6	.56	0.38	.39	.06	---	.47
Riboflavin, mg	0.6	.12	2.0	.08	.30	---	.38
Niacin, mg	(6)	4.0	1.0	2.8	.15	---	2.95
Pantothenate, mg	(7)	1.3		.9		---	(17)
Pyridoxine, mg	0.15	.21		.15		---	> .15
Folic acid, mg	(7)	.19		.13		---	(17)
Biotin, μg	(7)	5.0		3.5		---	(17)
Cyanocobalamin, μg (2)	(7)					---	(17)
Inositol, mg	(7)	170		120		---	(17)
Choline, mg	75	90		63		---	> 63

(1) 1 I.U. is equivalent to 0.2 micrograms vitamin A alcohol or 0.6 micrograms beta-carotene.
(2) Vitamin B_{12}.
(3) Reference: Farris, E. J., and Griffith, J. Q., Jr., ed., The Rat in Laboratory Investigation, (by R. H. McCoy), Chapter 5, J. B. Lippincott Company, 1949.
(4) There is evidence that rats can show slightly better growth with 40% protein, but 30% is considered adequate.
(5) Rats do not require vitamin D in their diet if the Ca:P ratio is 1:1 to 2:1 and if the phosphorus level is 0.4%. The wheat diet contains the necessary amounts of calcium and phosphorus.
(6) Niacin or nicotinic acid is apparently not required by the rat.
(7) Requirements not established.
(8) The oil of the wheat germ is an excellent source of vitamin E.
(9) Mostly lactose.
(10) The values are not available but are known to be extremely low in milk.
(11) The fat-soluble vitamins are mostly removed with the butterfat.
(12) Furnished by 14 g Borden's LabCo Brand biologically assayed vitamin-free casein per 100-g diet.
(13) Furnished by 0.75 g C.P. calcium carbonate per 100-g diet.
(14) Furnished by 0.25 g C.P. sodium chloride per 100-g diet.
(15) Furnished by 90 mg of ferric citrate per 100-g diet.
(16) Supplied weekly by 0.1 ml of a solution consisting of 2,000 I.U. vitamin A acetate per cc of corn oil.
(17) Although these totals are not known at present, experience has shown that if several of the B vitamins are supplied in adequate amounts by a dietary source such as cereals and milk, the remainder of the B vitamins are also supplied at adequate levels.

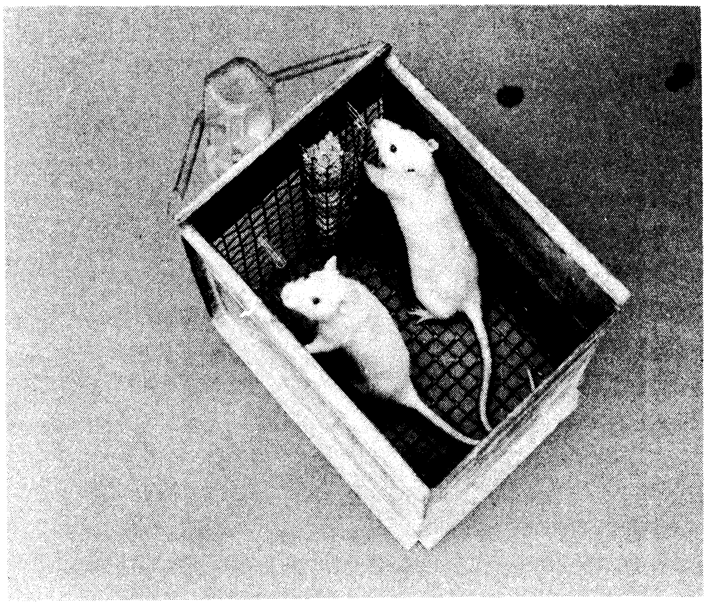

Fig. 14. View of cage with special container for the wheat diet. These two rats on the wheat experiment are able to eat the diet through the wire. This arrangement facilitates feeding and keeps the diet off the cage bottom.

3. GROWTH CURVES TO DATE

Figure 15 shows the growth of these animals from the beginning of the experiment to the time of writing of this report. The males and females on each of the two diets are shown in separate curves. There is no distinguishable difference in the rate of growth between the animals on the two diets.

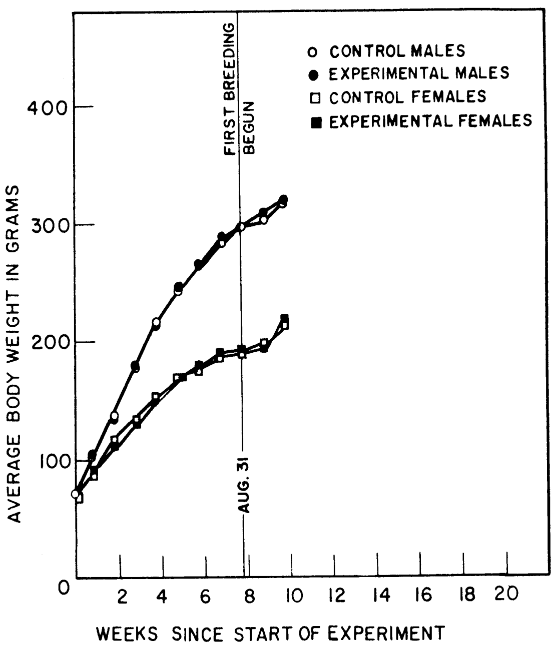

Fig. 15. Growth curves of the male and female rats on the control and irradiated wheat diet.

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