ENGINEERING RESEARCH INSTITUTE
THE UNIVERSITY OF MICHIGAN
ANN ARBOR

Progress Report No. 10

WHOLESOMENESS OF GAMMA-IRRADIATED DIETS

Period December 15, 1956 to October 15, 1957

L. E. Brownell
C. H. Burns
K. A. Kleyn
R. L. Kruger
Fission Products Laboratory

Project 2307

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

October 1957

NOTE

Final reports on the following four feeding experiments will appear in a separate report during the month of December, 1957:

(1) Long-term feeding experiment on 4-megarep irradiated canned beef, using rats;

(2) The Mouse Reproduction Study, using the irradiated beef diet above;

(3) The Rat Reproduction Study on Irradiated Wheat; and

(4) The long-term experiment on 3-megarep irradiated wet mash, using chickens.

ABSTRACT

Progress is reported herein on the long-term feeding experiment on irradiated potatoes using rats for the period 15 December 1956 to 15 October 1957. No important differences have been noted during this period with respect to irradiation of the potatoes in the diet on the basis of the following criteria: (a) growth rate and maintenance of body weight of three generations of animals, (b) efficiency of food utilization by second-generation animals, (c) reproductive performance through two breedings of second-generation animals, (d) blood cell counts of first-generation animals during the tenth month of life, and (e) observation of gross pathological abnormalities.

With respect to mortality, a vascular disease has been observed in about half of the animals which have died or were sacrificed, and in most of these cases the disease was severe enough to have caused death. This disease (a) bears no relation to irradiation of the potatoes in the diet, (b) is aggravated by the potato diet, but not by potato decay present in the diet, and (c) is genetically influenced.

With respect to mortality of animals not involved with vascular disease, the incidence among animals fed diets containing irradiated potatoes is two to three times that of animals fed the control diet. No particular pathology appears involved, nor can all this mortality be attributed to the inclusion of some potato decay in the diets during the early phase of the experiment.

OBJECTIVE

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

POTATO-FEEDING EXPERIMENT

A. PRESENT STATUS OF THE EXPERIMENT

The first-generation rats on the two-year sub-acute toxicity study of irradiated potatoes are in their nineteenth and twentieth months. The third round of blood cell counts on the animals regularly used for this purpose is now being made.

Tissue slides, paraffin blocks, and complete reports are available for 33 animals in this generation which have died or were sacrificed, not including 12 extra-littermate animals. Tissue slides and blocks, but not reports, are available for 25 additional animals, not including 5 extra-littermates. This material, except for some of the paraffin blocks, will be sent to the A.F.I.P. during the first week of November. Of the above 58 animals, 27 showed a vascular disease, and the paraffin blocks of tissues from these animals are being sent to MNL in Denver.

Because the stores of irradiated potatoes for this experiment will not be sufficient to complete the experiment as planned, it is proposed that replicate groups of animals be terminated systematically when (a) mortality reaches 60% in any one replicate, in which case the survivors in that replicate will be sacrificed, and (b) when mortality reaches 100% in any one group in one replicate, in which case the survivors of the same sex in that replicate will be sacrificed. It is also proposed that, when the stores of Maine Katahdin 2X potatoes are exhausted, to eliminate this variety from all diets, and when the 2X Idaho Russets are exhausted, to sacrifice all survivors on the 2X diet.

Robert Berkman, D.V.M., graduate student in veterinary pathology at Michigan State University, will conduct most of the autopsies involved in the termination of this experiment. Blood cell counts will be made on all animals as shortly before sacrifice as possible.

The second-generation animals have been retained on the experiment and are being fed the potato diet. The potatoes are either Maine Katahdin or Maine Russet Burbanks and are not irradiated.

In June the second-generation animals completed both breeding periods, and members of the third generation were selected from their second litters. Tissue slides, paraffin blocks, and complete reports are available for the seven animals which died or were sacrificed while on the irradiated potato diets. The paraffin blocks have been sent to MNL in Denver and the slides and reports will be sent to the A.F.I.P. in the first week of November.

Following this period, these animals were continued on the potato diet, but nonirradiated Maine varieties from the 1956 crop were substituted for the irradiated potatoes. These animals are being used for experimental studies on the vascular disease.

The third-generation animals are now undergoing their first breeding. Efficiency-of-food-utilization measurements were not made on these animals as on the first two generations. Instead, members from the first litters of these animals will be placed in standard metabolism cages and food-efficiency measurements will be made with greater accuracy than before.

B. QUANTITATIVE DATA

1. Body Weight of First-, Second-, and Third-Generation Animals

Figure 1 shows the growth curves for the three generations of animals in the seventeenth month of the experiment. The mortality among first-generation males makes comparisons difficult. The 1X and 2X groups, but not the control, may be entering the period of gradual weight loss which continues until death occurs. The first-generation females continue to gain weight. In the second generation, the 1X males appear to be doing the best. The male and female groups in the third generation show no differences.

2. Efficiency of Food Utilization by Second-Generation Animals

Table I shows data for ten males and twenty females fed each of the three diets for the last six of the twelve weeks during which food efficiency was measured. Data for the first six weeks will be presented later.

The efficiency of the 1X males and females is somewhat greater than that of the 2X animals, but with respect to the controls there appear to be no important differences due to irradiation of the diet.

3. Reproductive Performance of Second-Generation Animals

Table II gives the data on the second-generation animals through both breeding periods. Only 70% of the 1X females during each breeding actually gave birth, whereas at least 95% of the 0X and 2X females did so. In all three groups, the percent of pups alive one day after birth which were weaned was very low during the first breeding and only a little better during the second. All other values appear to be normal. Weaning weights were high, due partly to the fact that the pups have access to the mother's diet before being weaned.

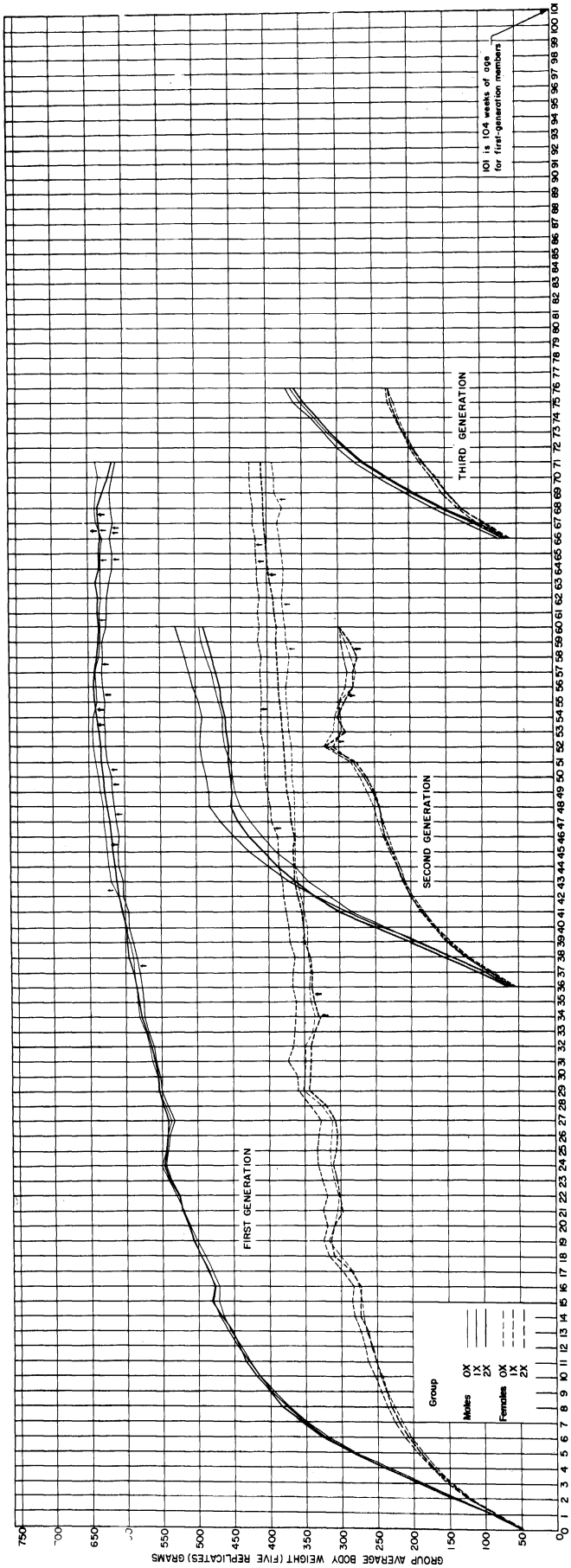

Fig. 1. Growth curves of three generations of rats on the long-term feeding experiment with irradiated potatoes.

TABLE I

Efficiency of Food Utilization by Second-Generation Animals*

Week Following Weaning	Males			Females		
	0X	1X	2X	0X	1X	2X
7	.20	.25	.20	.11	.12	.11
8	.13	.21	.17	.07	.08	.08
9	.19	.19	.15	.12	.09	.10
10	.15	.15	.14	.10	.08	.10
11	.12	.14	.11	.07	.08	.03
12	.11	.10	.07	.06	.05	.04
Average	.15	.17	.14	.088	.083	.077

*Figures are grams gain in body weight per gram diet solids consumed, and are averages of ten males or twenty females.

C. PATHOLOGY DATA

1. Observations Made at Weekly Inspection of Animals

Table III is a summary of the observations made from the thirty-sixth to the seventy-second week as a result of the weekly inspection of each first-generation animal for gross pathology. The maximum-minimum values help to interpret the average; for example, one animal exhibiting a middle-ear infection for ten weeks contributes to the average the same value as ten animals having the infection at one time.

None of these abnormalities appears to be related to irradiation of the potatoes in the diets. In most cases, the incidences among control males are highest; in half of the cases, incidences among control females are the highest.

2. Blood Cell Counts on First-Generation Animals, Second Round, February, 1957

Table IV presents blood cell counts made in February, 1957, on the same animals used in August, 1956, except for one control male which died. Another control male had an abscess at the time; its white blood cell count was 23,000/mm^3 and this was not included in the average for the four other males in that group.

There are no important differences among the groups on the basis of these counts.

TABLE II

Reproductive Performance of Second-Generation Animals

Group:	0X		1X		2X	
Breeding:	1st	2nd	1st	2nd	1st	2nd
1. Number of females bred	20	19(3)	19(1)	18(2)	20	18(4,5)
2. Number of males used	10	10	10	10	10	9(6)
3. Percent of females bred which appeared to become pregnant	100	94.7	84.2	77.7	100	94.4
4. Percent of apparently pregnant females which gave birth	100	100	87.5	85.7	95.0	100
5. Average number of pups born per litter	10.9	9.8	10.3	9.2	11.1	9.8
6. Percent of pups born surviving birth and the first day after birth	91.3	93.8	92.4	92.7	90.0	97.6
7. Percent of pups surviving the first day which reached 21 days (weaning)	57.0	83.2	62.6	71.8	65.4	72.5
8. Average body weight of pups at 7 days		15.0		14.5		15.1
9. Average body weight of pups at 14 days		30.4		30.6		31.7
10. Average body weight of pups at weaning	47.3	51.1	49.6	53.5	44.9	54.4

1st breeding Number of pups disposed on the 5th day:
- 0X - 15
- 1X - 3
- 2X - 18

2nd breeding Number of pups disposed or added to litters of less than 10 pups on the 7th day:
- 0X - 7
- 1X - 3
- 2X - 12

(1) 1X ♀ 16R4 sacrificed 570313 after bred for 3 weeks and 3 days—1st breeding.
(2) 1X ♀ 3R1 sacrificed 570515 after bred for 3 weeks and 4 days—2nd breeding.
(3) 0X ♀ 27R1 died 570509 after bred for 2 weeks and 6 days—2nd breeding.
(4) 2X ♀ 56R2 sacrificed 570419—at the first day of 2nd breeding.
(5) 2X ♀ 74R5 sacrificed 570415—after 1st breeding.
(6) 2X ♂ 74R5 died 570519 after bred for 1 week and 2 days—2nd breeding.

TABLE III

Average, Maximum, and Minimum Incidence of Gross Pathology
from 36 to 72 Weeks of Experiment

Gross Observation	Diet	Males			Females		
		36-wk avg	Min during 36-wk period	Max during 36-wk period	36-wk avg	Min during 36-wk period	Max during 36-wk period
Tumors and/or unidentified swellings	0X	1.6	0	3	0.9	0	3
	1X	0.3	0	3	2.1	0	4
	2X	0.0	0	0	0.2	0	1
Respiratory Infection							
Severe	0X	1.0	0	3	1.3	0	2
	1X	2.6	0	5	1.6	0	3
	2X	3.4	2	5	1.6	0	4
Moderate	0X	3.8	1	7	0.7	0	2
	1X	3.7	2	6	2.3	1	4
	2X	2.7	1	6	1.6	0	4
Other Inflamations							
Eyes	0X	3.1	0	6	2.5	1	7
	1X	1.1	0	5	1.3	0	3
	2X	1.5	0	3	1.9	0	4
Middle ear	0X	0.0	0	0	0.0	0	0
	1X	0.4	0	1	0.0	0	0
	2X	0.0	0	0	0.0	0	0
Nose	0X	0.3	0	1	0.10	1	2
	1X	0.4	0	2	0.08	1	1
	2X	0.8	0	3	0.06	1	1
Urogenital	0X	1.6	0	4	0.1	1	1
	1X	1.0	0	3	0.0	0	0
	2X	0.7	0	2	0.0	0	0
Abscesses and Sores	0X	2.7	0	10	2.0	0	5
	1X	2.8	1	5	1.9	0	5
	2X	1.9	0	6	2.1	0	6
Sensitivity to touch	0X	0.8	0	2	0.06	0	2
	1X	0.7	0	3	0.08	0	1
	2X	0.7	0	3	0.03	0	1
Other Abnormalities	0X	0.6	0	2	1.8	0	3
	1X	0.6	0	4	0.1	0	1
	2X	0.4	0	4	1.1	0	3

TABLE IV

Blood Cell Counts - Potato Rats - Second Round - February, 1957

(Each value is an average for 5 animals; figures in parentheses are minimum and maximum values in the group of 5 animals.)

Group	Hemato-crit, %	Hemoglobin, Grams %	Corpuscular Hemoglobin, %	White Blood Cells, Thou-sands per mm³	Differential Count: % of White Cell Count						Platelets
					P(1)	SL(2)	LL(3)	M(4)	E(5)	B(6)	
Males											
0X	50.0	15.9 (14.8-17.4)	31.8	13,150 (9800-18,400)	22 (17-30)	65 (63-73)	8 (2-15)	2 (1-3)	3 (2-5)	0	Adequate
1X	49.8	16.5 (15.3-17.4)	33.2	12,110 (8900-15,400)	26 (23-29)	60 (56-67)	8 (4-12)	4 (1-7)	2 (1-4)	0	Adequate
2X	48.9	15.1 (13.7-16.3)	30.9	13,100 (8800-19,000)	24 (11-40)	62 (55-70)	9 (3-15)	4 (2-8)	2 (1-4)	0	Adequate
Females											
0X	46.6	15.1 (14.4-16.3)	32.4	6280 (4000-9200)	32 (12-49)	58 (37-81)	6 (4-8)	2 (1-3)	2 (0-4)	0	Adequate
1X	47.4	15.4 (14.6-17.2)	32.5	6480 (4400-9100)	20 (12-31)	65 (51-78)	9 (3-14)	4 (0-7)	2 (0-5)	0	Adequate
2X	45.9	14.7 (12.7-15.7)	32.1	5850 (3600-8300)	25 (11-32)	65 (55-74)	8 (1-19)	1 (0-2)	2 (0-3)	0	Adequate

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

3. Mortality

a. Total incidence.—The incidence of mortality among first- and second-generation animals for nineteen months (up to 8 October 1957) is shown in Fig. 2. The mortality rate for all three groups of females in the first generation is essentially the same and ranges from 24 to 32%. Also, the mortality rate for control males is about the same as that for the females. But the rate for the males, on the 1X and 2X diets, is 2 to 2-1/2 times higher than that for the controls (52-60%). A linear projection of the mortality rate to 104 weeks of age for each of these groups would show no animals surviving at the time this experiment is to be terminated. For comparison it may be mentioned that the mortality rate for the extra-littermate males and females fed Purina Laboratory Chow (which is not shown in Fig. 2) is slightly lower than that for the males and females fed the nonirradiated potato diets.

The mortality rate for second-generation animals is also shown in Fig. 2. For the period during which they were fed the regular potato diets, one control animal (a female), three 1X animals (one male, two females), and three 2X animals (one male, two females) were lost. When the third generation had been weaned from the second, the entire second-generation colony was switched to a single potato diet containing one nonirradiated potato variety (Maine Katahdins or Maine Russet Burbanks from the same harvest as those used in the experiment). The mortality during the second phase (shown to the right of the vertical line in Fig. 2) has been one female formerly on the 0X diet, two males and three females formerly on the 1X diet, and two males and three females formerly on the 2X diet

The third generation of animals are 22 to 26 weeks of age, and none has been lost.

b. Incidence of a vascular disease.—Of the 52 first-generation animals lost to date, 29 or 56% were afflicted with lesions of the vascular system closely resembling what is called "peri-arteritis nodosa" when seen in humans. The severity of the disease at the time of death or sacrifice ranged from such mild cases as to be barely apparent at autopsy to severe cases causing a sudden and fatal hemorrhage. The occurrence of this disease apparently bears no relation to irradiation of the potatoes in the diet. Five of the six males in the control group (the sixth having died long before the disease was first noted in the colony), seven of the fifteen males in the 1X group, and eight of the thirteen males in the 2X group were found with the disease. Among the females, the incidence to date is four out of six in the control group, two out of nine in the 1X group, and four out of eight in the 2X group.

Except for a recent case, none of the sixteen members of the extra-littermate colony which died or were sacrificed was found to have this disease on the basis of either the gross or microscopic examination. In the second generation, on the other hand, fifteen of the eighteen animals lost were found to be afflicted with it, most of them severely enough to cause sudden death.

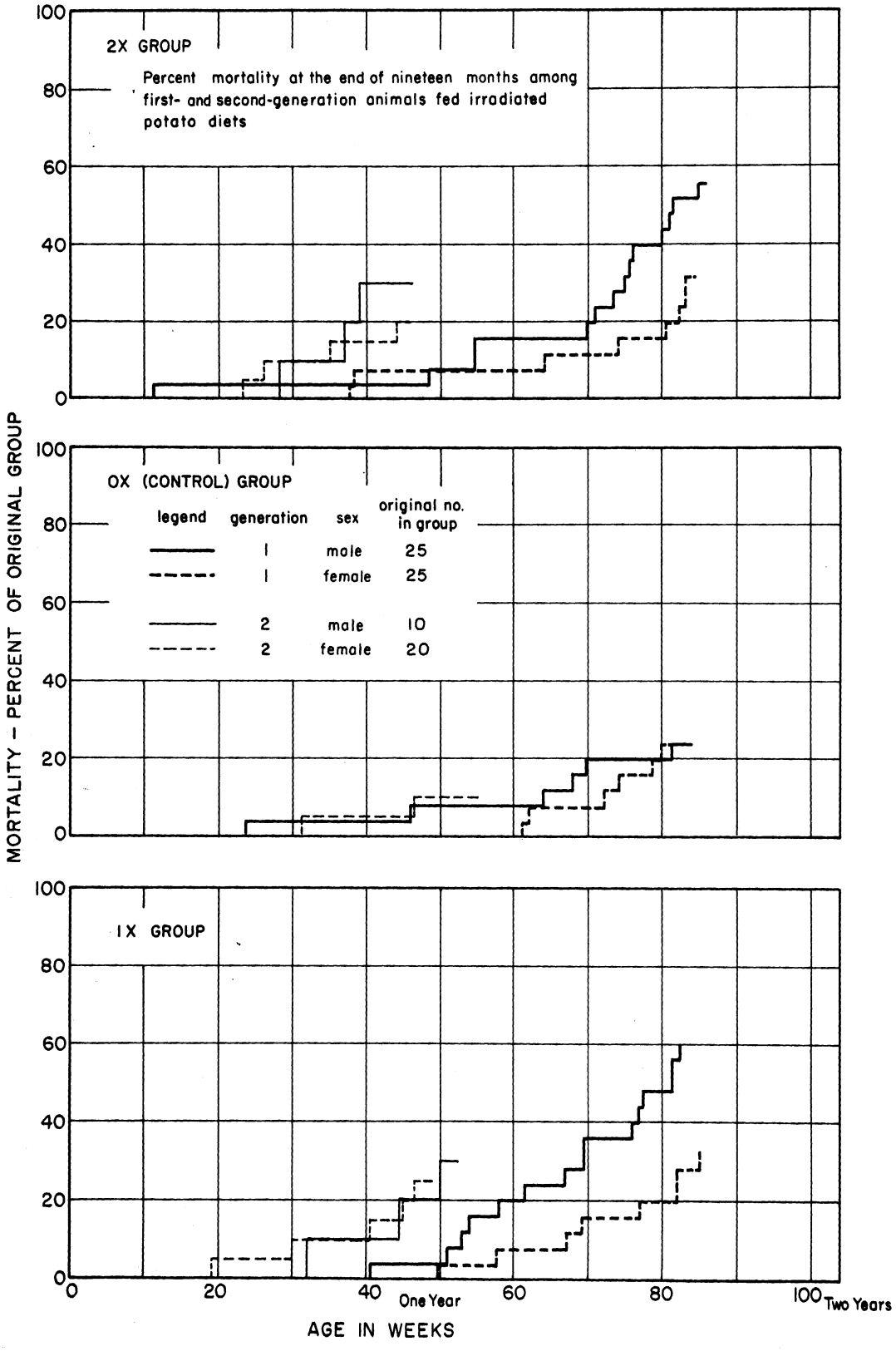

Fig. 2. Mortality among first- and second- generation rats in the long-term experiment with irradiated potatoes. The frame of each graph corresponds, vertically, to the number of animals in the group, and horizontally, to the proposed duration of the experiment. Each step upward in the graphs represents an incidence of mortality.

The disease involves the arteries of the gastro-intestinal tract, the focal point being the pyloric end of the duodenum, a point common to the portal vein, pancreas, stomach, and duodenum. From this point it usually extends down the small intestine and occasionally involves the colon; it frequently involves the arteries of the mesentery. It usually involves the arteries of the pancreas, microscopically if not grossly. The vascular systems of the stomach, the spleen, kidneys, and particularly the testes also have been involved. In a few cases the disease involved the diaphragm, esophagus, heart, trachea, and the adrenal glands.

The disease consists of nodular developments along an artery, giving the vessels a "beaded" appearance. The nodules vary in size from less than 1 to 5 mm in diameter. They are bluish or purplish red, and when crowded together have the appearance of a cluster of miniature grapes. Although invariably present on the duodenum in diseased animals, the nodule is never part of the intestinal wall itself. In older animals, some nodules become greatly enlarged (up to 1 x 2 cm) and assume bizarre shapes. Occasionally a series of nodules along a portion of the small intestine fuse to form a tortuous tissue running parallel to the intestine, often larger in volume.

Of the twenty-three cases of this disease found in both first- and second-generation animals for which the histopathological report has been made, the following table lists the frequency with which this disease has involved the blood vessels of various organs from both a gross examination at autopsy and microscopic examination of tissue sections.

TABLE V

Incidence of Vascular Lesions by Organs

Vascular System of	Gross	Microscopic
Heart	0	2
Trachea	0	1
Diaphragm	0	1
Spleen	2	2
Esophagus	0	1
Stomach	1	6
Pancreas	10	18
Duodenum	18	14
Jejunum	13	
Ileum		
Mesentery	8	8
Colon	2	6
Adrenal	0	1
Kidney	0	8
Testis	2	6
Uterus	0	2

Figure 3 illustrates the tendency for this disease to run in families. The eight animals in each horizontal row are littermates. The diet fed to each animal is shown across the top. The twenty-five rows or families are divided into five replicates of five litters each, the animals of one replicate being one week older than those of the next. The figure shows the animals which have died, their age at death in weeks, the number of animals in the second generation sired or cast by each first-generation animal, and of these how many have died, and the presence or absence of vascular lesions.

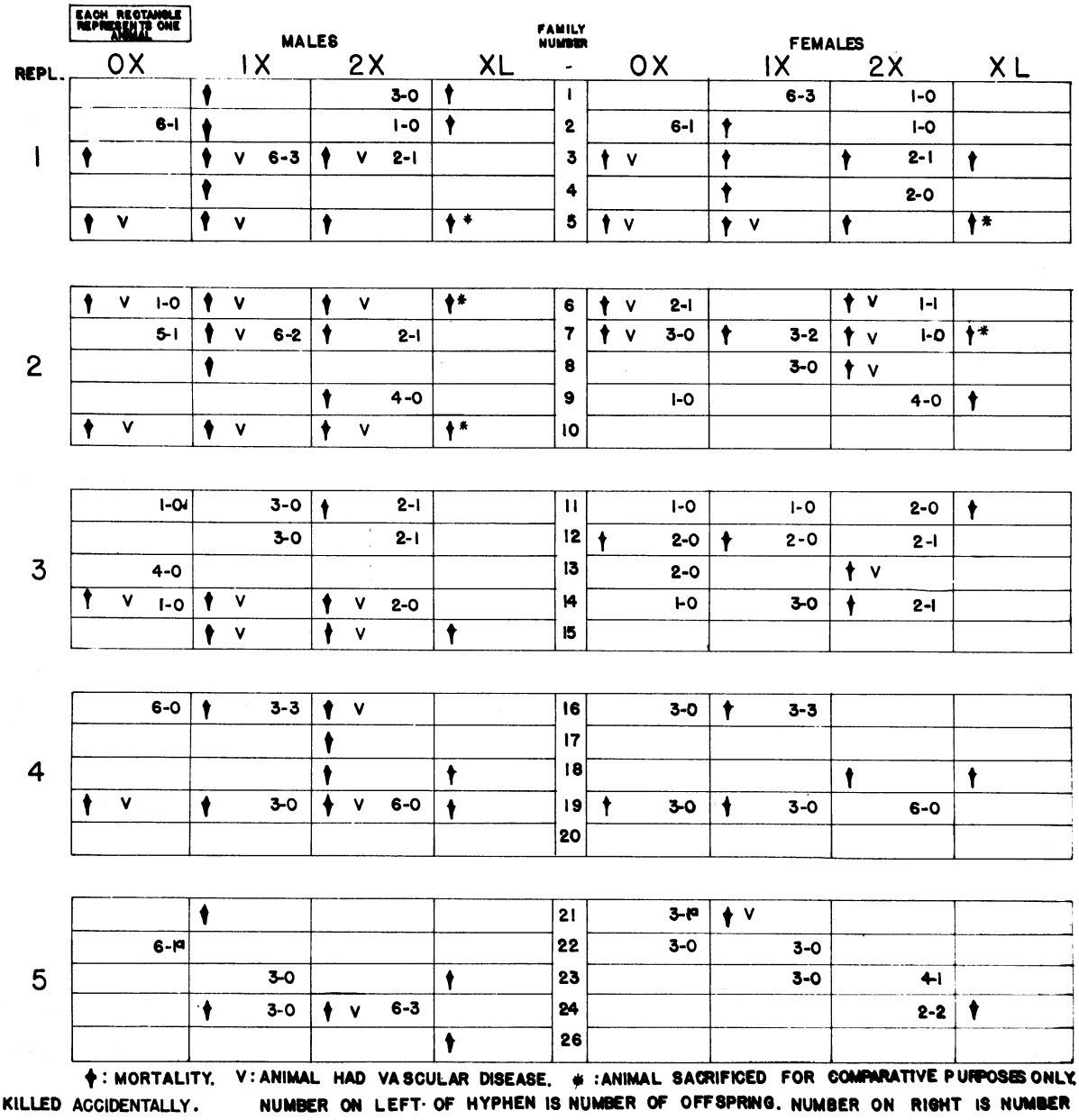

Fig. 3. Long-term rat-feeding experiment with irradiated potatoes. Mortality, incidence of vascular disease, offspring and mortality of offspring, by family and dietary group. 15 October 1957 (FPL Nos. 1-100 incl.).

It is apparent that the disease shows a tendency to run in families. Of the twenty-five families, five alone account for 21 of 28 cases. A random distribution of this number of cases in such a colony would show a mean of less than 1-1/2 members per family.

Further evidence of the genetic tendency is revealed by the fact that of the eighteen second-generation animals lost, sixteen of them had a parent which later died usually with the disease. In fact, examination showed that nearly every one of them was involved with the vascular disease, and the parent was usually a male (this corresponds to the fact that 71% of the cases of vascular disease in the parent generation involved males). Table VI shows the relation between paternity and second-generation mortality and how it varied with dietary group.

TABLE VI

Mortality in and Parentage of Second-Generation Colony

Group	% Mortality	% Having Fathers Since Dead of Vascular Disease	% Having Mothers Since Dead of Vascular Disease
0X	7	7	17
1X	27	40	0
2X	27	53	7

Of the first-generation males which happened to become fathers, a higher percentage in the 1X and 2X groups happened also to have the vascular disease. This correlates with the higher mortality among second-generation animals in these groups, almost all of which was due to vascular lesions. The percentage of mothers which had vascular disease at death was lower and did not correlate with the G-2 mortality.

In addition to the genetic factor involved in this disease, there appears to be a dietary one. It was mentioned that sixteen animals in the extra-littermate colony fed Purina Laboratory Chow died or were sacrificed but that none of these was found with vascular lesions, except possibly the most recent one. Of the sixteen, five were sacrificed because two or more of their brothers or sisters on the potato diets had been found with the disease. Since the disease runs in families, they were sacrificed to provide confirmation of its absence in animals fed Purina Laboratory Chow. An animal in this group recently died of a hemorrhage which originated from two giant nodules on the duodenum. The gross morphology of these lesions was somewhat different from vascular lesions seen previously; no other animal in this family has been lost.

In regard to the dietary variable, it is worth noting that the disease began occurring in the second-generation colony only a month or two after it appeared in the first generation, whereas the age difference is about eight months. This suggests that something in the potato diets sensitized genetically susceptible animals to the vascular lesions. That this dietary factor was not the potato decay included in the diets fed during the sixth and seventh months of the experiment is shown by the fact that the second-generation animals were born at least four or five weeks after potato decay was no longer included. The diet has remained substantially identical in composition and manner of preparation except for changes in the potatoes themselves, in the use of cod liver oil or Vitamin A acetate as a Vitamin A source, and in the replacement of the calcium carbonate in the diet by an equivalent amount of additional calcium phosphate.

This disease has been recorded in the literature, but the causes of it, such as advanced age and toxic Vitamin D levels, are far removed from conditions prevailing in this experiment. The predisposing factor may be nondietary, such as uremia (accumulation of nitrogenous waste products in the blood) due to kidney infection as is often the case with humans having peri-arteritis nodosa, or as viral pneumonia which is present in the great majority of animals at death. The second-generation animals, which were no longer necessary for the experiment once they had produced the third, were nevertheless retained in the experiment for the purpose of learning more about the vascular disease.

c. Incidence of other pathology.—Table VII is a brief summary of the prominent pathology observed in the first-generation animals at death. The number which was sacrificed and that which died are included to reflect the difficulty that has been encountered in anticipating death; the sudden hemorrhages caused by severe vascular lesions have accounted for the majority of deaths. The animal colony is checked frequently each 24-hour period, and thereby only a very few tissue specimens have been rendered useless by postmortem change.

Although the distinction between severe and mild vascular lesions is arbitrary, there is little to distinguish the three groups of males and the three of females fed the potato diets. The large difference among the dietary groups is with those which do not have the vascular disease. Only five of these animals died before the disease first appeared (one 0X, one 1X, and one 2X males and two 2X females). The remainder of the table is based on a somewhat arbitrary classification of pathology, and it includes animals that were involved with vascular lesions. Severe respiratory disease was present in males on the irradiated diet to a greater extent than in control males, but this distinction did not apply to the females. The incidences of middle-ear infection, nephritis, and tumors were not consistently greater among animals on irradiated diets. The usual observation in chronic toxicity experiments is that the toxic constituent aggravates the "natural" pathology of the test animal but does not cause a unique pathology of its own.

TABLE VII

Brief Summary of Prominent Pathology at Death of First-Generation Animals
Fed Irradiated Potato Diets
15 October 1957 (FPL Nos. 1-100 incl.)

	Males				Females			
	0X	1X	2X	XL	0X	1X	2X	XL
Original group size	25	25	25	25	25	25	25	25
Total mortality	6	15	14	10(1)	6	9	8	7(2)
Number sacrificed	4	6	7	6(1)	3	9	6	4(2)
Number which died	2	9	7	4	3	0	2	3
Number with:								
Vascular disease to some degree	5	7	8	1 ?	4	2	4	0
Vascular disease to a severe degree	4	5	6	1 ?	3	2	3	0
No vascular disease	1	8	6	9(1)	2	7	4	7(2)
Severe respiratory disease	1	6	5	4	2	1	3	3
Middle-ear infection	1	5	1	0	1	3	3	2
Nephritis and urinary tract infection	1	3	3	?	1			1
Tumor	0	1 ?	0	1	2	4		2
Other	1	1	1		1	3	3	

(1) Three of these were sacrificed for comparative purposes only.

(2) Two of these were sacrificed for comparative purposes only.

Some of this pathology may be a consequence of having included potato decay in the diets during the first six months of the experiment. During the last 10 weeks of that period, the percent of decay included was at a maximum of 0.9, 3.3, and 5.3 for the 0X, 1X, and 2X diets, respectively. The percent mortality so far not found with vascular lesions is 4, 32, and 24, respectively, for males, and 8, 28, and 16, respectively, for females. However, the higher mortality of animals fed the 1X diet indicates that not all the results can be attributed to having included some potato decay in the diet.