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WHOLESOMENESS OF A GAMMA-IRRADIATED DIET FED TO CHICKENS

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ABSTRACT

A review was made of other studies performed at the University of Michigan on the wholesomeness of gamma-irradiated food including: (1) the possibility of nuclear activation by gamma irradiation of elements, (2) preliminary animal feeding tests, (3) formation of peroxides in irradiated fats, (4) effect of gamma radiation on nutrilites as determined by growth of *Tetrahymena pyriformis*, (5) pilot studies using albino rats fed a semisynthetic diet (50% canned meat) given doses 2, 20, and 45 megarep, (6) pilot studies using two strains of mice, and (7) data for one year on a long-term rat feeding and breeding study using the semisynthetic diet (4-megarep dose).

The results of these studies indicate that no radioactivity was induced by cobalt-60 gamma radiation in 24 elements that occur in foods. Gamma radiation produces peroxides in animal fats and to a greater extent in fats with less highly saturated fatty acids.

Tests with protozoa showed that thiamine, riboflavin, pantothenate, pyridoxine, folic acid, and thioctic acid were altered structurally by a dose of gamma radiation of less than 1 megarep, while a dose of 2 megarep was required to produce changes in niacin. Most amino acids proved to be relatively radiation-resistant. At the high radiation levels of 10 megarep and 23 megarep, respectively, only serine and methionine were damaged. All other amino acids remained biologically active even after receiving 23 megarep, the highest level of radiation employed.

In short-term feeding studies using albino rats no significant differences were noted between the controls and the animals fed a diet irradiated at 2 megarep. However, a marked vitamin deficiency was observed when the complete diet was irradiated with a dosage of 20 megarep. This deficiency was removed by supplementing the diet with the water-soluble vitamins.

No evidence of toxicity was observed in tube-fed rats on a diet irradiated with up to 45 megarep. In other pilot studies using small numbers of mice the second generation of Bagg-strain albino mice on the 4-megarep irradiated diet failed to reproduce, whereas a mixed pigmented strain of mice on the same diet reproduced as did control groups for both strains, which indicates that Bagg-strain albino mice may be more sensitive to the effect of radiation on the experimental diet than are Holtzman rats.

With regard to growth, reproduction, and lactation performance in a long-term feeding and breeding experiment (4-megarep diet) the albino rats on the control diet appear to be slightly superior to the animals on the irradiated diet. Additional data are required to determine whether or not these results are significant.

ABSTRACT (continued)

A long-term chicken feeding experiment was initiated using 190 Dryden-strain White Leghorns. No difference in general appearance or weight growth was observed during the first two weeks of the experiment.

Results to date indicate that as compared with changes in palatability and vitamin content, toxicity appears to be an unimportant aspect of food irradiation even at much higher levels than would be encountered in future commercial pasteurization or sterilization with gamma radiation.

WHOLESOMENESS OF A GAMMA-IRRADIATED DIET FED TO CHICKENS

I. REVIEW OF OTHER STUDIES PERFORMED AT THE UNIVERSITY OF MICHIGAN ON THE WHOLESOMENESS OF IRRADIATED FOOD

A. A Study of the Possibility of Nuclear Activation by Gamma Radiation of Elements Found in Food

1. Introduction. The most frequent question asked by the layman regarding the gamma irradiation of food is whether or not the irradiated food has an induced radioactivity. It has been assumed by many who have studied the effects of x-rays and gamma rays on food sterilization, chemical reactions, etc., that these radiations induce no radioactivity. This assumption is based on the fact that the irradiated materials show no activity when tested with ordinary radiation monitors. In most cases, however, the background radiation level is such that small amounts of induced activity could be easily overlooked.

W. W. Meinke[1,2] has recently reported a preliminary investigation of the possibility of nuclear activation by gamma radiation. This study was supported by contract AT(11-1)-162 with the U.S. Atomic Energy Commission. The Nuclear Instrument and Chemical Corporation scintillation well counter used in the study was made available from an AEC nuclear chemistry grant under contract AT(11-1)-70. Since this study is of importance with regard to the question of wholesomeness of gamma-irradiated food it is included in this review.

An independent experimental program was conceived as the result of discussions at the Symposium on Radiation Sterilization of Foods sponsored by the Committee on Foods of the Advisory Board on Quartermaster Research and Development held on June 26, 1953, at Massachusetts Institute of Technology under the general auspices of the National Research Council. This program was not designed to evaluate the tolerance limits of various activities in foods. Its purpose is only to determine within the limits of detection available the amount of activity (if any) induced in a number of elements by gamma rays from cobalt-60 and later from gross fission products. With such extensive effort being expended by a number of laboratories in trying to perfect the technique of food sterilization with gamma radiation, it was felt that some numerical limits for the amounts of activity induced by gamma radiation should be experimentally determined. Once determined these results could then be evaluated as to their hazard by the proper authorities.

A search of the literature indicated that x-rays induce low levels of activity in certain elements. A 4.1-hour indium activity was reported[3] first in 1939 by x-ray excitation and was shown[4] to have a threshold for production of 1.2 ± 0.1 mev. Later it was found that lead[5] and silver[6] activities could also be produced by x-rays with energies up to 1.5 mev. Wiedenbeck[7,8,9] studied the excitation of these elements as well as other elements such as rhodium, cadmium, gold, and krypton. In each of these cases the ground state isotope was excited to an isomeric state by the x-radiation. The isomer then decayed by its characteristic half life and energy as shown in Table 1[10].

Guth[11] has shown that this excitation by x-rays is a "line absorption" in which x-rays of only a certain limited energy will induce the activity. It was found that the widths of the lowest nuclear levels of lead and indium are of the order of a few millivolts. Hence, in the continuous spectrum of 1.5-mev x-rays, only about one in ten^{10} x-ray quanta has an energy suitable to induce activity.

TABLE 1[10]

NUCLEAR DATA FOR ISOMERS

Isomer	Half Life, hr	min	sec	Radiation	Energy of Photons, (mev)	Gamma Rays Converted
Rh 103 m		57		γ	0.040	highly
Ag 107 m			44.3	γ	0.094	e^-/γ =~16
Cd 111 m_2		48.6		γ	0.150	e^-/γ = 2.3
					0.246	e^-/γ = 0.064
In 115 m	4.50			γ (95%)	0.335	e^-/γ = 0.98
				β^-(5%)	0.83	
Au 197 m			7.4	γ	0.130	$e_{K}^-/\gamma \leq 2.0$
						K/L/M = 1/7.5/3.6
					0.279	$e_k/\gamma \sim 0.3$
Pb 204 m_2				γ	0.905	$e^-/\gamma \sim 0.1$
					0.374	$e^-/\gamma \sim 0.05$

A logical extension of this work was to determine whether indium activities could be produced by the gamma rays from radium and its daughters.

Guth[11] reports that Goldhaber, Hill, and Szilard irradiated indium with the gamma rays from 1/2 gm of radium without producing any activity. Since it is known that the energy of these gamma rays is sufficient to excite the indium, the absence of activity must be attributed to the fact that none of its known gamma-ray energies is equal to the energy of the activation level. The probability of excitation is therefore small and Guth concludes that only irradiation by a continuous x-ray spectrum is likely to lead to a line absorption.

With the advent of multikilocurie sources of cobalt-60 and eventual megacurie sources of fission products, gamma-ray intensities many orders of magnitude greater than that from 1/2 gm of radium are available. Furthermore, the thickness of these sources tends to "smear" out the energy of the gamma rays emitted by the source until the gamma energy spectrum no longer shows sharp, nearly monoenergetic rays but instead a gamma-ray distribution which approaches a continuous spectrum. This spectrum should then be nearly as effective as the continuous x-ray spectrum for activation. Harbottle[12] has recently published a report of the activation of indium and cadmium isomers with cobalt-60 gamma rays.

From the literature references it was apparent that if any activity were formed it would be low-level and would require a sensitive detector. In addition, Table 1 shows that, in general, the isomeric states formed by the activation decay by the emission of gamma rays. An ordinary Geiger tube, whose counting efficiency for gamma rays is only about 1 percent, is ineffective in detecting such radiation. A scintillation well counter had been obtained, however, from the Nuclear Instrument and Chemical Company for other low-level gamma-ray counting work on a nuclear chemistry grant from the Atomic Energy Commission. This counter, which was made available for the gamma activation work, enabled the sensitivity of the measurements to be increased.

The well counter consists of a crystal of thallium-activated sodium iodide, 1-3/4 inches in diameter and 2 inches thick. A 3/4-inch hole, 1-1/2 inches deep, was drilled in the center of this crystal and the entire crystal was encased in 1/32-inch aluminum. A test tube containing a sample was inserted in the hold for measurement. Radiation (beta and gamma) which penetrates the wall of the tube and the thin aluminum casing of the crystal was absorbed in the crystal giving a flash of light. This scintillation was then "seen" by a Dumont 6292 (K-1186) photomultiplier tube which feeds a scaler and register. The particular well counter used had a counting efficiency of about 43 percent for cobalt-60 rays and about 51 percent for those of iodine-131.

2. Procedure and Results. Since meat is one product for which radiation sterilization may be used, the trace elements it contains were checked for possible activation by the cobalt-60 gamma-ray sources. The

amount of various trace elements which occur in meat (beef) has been given in American Scientist based on a method developed by Arthur J. Mittledorf of the Armour Foundation.[12] He estimates that during a year the average American eats about 60 lb of beef. The amount in ounces of trace elements a person obtains in eating these 60 lb of beef is given in Table 2.

TABLE 2[12]

OUNCES OF TRACE ELEMENTS IN ONE YEAR'S CONSUMPTION (60 lb) OF BEEF

Aluminum	0.00001	Molybdenum	0.000014
Boron	0.00006	Nickel	0.000003
Calcium	0.0025	Phosphorus	1.6
Chromium	0.000003	Potassium	4.7
Cobalt	0.000002	Silicon	0.00006
Copper	0.00043	Silver	0.000013
Iron	0.0306	Sodium	0.40
Lead	0.00001	Tin	0.000002
Manganese	0.00002	Zinc	0.045
Magnesium	0.204		

Pure samples of each of these elements (as well as carbon, oxygen, sulfur, and iodine) were placed in test tubes 1.6 cm in diameter and 15 cm long which just fit into the counting well. The test tubes were filled with at least 2 inches of sample so that the well would be completely filled with the sample for maximum detection sensitivity. These samples were irradiated by the multikilocurie cobalt-60 source for varying periods of time and then counted in the scintillation well counter. Because of a large detection area this counter has a high background counting rate. This background was reduced to about 300 counts per minute however by enclosing the counter in about 4 inches of lead. Counts of 1-hour duration both for background and sample gave reasonable statistical precision. A count of this length gives a "9/10 error" of about 1.2 percent for the determination.

The method of activation by gamma radiation was first tested by the irradiation of indium in the cobalt-60 source. It was felt that if the source had enough intensity to produce activity in anything it would activate the indium. The tube was filled with about 85 gm of indium and irradiated in a flux position of about 220,000 rep per hour, nearly to saturation of the 4.5-hour activity. When counted, the sample showed activity about forty times the background of the well counter. The activity decayed with a half life of 4.41 hours with a 9/10 reliability factor of 0.05 hours. This value agrees with accepted literature values for In^{115m}. A rough attempt to evaluate the efficiency of the well counter gave a value of about 50 percent for the In^{115m} gamma ray. In addition a rough evaluation of self absorption of the radiations in the indium metal indicates that about 1000 γdis/min/gm of indium were formed in the irradiation or roughly 4.5 γdis/min/gm In per 1000 rep per hour saturation flux. Harbottle[13] has recently reported similar results in an independent investigation at Brookhaven National Laboratory. He reports an order of magnitude value for the cross section for formation of indium from cobalt-60 gamma rays of 10^{-9} barns.

The work with indium was admittedly weighted heavily in favor of success but it established that the method was satisfactory to detect small amounts of activation induced by gamma radiation. Twenty-four samples of pure elements or compounds were then prepared and irradiated. The elements, their sources, and weights are listed in Table 3.

TABLE 3

FORM, SOURCE, AND WEIGHT OF ELEMENTS IRRADIATED IN GAMMA-RAY SOURCE*

1. Aluminum powder - high purity - Alcoa - 2.7 gm
2. Elemental boron powder - student prep - small amount of chemical contamination - 4.8 gm
3. Calcium metal turning - B and A reagent grade - 2.1 gm
4. Carbon - graphite flakes - Fisher - 2.9 gm
5. Chromium metal powder - Charles Hardy - 15.1 gm
6. Cobalt metal powder - old Kahlbaum material - contains some Cl - 5.9 gm
7. Copper metal pellets - Standard Laboratory grade - 16.3 gm
8. Iron metal powder - Standard Laboratory grade 12.2 gm
9. Elemental iodine crystals - Mallinckrodt analytical reagent - 11.0 gm
10. Lead metal filings - Mallinckrodt analytical reagent - 21.5 gm
11. Magnesium metal powder - Dow - high purity - 5.5 gm
12. Manganese metal powder - Charles Hardy - 15.1 gm
13. Molybdenum metal powder - Primus Chemical Company (1912) - 14.5 gm
14. Nickel metal shot - Mallinckrodt analytical reagent - 16.1 gm
15. Oxygen - (Al_2O_3) - Harshaw - 5.3 gm
16. Phosphorus - $(NH_4)_2HPO_4$ - Mallinckrodt analytical reagent - 3.7 gm
17. Potassium - KCl - General Chemical Company - reagent grade - 5.3 gm
18. Elemental silicon powder - Fairmount Chemical Company - 4.0 gm
19. Silver metal powder - G. Frederick Smith - reagent grade - 16.0 gm
20. Sodium chloride (NaCl) Merck analytical reagent - 5.9 gm
21. Sodium fluoride (NaF) - cp - 3.3 gm
22. Sulfur (roll) - cast - 4.38 gm
23. Tin metal granules - General Chemical Company - reagent grade - 15.1 gm
24. Zinc metal granules - cp - 10.6 gm

*The weight listed in the table is the amount of material contained in the bottom 1 cm of the test tube used in the irradiation and counting.

These elements were subjected to two series of experiments. In one the samples were irradiated simultaneously for a day or so and then rapidly removed from the source room and counted. It was possible by rapid manipulation of the source to have a sample in the well counter between 40 to 45 seconds after the end of irradiation. By raising the source immediately after removal of one sample, it was assured that any short-lived activity in the remaining samples would remain at saturation. Short-lived activities with half lives down to the order of 20 seconds could have been detected if formed in even 1/10 the yield of the indium. However, no activity greater than 5 percent of background was found in any of these samples in 20-minute counts.

In a second set of experiments the samples were irradiated in the high flux position of the source at a flux level of 220,000 rep per hour for about 2 weeks. The samples were then removed individually and counted. They were compared with a background count of the same day and also with a blank sample count before irradiation. An hour background count fluctuated $\pm$ 1 percent over the 3-week period of the measurements. It was found that only two samples varied from this background region by more than $\pm$ 1 percent. One was potassium chloride whose natural radioactivity increased the blank count to 2/3 more than background. The irradiated sample gave the same count as the blank sample, however. The second sample was the chromium metal powder. An increase was found in this sample after irradiation but the activity did not decay over a period of several weeks and must be attributed to contamination.

It should be stressed that in the method used, the radiation must pass through the test tube, about 300 mg/cm^2 thick, and the aluminum casing of the crystal before it is detected. This then prevents the detection of gamma rays of less than about 30 kev energy or of most conversion electrons from highly converted gamma rays. Since early work in this field pointed out several elements whose isotopes on activation decayed with such weak radiations, the present work is somewhat limited. Nevertheless, it was thought worthwhile to perform the experiment with the equipment on hand and to recognize the fact that nuclides emitting certain very weak radiations would escape detection.

Activity found in a sample might also be accounted for by conditions other than gamma-ray activation. One of the elements tested, potassium, has a low abundance isotope that is naturally radioactive and was detectable in the scintillation crystal. Natural radium also often occurs to a small extent in many materials. Shandley,[14] at the University of Rochester, has reported a highly sensitive method for determining the radium content of common foods by deemanation. He found that most of the foods tested fell within the range of 0.74 to 6.5×10^{-15} gm radium per gm of starting material. This amounts to about 10^{-9} microcurie of radium or about 2×10^{-3} disintegrations per minute per gm of food. The use of high-purity samples of elements in the experiments reduced the possibility of such contamination.

In addition as a check on the above items, careful background blank counts were taken on each sample and compared with the activity of the sample after irradiation.

3. Summary. This work substantiates the belief that no radioactivity is formed when 24 elements common as trace elements or substituents in food are irradiated in a 10-kilocurie cobalt-60 source. Two extensions of the work however should be made. For one a sensitive method should be utilized for detecting the weakest radiations without self-absorption and container-absorption effects. The use of a relatively large volume of a liquid scintillator such as used in low-level carbon-14 and tritium tracer work might give the required detection sensitivity in this weak radiation range.[15,16,17] Chemical problems of dissolving the element and scintillator in a mutual solvent would, however, add difficulties to this approach.

In addition, this work was further limited by the fact that it was done with cobalt-60, whose two gamma rays have an energy of 1.33 and 1.13 mev. Any commercial method of food sterilization would probably use fission products in some form. The gamma spectrum emitted by such a source depends on the age of the fission products and the energy of the neutrons producing the fission. The contribution of radiations from particular fission-product isotopes has been presented as a function of age for uranium-235 thermal neutron fission by Hunter and Ballou.[18] From their work it can be assumed that many of the gamma rays will have energies equal to or lower than those of the cobalt-60 radiations.

However, small percentages of higher energy gamma rays may be present in the fission products from an operating reactor after a few weeks or months of "cooling". The energy spectrum of "thick" sources of these products thus could start at a much higher energy than the cobalt spectrum. Since the early x-ray work showed that a number of elements was capable of excitation at energies between 1.5 and 3.0 mev, the experiments reported here should be repeated with kilocurie or larger sources of fission products. Suitable sources now exist in the gamma-ray facility of the Materials Testing Reactor, Arco, Idaho, where spent fuel rods from the reactor are allowed to "cool down" before processing. Samples can be irradiated there in an area of high-flux fission-product radiation.

B. Preliminary Animal Feeding Tests

The Fission Products Laboratory of the University of Michigan received a cobalt-60 "one-kilocurie" source of the Brookhaven design in June, 1951. Shortly thereafter some experimental studies were conducted on the preservation of foods with gamma radiation. However, before any irradiated food was eaten by human beings some preliminary tests were made to check for possible acute toxicity. These preliminary animal feeding experiments with food exposed to gamma radiation were conducted in September, 1951, by F. H. Bethell, M.D., and A. H. Kretchmar, M.D., in the AEC Biological Effects of Irradiation Laboratory.[19] The results showed no difference in health, growth, etc., between the animals fed irradiated reconstituted whole milk and those fed the control milk. All milk samples were packaged in polyethylene bags and those to be irradiated were placed in the "kilocurie" cobalt-60 source and were given a dose of slightly over 2 megarep. The milk was reconstituted Klim prepared as a four-times concentrate and diluted just before feeding by the addition of a salt solution. This milk constituted the entire diet, with the exception of one nonirradiated leaf of lettuce added each week. It was emphasized[19] that this experiment was only exploratory and that any conclusions from the data were preliminary and contingent on further testing, both with feeding experiments and with more refined methods of assay for individual components of the diet.

C. Some Effects of Gamma Radiation on Chemical Constituents of Foods

1. Formation of Peroxides in Irradiated Fats. Although the preliminary feeding experiments were negative, other experimental studies, supported by Michigan Memorial-Phoenix Project No. 41 and reported by Graikoski, Kempe, and Brownell,[20] indicated that appreciable quantities of peroxides were formed as a result of irradiation of fats and as might be expected the amount of peroxide was greater for fats containing larger percentages of unsaturated fatty acids (see Table 4).

TABLE 4

EFFECT OF GAMMA RADIATION ON SOME ANIMAL FATS[20]

Fat Sample	Dose, megarep	Peroxide Value* m.e. O_2/kg fat	
		Control	Irradiated
Pork No. 1	1.83	0	48.54
Pork No. 2	2.07	0	58.75
Beef No. 1	1.83	0	26.62
Beef No. 2	1.83	0	1.97
Bacon Fat	2.07	0	34.21
Lard	2.07	5.01	15.04

*Analysis conducted soon after irradiation

A definite and continuous increase in peroxide oxygen on storage was observed with irradiated beef and pork fats.[20] Data on stored irradiated pork fat are given in Table 5.

TABLE 5

PEROXIDE VALUES OF PORK FAT STORED AT 4°C
AFTER IRRADIATION WITH VARIOUS DOSAGES

Dose, megarep	Days at 4°C					
	0	4	8	13	17	22
	Peroxide Value m.e. O_2/kg fat					
0	0	0	0	0	0	0
0.038	0.90	2.18	2.24	6.77	9.98	26.57
0.077	7.23	12.18	13.93	31.64	48.68	60.35
1.690	91.76	99.67	97.87	99.67	108.02	120.25

In the interest of utilizing radiation for food sterilization, the stability of irradiated fats must be determined and the relationship of stability to toxicity for such products must be ascertained. Toxicity studies by Whipple[21,22] have shown that oxidized fats produce a disease called "oxidized fat syndrome" in dogs and rats.

2. Effect of Gamma Radiation on Nutrilites as Determined by Growth of *Tetrahymena Pyriformis*. Long-term feeding and breeding experiments are required to explore the effect of gamma radiation on nutrilites when mammals are used as the test subjects. However, a great quantity of information can be obtained in a short while if protozoa are used. Certain of these one-celled animals such as *Tetrahymena pyriformis* have approximately the same requirements for the essential amino acids and water-soluble vitamins as mammals. Using this microorganism, Elliott, Gross, and Brownell[23,24] reported the effect of gamma irradiation on the nutrilites of a completely synthetic medium used to grow the protozoa. This study was supported by Michigan Memorial-Phoenix Project No. 73. The analysis of the medium used is given in Table 6.[24]

A preliminary irradiation of the medium described in Table 6 was performed to determine the range of radiation effects and the comparative radiation effects on a dry and on a liquid medium. A range of irradiation from 1/2 to 4 megarep was employed in the initial screening experiment for both the dry and liquid medium. The highest radiation dose had no apparent effect on the dry components since the protozoa grew equally well in a medium prepared from the irradiated mix and in the nonirradiated medium. On the

TABLE 6[24]

COMPOSITION OF THE BASAL CHEMICALLY DEFINED MEDIUM USED TO GROW *TETRAHYMENA PYRIFORMIS*

Compounds	Molarity	Final Concentration in Medium, 1 megarep
<u>Amino Acids</u>		
L-Arginine · HCl	0.172	150
L-Histidine · HCl	0.142	110
D_xL-Isoleucine	0.152	100
L-Leucine	0.106	70
L-Lysine	0.048	35
D_xL-Methionine	0.046	35
D_xL-Phenylalanine	0.061	100
D_xL-Serine	0.316	180
D_xL-Threonine	0.343	180
L-Tryptophan	0.019	20
D_xL-Valine	0.102	60
<u>Carbon Sources</u>		
Glucose	1.4	1000
Na acetate	3.0	1000
<u>Nucleic Acid Components</u>		
Adenylic acid	0.0088	25
Cytidylic acid	0.0096	25
Guanylic acid	0.0087	25
Uracil	0.0223	25
<u>Vitamins</u>		
Thiamine-HCl	8.25×10^{-4}	1
Riboflavin	7.5×10^{-5}	0.1
Ca pantothenate	0.96×10^{-4}	0.1
Niacin	2.03×10^{-4}	0.1
Pyridoxine · HCl	2.33×10^{-3}	2
Folic acid (PGA)	5.75×10^{-6}	0.01
Thioctic acid	4.3×10^{-8}	0.001
<u>Inorganic Salts</u>		
K_2HPO_4	0.144	100
$MgSO_4 \cdot 7H_2O$	8.1×10^{-3}	10
$Zn(NO_3)_2 \cdot 6H_2O$	3.9×10^{-3}	5
$FeSO_4 \cdot 7H_2O$	4.2×10^{-4}	0.5
$CuCl_2 \cdot 2H_2O$	7.3×10^{-4}	0.5

other hand, the irradiated liquid medium failed to support growth of the ciliates when the irradiation dose was 1/2 megarep or over. When nonirradiated medium was added to these inhibited cultures, growth recovery was achieved at a rate which was inversely related to the radiation level; i.e., recovery was greater when fresh medium was added to that which received only 1/2 megarep than when added to medium irradiated by 4 megarep. A dose-response curve based on the experimental data showed that the critical range of irradiation for the complete liquid medium was from 0.15 to 0.30 megarep.

In the first experimental series in which separate components were tested for the effects of irradiation, each was irradiated in the concentration which usually is employed in the preparation of the medium as given in Table 6. Media were prepared by omitting one or several of the components in groups. The five groupings used were: (1) amino acids, (2) vitamins, (3) nucleic acid components, (4) glucose, and (5) sodium acetate. Each component was individually irradiated for 1.0 megarep. The irradiated compounds were then pooled in the described groups and added to that experimental medium which was deficient in the test group. On testing the growth of Tetrahymena in such media it was found that only the medium containing irradiated vitamins failed to support growth. All other media yielded normal growth except a control medium prepared entirely from irradiated components.

In order to determine which of the vitamins were destroyed at this level of irradiation (1 megarep) an omission experiment was performed in which single vitamins prepared as shown in Table 6 were left out of the medium. The vitamin which was omitted was then replaced by the irradiated vitamin. Growth experiments in this series indicated that pyridoxine and thiamine did not decrease in efficiency as a result of irradiation, whereas thioctate, folic acid, riboflavin, niacin, and pantothenate were all partially destroyed. A lack of toxicity in all the irradiated vitamins was demonstrated by supplementing the nonirradiated control medium with single irradiated vitamins and obtaining growth which was equivalent to that in the control medium. Additional tests were made with irradiated individual components to determine the dosage of gamma radiation required to affect the various vitamins. High-level dosages in other omission experiments were observed to produce effects on some of the other nutrilites. The results can be summarized as follows.

Thiamine, riboflavin, pantothenate, pyridoxine, folic acid, and thioctic acid were shown to be altered structurally by less than 1 megarep, while 2 megarep were required to produce changes in niacin. Most amino acids proved to be relatively radiation-resistant. At the high radiation levels of 10 megarep and 23 megarep, respectively, only serine and methionine were damaged. All other amino acids remained biologically active even after receiving 23 megarep, the highest level of radiation employed.

However, organoleptic observations showed that irradiation had caused color and odor changes in many of those amino acids whose biological activity for Tetrahymena remained unaffected. The nucleotide guanylic acid was also inactivated at 23 megarep.

D. Pilot Animal Feeding Studies

Although the tests with protozoa were very informative the use of this organism for toxicity studies has not yet been established as an accepted procedure. Discussions in 1953 with representatives of the Food and Drug Administration indicated the advisability of conducting long-term feeding and breeding experiments using more than one diet and more than one species of animal.

To establish the wholesomeness of irradiated food by means of an animal feeding and breeding experiment, it was decided that a diet should be used in which the main caloric intake consisted of a suitable mixture of irradiated carbohydrates, fats, and proteins. In addition, a portion of the water intake should be in the irradiated food. Some of the effects of irradiation are believed to result from the formation of hydroxyl radicals from water; therefore, it is important that the food to be irradiated should not be in the anhydrous state. This point was demonstrated with irradiated (4 megarep) anhydrous complete medium for Tetrahymena pyriformis which was unaffected by this dose of irradiation. Meat was considered to be a more suitable and convenient food than the reconstituted milk used in the first feeding experiment because of the difficulties of obtaining normal feeding with a complete liquid diet.

A series of pilot studies were made, prior to commencing long-term experiments, to check the adequacy of the diet selected and to explore the effects of different dosage levels of gamma irradiation. Three series of pilot experiments were conducted with Holtzman-strain albino rats using radiation dosages for the diet of 2, 20, and 45 megarep. One pilot experiment was conducted with mice using a pigmented strain and a Bagg albino strain and a diet receiving a radiation dosage of 4 megarep.

1. The 2-Megarep Rat Pilot Study. This pilot study was reported by Uhlendorf, Eckstein, and Brownell[25] and was supported by Michigan Memorial-Phoenix Project No. 41. It was the first of a series of small-scale pilot experiments using Holtzman-strain albino rats to explore the effects of dosage level on gamma-irradiated food. It was decided that the diet should be a complete ration and yet such that quantitative experiments such as vitamin assays can be performed. From the point of view of applying these results to human consumption, a diet containing foods consumed by humans would be preferred to a completely synthetic diet. The food mixture selected was a semisynthetic diet in which a large proportion of the protein was in the form of a canned meat product, Swift's Beef for Babies. The use

of a semisynthetic diet containing 50% heat-sterilized meat minimized the problems of storage and provided more adequate controls than would be possible using raw meat. No attempt was made to study the effect of storage on irradiated food.

In the pilot experiment a total of 27 Holtzman rats was divided into three groups and fed diets A, B, and C. The control diet (A) consisted of nonirradiated diet. In the case of experimental diet (B) all but the vitamins, fats, and salts were irradiated, whereas all components of the experimental diet (C) were irradiated at a radiation dose of 2 megarep. Unfortunately, due to various unanticipated delays these animals were not fed the experimental diets during the period of most rapid growth. The growth curves for a period of over two months are shown in Figs. 1, 2, and 3 where the average weight of each group is plotted as a function of time. It should be noted that the feeding of diets A, B, and C commenced on May 22, 1953, and that the animals were first mated on June 1, 1953. The average growth curves for the males shows no significant variation between the groups fed diets A, B, and C. These data indicated that for the period these animals were fed, the partly irradiated and the completely irradiated diets did not have a deleterious effect as far as can be ascertained from the rate of growth.

The reproduction in the first breeding of this pilot experiment was 100 percent in each group, group A containing 4 females, and groups B and C, 5 females each. The data on reproduction are given in Table 7. The experiment was continued until the females had weaned their second litters. No significant differences were observed between the control group and those fed the partly and completely irradiated (2 megarep) diets.

2. The 20-Megarep Rat Pilot Study. France, Eckstein, and Brownell[26] reported a pilot study supported by Michigan Memorial-Phoenix Project No. 41 which was designed to test the wholesomeness of food irradiated at 20 megarep. In the first experiment of this series six-week-old members of the first litters of the animals in the 2-megarep study were placed on diets, part or all of which had been irradiated at a dosage of 20 megarep. Three groups of 8 animals each, divided equally as to sex and littermates, received diets *ad libitum* prepared as previously described, XA being the control diet, XB the partly irradiated diet, and XC the completely irradiated diet. The growth data indicated a marked reduction in rate of weight gain for the animals on the partly (XB) and completely (XC) irradiated diets.

In an attempt to determine whether the decreased growth was caused by a change in the nutritional adequacy or in the palatability of the irradiated diets, a second 20-megarep experiment was initiated with 24 young rats of second litters of the animals used in the 2-megarep experiment. In order to control the factor of palatability in the new experiment all the animals were restricted in food intake to the amount consumed by the group

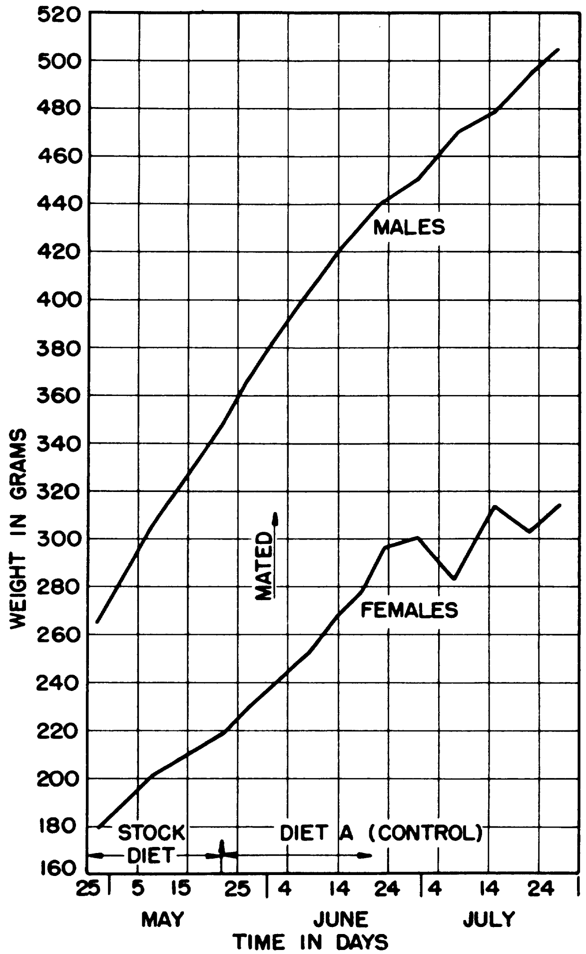

Fig. 1. Growth Curves for Rats Fed Diet A (Control) in 2-Megarep Pilot Study.

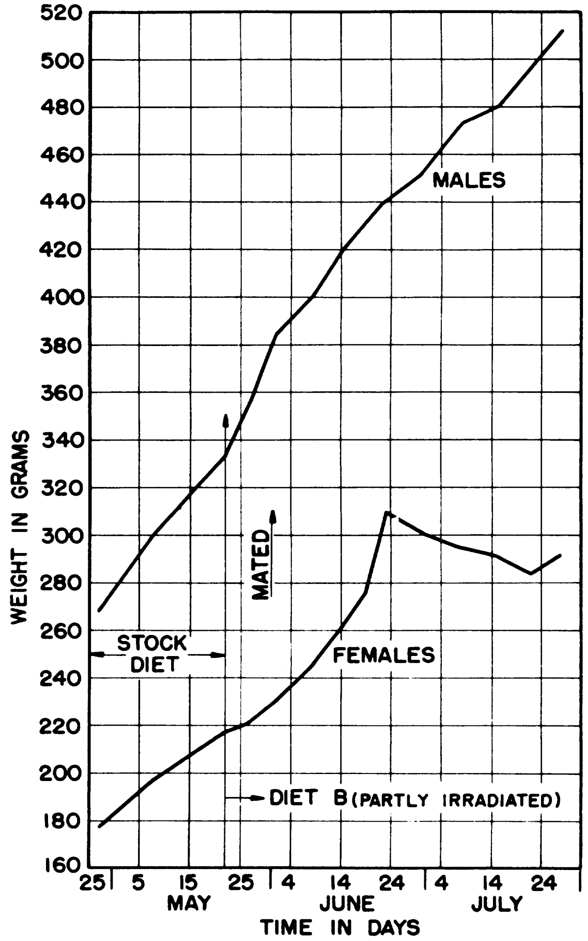

Fig. 2. Growth Curves for Rats Fed Diet B (Partly Irradiated) in 2-Megarep Pilot Study.

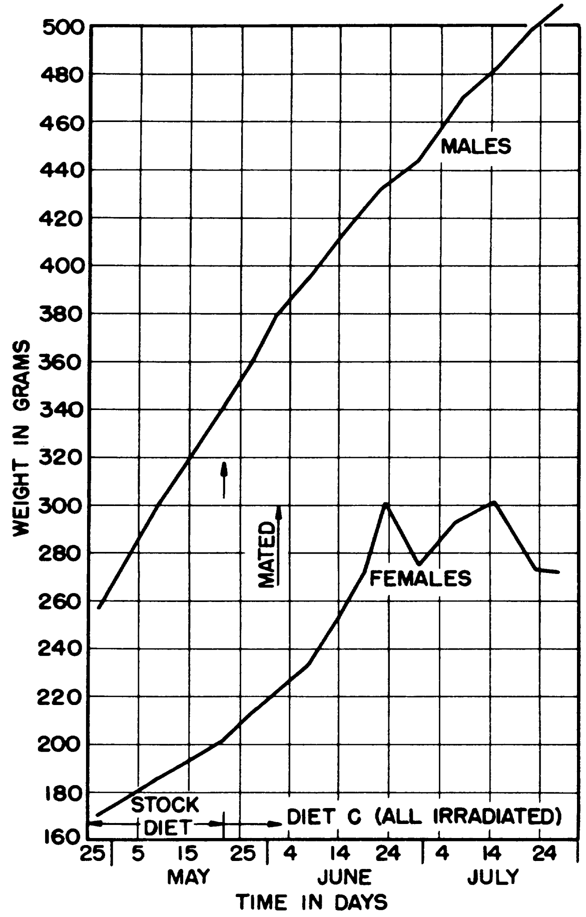

Fig. 3. Growth Curves for Rats Fed Diet C (All Irradiated) in 2-Megarep Pilot Study.

TABLE 7

REPRODUCTION DATA FOR FEMALE RATS IN 2-MEGAREP PILOT STUDY

	Group	No. of Females	No. of Litters	Total No. Born	Average No. per Litter	Percent Weaned	Average Weight at Weaning
First Breeding	A	4	4	47	11.75	97.4*	55.8
	B	5	4**	42	10.5	71.1***	45.2
	C	5	5	60	12.0	100.0*	55.4
Second Breeding	A	4	2	18	9.0	88.8	50.0
	B	5	4	39	9.8	100.0	41.5
	C	5	5	51	10.3	80.4	38.6

*Since the litters in the first breeding were reduced to 10 on the fifth day, the percentage weaned is calculated on the basis of the reduced litter.

**One female in this group fell approximately 4 feet to the floor. The next day she gave birth to 7 pups; 4 were born dead and the other 3 died a few hours later. Her performance as a foster mother was good. This rat is not included in the table.

***One litter of 10 pups died before the fifth day. This was apparently due to a failure in lactation. Of the pups in the other 3 litters, 96.4 percent were weaned. The rats have since been remated and this rat was again unsuccessful in lactating. This is probably a characteristic of the individual animal not caused by the diet.

with the lowest intake. An additional experimental group of animals was fed a diet in which all the protein and carbohydrate constituents were irradiated after mixing (the XB' diet) for comparison with the group fed XB diet, in which the canned Swift's Beef for Babies and the dry protein and carbohydrate ingredients were irradiated separately.

The weight changes of the four groups (each containing 3 males and 3 females except the XC group, which had 2 males and 4 females, with littermates distributed equally among the groups) on restricted dietary intake were followed for the first 3 weeks after weaning and are shown in Figs. 4 and 5. In this experiment the animals on the XB' diet showed an almost identical growth rate to the animals on the XA (control) diet. This suggests that the retardation in weight gain observed in the group fed (XB) 20-megarep irradiated diet ad libitum was due to lack of acceptability rather than to impaired nutritional value of the 20-megarep

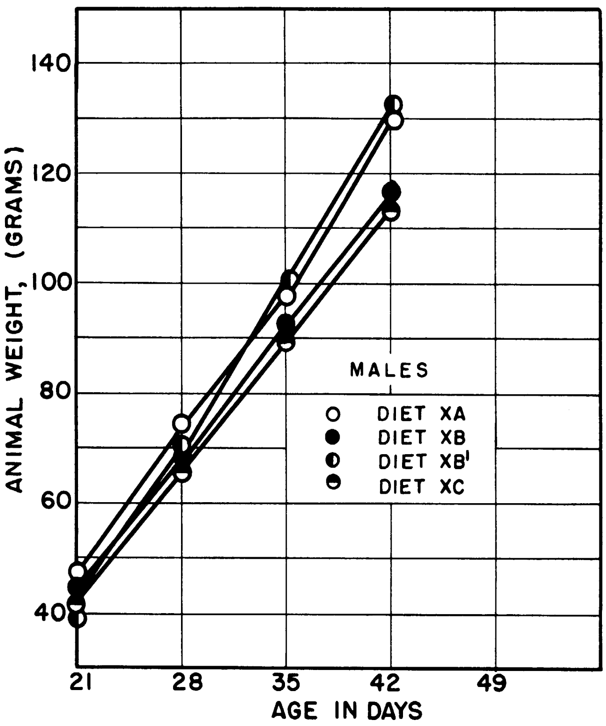

Fig. 4. Growth Curves for the First 3 Weeks after Weaning of Male Rats on Restricted Dietary Intake in the 20-Megarep Pilot Study.

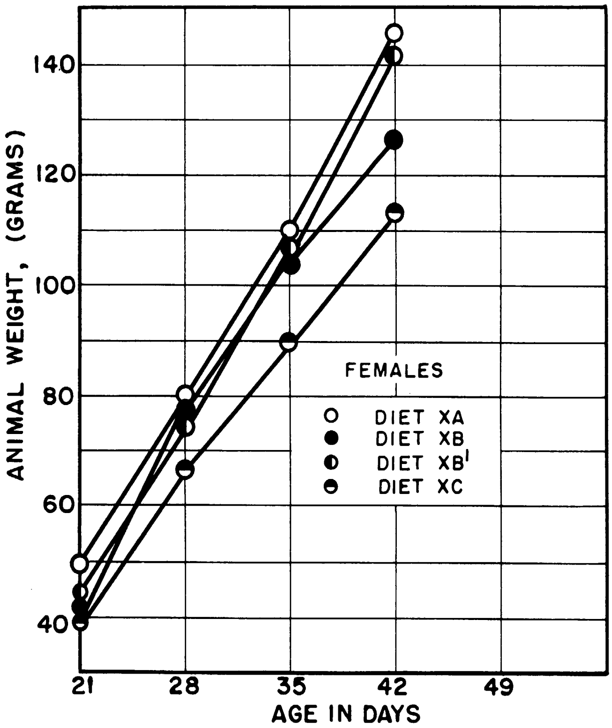

Fig. 5. Growth Curves for the First 3 Weeks after Weaning of Female Rats on Restricted Dietary Intake in the 20-Megarep Pilot Study.

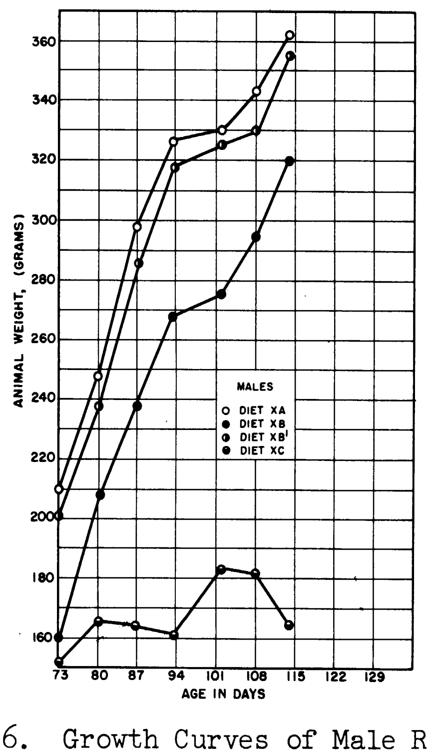

Fig. 6. Growth Curves of Male Rats in the 20-Megarep Pilot Study. (Food intake was restricted during first 7 weeks after weaning, then allowed *ad libitum*.)

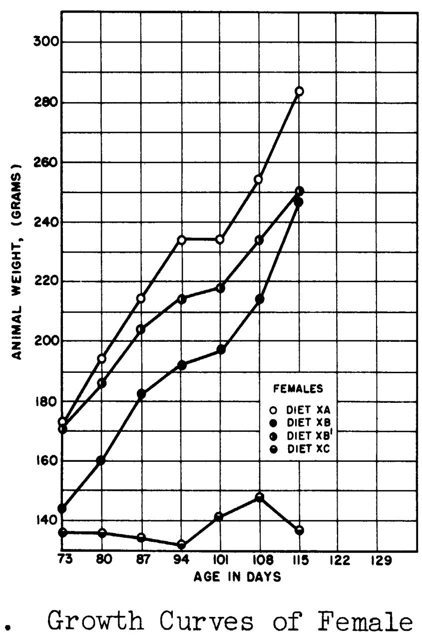

Fig. 7. Growth Curves of Female Rats in the 20-Megarep Pilot Study. (Food intake was restricted during first 7 weeks after weaning, then allowed *ad libitum*.)

XB diets. In the case of the restricted dietary intake experiments the animals on the XC diet (completely irradiated at 20 megarep) showed a smaller growth rate than the other groups of animals. However, this difference was not pronounced until the animals were about 70 days old. The animals in each group were fed ad libitum after they were 73 days old. The growth curves for this period are shown in Figs. 6 and 7. Note that the animals on the XA and XB' diets showed nearly the same growth rate whereas the animals on the XC diets showed very little growth.

The vaginas had not opened in the XC females at 110 days of age and no pregnancies occurred. The downhill course of these animals indicated by the weight curve was accompanied by the development of typical symtoms of thiamine deficiency. Figure 8 shows 3 animals in this condition. The female and one male were at this point placed on a completely irradiated diet to which nonirradiated, water-soluble vitamin mixture was added in the original quantities. The animals immediately started eating the diet despite its extreme rancidity. The fat content was 17 percent by weight on the dry basis. In less than 8 weeks the 4-month-old male increased in weight from 116 to 316 gm, and the female from 139 to 232 gm; 5 weeks after vitamin supplementation began the female gave birth to 7 young, of which 2 survived to weaning age.

The necessity for completing the pilot studies and starting the long-term experiment prevented the maintenance of a freshly irradiated supply of diet in sufficient quantity to feed more than this one pair of XC animals. The striking recovery of these two rats when the water-soluble vitamin premix was added to the completely irradiated 20-megarep diet (see Fig. 9) suggested that vitamin destruction rather than the formation of toxic substances in this high-fat ration was responsible for its adverse effect on growth and reproduction.

Twenty-eight of the animals used in the 20-megarep pilot study were sacrificed for histopathological examination. Organs were identified only by code number at the time of examination. Pathologists, Professor R. C. Hendrix, M.D., and Professor R. C. Wanstrom, M.D., of the University of Michigan Medical School, expressed the opinion that no significant tissue changes induced by the diet were indicated by the results.

At 110 days of age the animals in the second 20-megarep experiment were mated, each mate being rotated among the females with transfers at weekly intervals. The results are given in Table 8. With due allowances for any irregularities which may have occurred, the weight curves and reproduction data show that normal or near-normal growth is possible on diets containing protein and carbohydrate constituents irradiated at a 20-megarep dose, but that normal growth and reproduction were not shown by rats on a diet that was completely irradiated at this level.

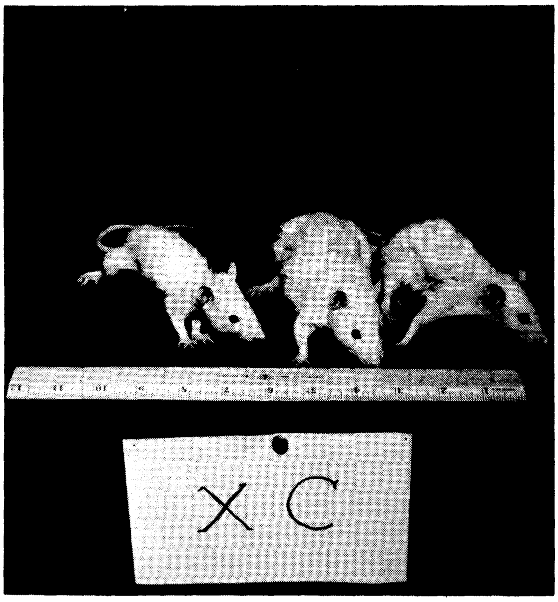

Fig. 8. Two Males on Left, Female on Right, Showing Effects of Feeding Diet Completely Irradiated at 20-Megarep Dose (Note Characteristics of Thiamin Deficiency).

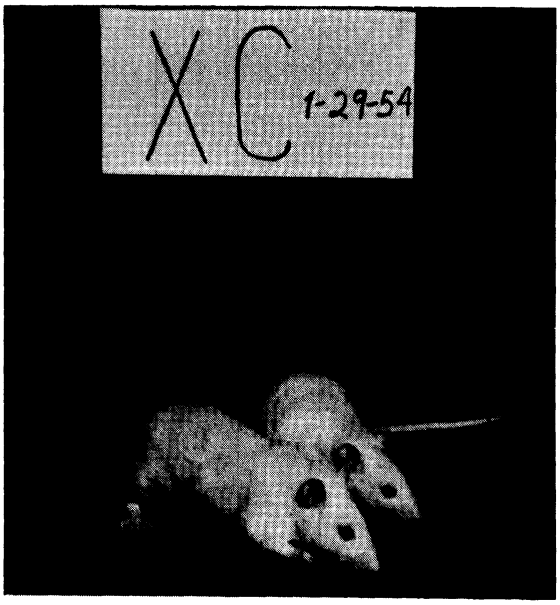

Fig. 9. Male (Left) and Female Fed Exclusively Completely Irradiated Diet (at 20 Megarep) for 10 Weeks, Then Fed the Same Diet Supplemented with the Water-Soluble Vitamin Premix for 3 Weeks. (Note: These animals are the same as the right two in Fig. 8 and show recovery from vitamin deficiency.)

TABLE 8

REPRODUCTION DATA FOR FEMALE RATS IN THE 20-MEGAREP PILOT STUDY

Group	Diet	No. of Females	No. of Successful Pregnancies	No. of Offspring at Birth	Total No. of Offspring at 21 Days	Mean Weight of Offspring at 21 Days
XA	Nonirradiated	3	3	26	24	43.0 gm
XB	Partially Irradiated*	3	3	16	16	31.8 gm
XB'	Partially Irradiated**	3	2	20	20	26.9 gm
XC	Completely Irradiated	2	0	0	0	----
XC'	Completely Irradiated; Nonirradiated Water-Soluble Vitamin Premix added	1	1	7	2	42.0 gm

*Canned meat and mixed dry protein and carbohydrate constituents were irradiated separately.

**All protein and carbohydrate constituents were mixed before irradiation; irradiated mix was stored up to two months before feeding.

The results obtained in this exploratory study indicate that vitamin destruction at a 20-megarep dose appears to be the principal deleterious effect insofar as growth and reproduction of rats is concerned. These results appear consistent with those obtained using Tetrahymena in which destruction of a few essential nutrilites at high levels of irradiation, rather than formation of any toxic substances, appears to be the principal consequence of irradiation.

3. The 45-Megarep Rat Pilot Study. This experiment, reported by France, Eckstein, and Brownell[27] and supported by the Michigan Memorial-Phoenix Project No. 41, was the final pilot experiment using Holtzman-strain albino rats. The purpose of the experiment was to determine the possible threshold for the development of acute toxicity in highly irradiated canned meat. Because the 20-megarep experiment indicated a decrease in acceptability of a highly irradiated diet this experiment was conducted by observing the changes in body weights of adult male rats. A previous exploratory study in which the rats were allowed to eat stock ration ad libitum between the forced feedings and in which part of the total food intake was given by stomach tube had indicated that tube-fed meat irradiated at 25 megarep did not produce any different effect on weight change than did nonirradiated meat. The tube feeding in itself, however, was sufficiently stressful to cause a marked reduction in weight gain when compared with controls not tube-fed.

A group of 50 Holtzman male rats averaging 355 gm at the start of the experiment were tube-fed the nonirradiated diet for 7 days. Then animals were eliminated because they showed signs of respiratory infection or responded unfavorably to the feeding procedure. The remaining 40 rats were divided into two equal groups of similar weight (about 312 gm). One group was used as a tube-fed control and the other group was tube-fed a diet containing canned meat irradiated at 23 megarep. The composition of the diet was canned meat (Swift's Beef for Babies), 1000 gm; sucrose, 70 gm; corn oil, 27.5 gm; HMW salt mixture, 10 gm; cellulose, 20 gm; tocopherol, 0.3 gm; choline chloride, 1.2 gm; and mixed vitamin supplement, 15 gm.

Water was added initially to give a solid content of about 20 percent to make the diet more fluid, as the canned meat exposed to this level of irradiation developed a rubbery consistency. After mixing in a Waring blender the diet containing the irradiated meat adhered tenaciously to the walls of the containers; large volumes of water were required to wash down the adhering meat. Even with this precaution the quantitative recovery of all the solids was difficult; therefore, the dry weight of each batch was determined and the solid content of both diets adjusted to between 16 and 18 percent. The fluid diet was carefully measured from a 30-ml hypodermic syringe and the rats were fed twice daily, except on Sundays when they were fed once. The amount given per feeding was 20 ml, a quantity that maintained body weight at a fairly constant level of about 275 gm from the third to the sixth week of tube feeding. Weight curves are shown in Fig. 10.

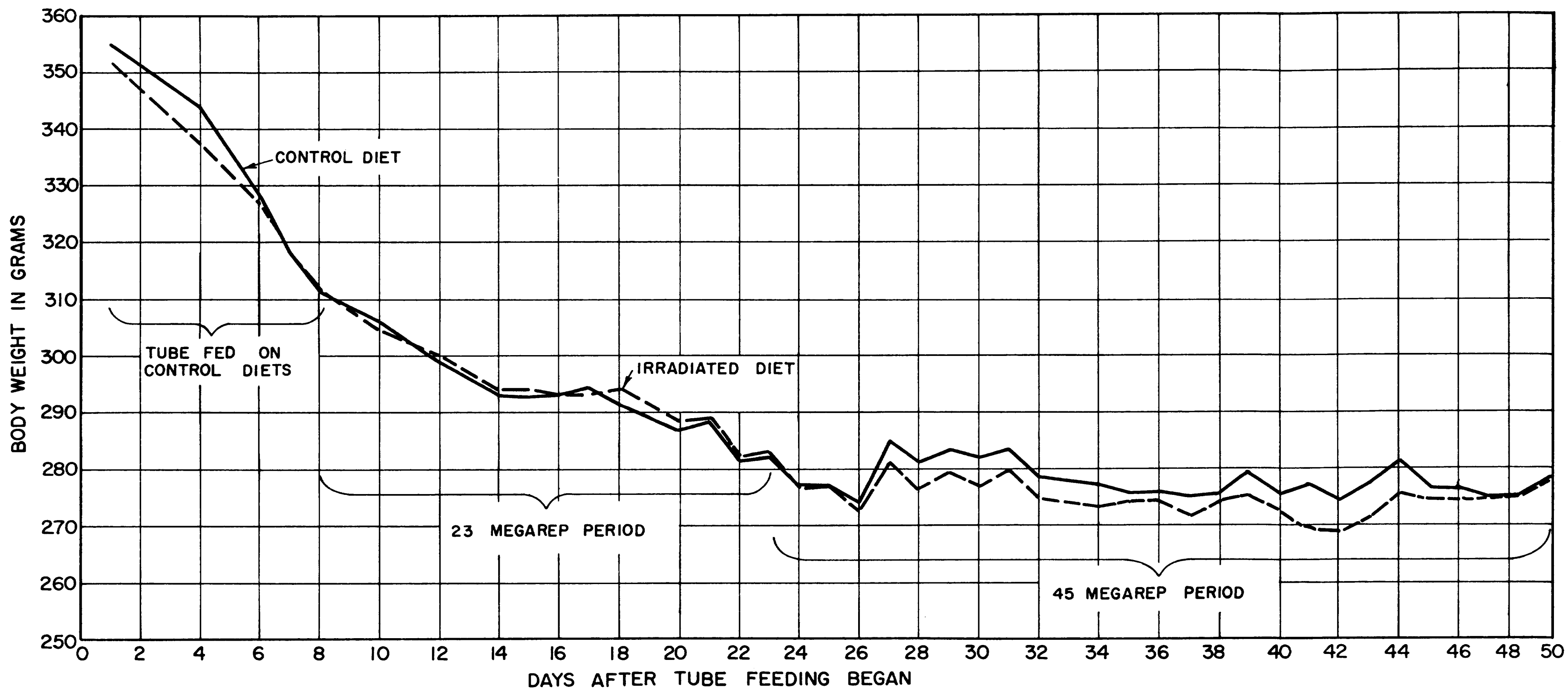

Fig. 10. Weight Curves in Tube-Feeding Pilot Experiment Using 23- to 45-Megarep Irradiated Meat.

The irradiation exposure of the meat was increased gradually to 45 megarep over the first 2 weeks and the size of control and experimental groups was reduced to 12 animals each on the nineteenth day of the experiment. The average weights of the animals in each of the groups of reduced size was the same as in the corresponding group prior to reduction of the group size.

The animals removed from the experimental diets were kept under observation for several weeks after their return to ad libitum feeding on the stock ration. Weight changes were followed for at least 2 weeks, at which time the original weight of both groups had been regained. No differences were observed between the two groups during the period of initial weight loss or during the recovery period. Diarrhea occurred in some of the animals fed the irradiated meat but an increase in the roughage content abolished this. Otherwise, there were no grossly observable symptoms of any deleterious action of forced feeding of a diet consisting largely of meat irradiated with doses up to 45 megarep.

4. The 4-Megarep Mouse Pilot Study. An irradiation dose of 4 megarep was selected for a long-term feeding and breeding experiment using albino rats. The original diet was modified slightly in an attempt to improve reproduction performance (see "The 4-Megarep Long-Term Rat Experiment"). As a check of the adequacy of the diet used in the 4-megarep experiments a pilot study, reported by France, Eckstein, and Brownell[28] and supported by Michigan Memorial-Phoenix Project No. 41, was made in which this diet was fed to mice.

In this pilot experiment reproduction studies and other observations were made on two generations of mice fed the same diets that the rats received in the initial phases of the long-term experiment. Two groups of mice, one composed of highly inbred animals of the Bagg albino strain and the other of mixed-strain, pigmented animals, were transferred from Purina Laboratory chow to the experimental diets at the time they were bred. Six Bagg albino and 16 mixed-strain female offspring of the animals on the non-irradiated diet were continued on the diets that their parents received. An additional control group was set up by transferring 6 albino and 16 mixed-strain females from the experimental rations to Purina Laboratory chow at the time they were mated for the production of the second filial generation.

Results of the reproduction studies on the first two generations are presented in Table 9. The breeding potential of the albino mice was too low to permit setting up groups of adequate size and a high incidence of sterility among the albino males of the first filial generation necessitated the replacement of the albino males with mixed-strain males of proven fertility. None of the albino females on the irradiated ration had produced young by the time they were 6 months old. The animals on this diet developed skin lesions with loss of hair and inflammation of the eyes.

TABLE 9

DATA ON REPRODUCTION IN THE 4-MEGAREP MOUSE PILOT STUDY

	Parent Generation				First Filial Generation					
	Nonirradiated Ration		Irradiated Ration		Nonirradiated Ration		Irradiated Ration		Purina Lab Chow	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Number of females	7	8	5	8	6	16	6	16	6	16
Number of litters born alive	5	6	3	8	5	15	0	16	6	12*
Total number of young born	21	37	14	46	45	121	0	113	43	89*
Average number of young born per litter	4.2	6.2	4.7	5.8	9.0	8.1	0	7.1	7.3	7.4*
Total number of young weaned	18	24	7	46	*	105	0	97	*	78*
Average number of young weaned per litter	3.6	4.0	2.0	5.8	*	7.0	0	6.1	*	6.5*
Average weight of young at 21 days (gm)	*	*	*	*	*	9.9	0	9.8	*	9.6*
Percent of weaned young having equilibrium disturbance	0	0	0	0	*	12.7	0	24.7	0	0*

(1) Bagg albino mice
(2) Hybrid, pigmented mice
* Records incomplete

Reproduction performance of the albino females fed the nonirradiated rations appeared to be normal. The data are included despite the small numbers of animals because the results suggest that the Bagg albino mice may be highly sensitive to dietary changes produced by irradiation, at least on the experimental ration fed.

Reproduction and lactation performance of the mice on the nonirradiated experimental ration compared favorably with that expected of animals raised on stock ration, in contrast with the performance of the rats in the long-term experiment. The mixed-strain animals on the irradiated diet showed appreciable impairment of ability to bear and raise their young. Some of the second filial mixed-strain generation young showed evidence of a neurological disorder characterized by an impaired ability to maintain equilibrium. The disorder was apparently attributable to the experimental diet and was twice as frequent in the offspring of mice fed irradiated ration as in those fed nonirradiated ration.

The weights of the young of the mixed-strain mice at the time of weaning were approximately the same for the animals on irradiated and nonirradiated experimental ration and on Purina Laboratory chow. Subsequent weight gains were also similar at least for the first two or three postweaning weeks, a result again in contrast with that observed for rats in the long-term experiment.

Although the number of animals involved was too small to be of statistical value, the fact that the second generation of Bagg-strain albino mice on the 4-megarep experimental diet failed to reproduce, whereas the mixed, pigmented strain of mice on the same diet reproduced as did control groups for both strains, indicated that Bagg-strain albino mice may be more sensitive to the effects of radiation on the experimental diet than are other animals studied to date.

E. The 4-Megarep Long-Term Rat Experiment

The tests with the protoza and the 20-megarep rat pilot study indicated that there is some loss in vitamins as a result of irradiation. There seemed to be no need to prove this again with an expensive long-term animal feeding and breeding experiment. However, there remains yet to be proven whether or not a diet in which the main caloric intake is from irradiated food is wholesome and nutritious and capable of supporting normal growth without any undesirable or toxic effect when used for long periods of time. There seems to be no information which would indicate the undesirability of irradiating the proteins, the principal food constituent responsible for growth, or the carbohydrate, usually the principal energy constituent of a diet. Therefore, it was decided that this is the question which should be investigated in the long-term feeding experiment.

1. Diet Adjustment and Preparation. With these considerations a long-term rat feeding and breeding experiment was commenced January 13, 1954, using a diet similar to that used in the pilot studies. These experiments are supported by Michigan Memorial-Phoenix Project No. 41 and the plan and early results have been reported by France, Rose, Eckstein, and Brownell.[26,27] At the beginning of the experiment the amount of edible oil in the diet was reduced from the 17% (dry basis) used in the 2-megarep rat pilot study to 12%. The original figure of 17% edible oil was considered to be high as fat accumulation occurred in some of the livers of the rats in the pilot studies. In July, 1954, the amount of cod-liver oil was reduced from the original 5% to 1% (dry basis) as the feeding of cod-liver oil at the 5% level was considered to be detrimental to good performance in reproduction. Roughage content was increased to 7% on a dry basis in order to eliminate occasional episodes of diarrhea which occurred in some of the animals in the exploratory studies. The Hubbel-Mendel-Wakeman salt mixture was then added at a 4% level on a dry basis, and the supplementary salts are omitted. Except for these changes and a slight adjustment in percentages the diet now being used in the long-term rat experiment is the same as used in the pilot studies. An analysis of the diet presently being used is given in Table 10.

The vitamin content of the diet, in particular the content of vitamins A and E, was considerably in excess of requirements for the rat. In the pilot studies the principal reason for exceeding minimum requirements, as well as for adding ascorbic acid and menadione which are not exogenous nutritive essentials in this species, was to detect possible toxicity from these sources in completely irradiated diets. To minimize the number of modifications in the diet these vitamins were not eliminated in the diet prepared for the long-term experiment even though the vitamin supplement is no longer irradiated. The water which the diet contains as a result of the incorporation of the canned meat products amounted to 38.5 percent. A small additional amount (55 gm/kilo) of water was added at the final mixing to improve the consistency of the diet.

A vitamin premix consisting of all the water-soluble vitamins except choline and a radiation premix composed of the canned meat, casein, starch, and cellulose are combined with the other ingredients and fed within two days of preparation. The premixes are stored at 4°C for not longer than 7 days. The oils are also refrigerated.

The irradiated portion of the diet includes all of the protein except any present in the liver and yeast preparations, all of the starch except a negligible amount added with the vitamin premix, about 12 percent of the fat, about 20 percent of the niacin, and small amounts of the various other vitamins and the salts which are added with the canned meat preparation.

TABLE 10

DIET USED IN 4-MEGAREP RAT EXPERIMENT AFTER JULY, 1954

Irradiated Constituents (4 Megarep)	Amount per Kgm of Diet, gm
1. Swift's Beef for Babies	500
2. Corn starch	290
3. Casein	75
4. Alpha cellulose	40
	905

Nonirradiated Supplements	Amount per Kgm of Diet, gm	mgm
5. Corn oil	54	
6. HMW salt mixture	24	
7. Yeast, brewers	11.28	
8. Liver concentrate	11.28	
9. Cod-liver oil	6.0	
10. Choline chloride	1.8	
11. Inositol	0.744	
12. P-Aminobenzoic acid	0.31	
13. α-Tocopherol	0.30	
14. Niacin		37.2
15. Ascorbic acid		19.2
16. Riboflavin		7.44
17. Pyridoxine HCl		7.44
18. Thiamin HCl		3.72
19. Menadione		1.92
20. Folic acid		0.53
21. Calcium pantothenate		0.30
22. Biotin		0.15

Bacteriological studies indicated that the radiation dose necessary to destroy high concentrations of resistant, spore-forming organisms may be in the neighborhood of 3 megarep. In order to allow a reasonable margin of safety the food is irradiated with approximately 4 megarep. The "irradiation flavor" induced by this dose can be expected to make the diet somewhat less acceptable to rats, a fact which must be taken into account in interpreting the data on the weight of the rats.

2. Animal Handling. Fourteen litters of Holtzman rats were obtained as weanlings on January 13, 1954. The 124 animals were divided to include 31 males and 31 females in each of two groups. One group received a nonirradiated diet and the other group received the partially irradiated diet in which essentially all of the proteins and the carbohydrates and a part of the fat have been irradiated (about 90 percent of the total diet).

The animal room was cleaned and fumigated during the week preceding the arrival of the animals and is reserved exclusively for this experiment. Only individuals and materials essential to the experiment are allowed entry into this room. A permanent record of temperature and humidity, which are maintained at constant levels by an air-conditioning unit, is provided by means of a hydrothermograph.

All variables not studied in the experiment are minimized and randomized in so far as possible; i.e., any local environmental differences in temperature, illumination, ventilation, and exposure to infection or to distracting influences were kept to a minimum. For this reason the control and experimental cages are placed in an alternate horizontal arrangement and males and females in an alternate vertical arrangement. Two nonlittermate animals are housed in each cage except for the odd animal in each group.

Carefully trained personnel are closely supervised by investigators with experience in animal nutrition studies. Animals are regularly fed once daily in the late afternoon, shortly before an automatic time switch turns out the light for 12 hours. Inspection at 8- to 12-hour intervals to ensure an adequate supply of food and fresh water at all times is part of a continuing effort to maintain optimal nutritional status prior to and during the reproduction studies. Both groups of rats receive food *ad libitum* and a record of the daily food consumption is kept. Fresh water is supplied daily. The growth of each animal is recorded after weighing on an accurate scale once a week at the same hour of the day.

3. Growth. Figure 11 shows the mean weight gain during the first year for the male and female rats that were fed from 22 days of age on the partially irradiated and nonirradiated semisynthetic diet. Ten weeks after weaning, the 31 males on the nonirradiated diet weighed an average of 384.2 gm and the 31 males on the partially irradiated diet weighed 370.6 gm. The

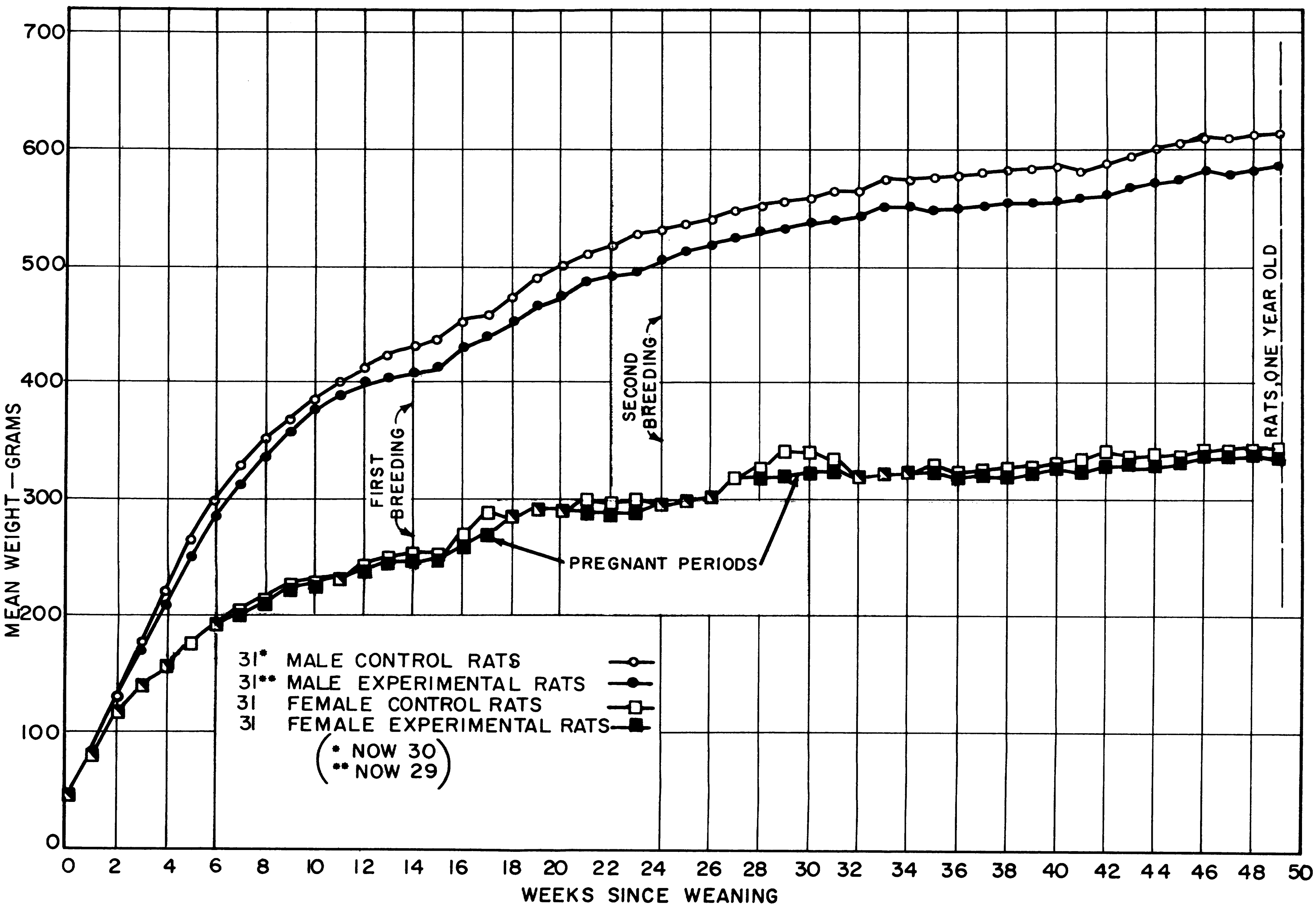

Fig. 11. Growth Curves for Male and Female Rats During First Year of 4-Megarep Long-Term Feeding Experiment.

12 male rats in the first pilot study, of the same strain and from the same supplier but fed Rockland rat diet, had a mean body weight of 260 gm at this age[25] approximately 2/3 of the weight of the long-term control males.

The growth rate obtained by Anderson and Smith[28] for the first 4 weeks after weaning with a natural diet supplemented with a paste high in fat, protein, and vitamin content was an average of 8 gm per day for the fastest growing male. This diet was stated by Dunn[29] to be near optimal for rat growth. The best growth rate attained by any individual parent-generation rat in the long-term experiment during the first 4 weeks after weaning was 8.2 gm per day observed for a male on the partially irradiated diet. The overall average daily weight gain for the male rats in the long-term experiment over this period was about 6.3 gm. The weight gain of the second filial-generation animals for the first 4 weeks after weaning was slightly superior to that of the parent animals for the corresponding period.

4. Reproduction. The rats used for the long-term feeding studies have been used as the parent generation for the reproduction studies. The parent-generation animals for the long-term experiment were first mated on April 21, 1954, when they were 121 days old. From each of the two groups of 62 rats, 20 males and 20 females were selected so they would be representative of the entire group in mean weight and in range of weights. The rats were subdivided into groups of 4 males and 4 females; each female in the subgroup was exposed in turn to each of the 4 males in the corresponding subgroup. For the first two pairings the animals were rotated at 2-week intervals and thereafter at weekly intervals. The principal reason for the longer interval between changes at the start of the breeding study, as well as for the delay beyond the scheduled age of 100 days for mating, was doubt concerning the condition of the vitamin premixes at the time originally planned for the start of the breeding period.

In the first breeding period each female was paired with up to 4 males; in the second breeding period pairing was continued with changes at weekly intervals either until pregnancy ensued or until the termination of the study.

The second breeding period was begun on June 28, 1954. In view of the poor record exhibited by the animals in the first breeding period, all the 124 animals in the colony were assigned to the second breeding experiment. Thus, for 11 pairs of animals in each group, the breeding period starting June 28 was the first time they had been mated. Each female that had produced a litter during the first breeding period was allowed at least 10 days rest before being remated. Figure 12 outlines the history to date and the plan being followed in the reproduction studies.

The results of the reporduction and lactation performance of control and experimental rats in the first and second breeding of the parent

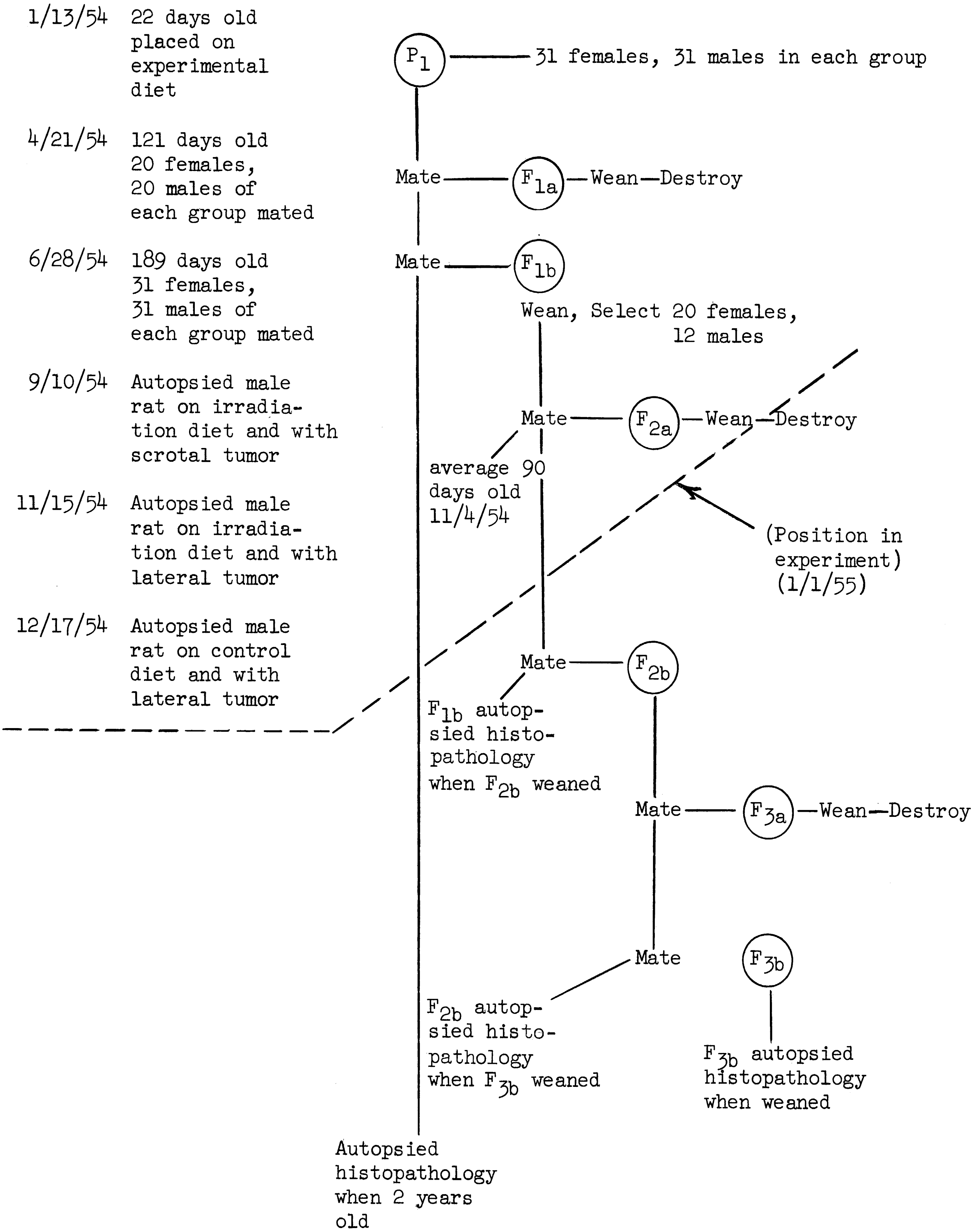

Fig. 12. Schematic Plan of 4-Megarep Long-Term Rat Experiment and Brief History to Date.

generation are given in Tables 11 and 12. The latter table also includes data on the performance of rats mated for the first time in the second breeding period. At the time the data were compiled, 18 of the 62 females had not given birth to young; consequently, the results for the second breeding period are incomplete.

Male sterility was high; in the first breeding period 40 percent of the control and 45 percent of the experimental males were exposed without success to 1 or more females later proven fertile. The corresponding figures for the second breeding period were 45 percent for the control and 35 percent for the experimental males.

The incidence of sterility in females was lower than in males; 10 percent of the control females and 25 percent of the experimental females apparently did not conceive after exposure to 4 males in the first breeding period. Data for the second breeding period are incomplete. At the time of writing a combined total of 76 pregnancies of a possible 102 occurred. The females of each group had 51 opportunities to produce litters; the control females succeeded 37 times, and the experimentals 33 times. Resorption gestation was responsible for a total of 6 reproduction failures.

The combined total of young produced by the control females was 339 and for the experimental females, 271. Early mortality was high with practically all the deaths occurring within 24 hours of birth. In the first breeding period about 18 percent of the young of the control females died before 21 days of age; in the second breeding period 32 percent of the young of multiparas and 43 percent of the young of primaparas died before weaning. The corresponding figures for the experimental animals were about 21, 29, and 76 percent, respectively. Combined early mortality was about 28 percent for the control group and 33 percent for the experimental group.

The total weight at weaning of the offspring of the 20 control females bred twice was 5.9 kg for first litters and 5.6 kg for second litters. For the offspring of the corresponding experimental females the total first litter weight was 3.7 kg; total second litter weight was 4.3 kg.

The results for both groups indicate high incidences of male sterility and early deaths in the young. Overall performance of experimental animals in bearing and raising second litter young was improved in most respects over the first breeding-period performance. Control animals were less successful in all categories except weight of young at weaning. If the change made in the vitamin E and cod-liver oil supplementation at the end of the first breeding period had any beneficial influence, this was reflected principally in the increased weights of the young at weaning. Animals fed partially irradiated diets were somewhat inferior in reproduction

TABLE 11

REPRODUCTION AND LACTATION PERFORMANCE IN FIRST BREEDING (F_{1a}) OF PARENT RATS IN THE 4-MEGAREP LONG-TERM EXPERIMENT

	Control Diet	Irradited Diet
Number of females	20	20
Number of males	20	20
Number of females not conceiving after four matings	2	5
*Number of males probably sterile	8	9
**Number of males not proven	2	3
Number of females conceiving the first week	8	3
Number of females conceiving the second week	1	3
Number of females conceiving the third week	3	4
Number of females conceiving the fourth week	0	0
Number of females conceiving after the fourth week	4	5
Number of females resorbing fetuses	2	0
Number of litters born	16	15
Number of litters born dead	1	1
Number of litters born alive of which none survived until weaning	1	1
Number of pups born	157	119
Average number of pups born per litter	9.8	7.9
Average number of pups per litter at 5 days	8	6.6
Average number of pups per litter at 21 days	8	7.4
Number of pups reaching weaning	127	82
Number of pups born dead	5	3
Number of pups born alive not surviving until weaning	25	21
Average weight of pups at 21 days (gm)	46.8	45.5
Average number of pups weaned per female bred	6.4	4.1

*Mated unsuccessfully with at least one female which later became pregnant by another male.

**Mated only with animals which later proved sterile or which were pregnant at time of mating.

TABLE 12

REPRODUCTION AND LACTATION PERFORMANCE IN SECOND BREEDING (F_{1b}) OF PARENT RATS IN THE 4-MEGAREP LONG-TERM EXPERIMENT

(Incomplete)

	Animals Bred for the Second Time at 189 Days of Age		Animals Bred for the First Time at 189 Days of Age		Combined Results of the Second Breeding Period	
	Control	Experimental	Control	Experimental	Control	Experimental
Number of females	20	20	11	11	31	31
Number of males	20	20	11	11	31	31
Number of females not conceiving after at least six matings	2	6	4	6	6	12
Number of males probably sterile	10	5	4	6	14	11
Number of males not proven	2	2	3	7	5	9
Number of females conceiving the first week	5	4	0	1	5	5
Number of females conceiving the second week	3	4	1	1	4	5
Number of females conceiving the third week	4	3	0	1	4	4
Number of females conceiving the fourth week	3	2	1	0	4	2
Number of females conceiving after the fourth week	1	1	3	1	4	2
Number of females resorbing fetuses	2	0	1	1	3	1
Number of litters born	16	14	5	4	21	18
Number of litters born entirely dead at birth	1	0	0	0	1	0
Total number of litters born alive of which none survived until weaning	1	3	3	2	4	5
Total number of pups born	145	115	37	37	182	152
Average number of pups born per litter	9.1	8.2	7.4	9.2	8.7	8.5
Average number of pups per litter at 5 days	6.1	5.6	4.4	2.3	5.4	5.1
Average number of pups per litter at 21 days	6.1	5.6	4.4	2.3	5.4	5.1
Number of pups reaching weaning	97	81	22	9	119	90
Number of pups born dead	17	11	2	4	19	15
Number of pups born alive not surviving until weaning	30	23	16	28	46	51
Average weight of pups at 21 days (gm)	57.8	52.7	55.0	56.1	58.0	55.4
Average number of pups weaned per female bred	4.9	4.1	2.0	0.8	3.6	2.8

and lactation performance to those fed nonirradiated diets in almost all categories.

5. Efficiency of Food Utilization. In an effort to utilize the mass of data on food intake which has been accumulating since the long-term animal feeding experiment began and at the same time to attempt to ascertain, at least to some degree of accuracy, the comparative efficiency of food utilization by the control and experimental animals, an analysis has been made of the efficiency of food utilization for the two diets based upon: (1) the record of diet fed each day, (2) the record and total refuse (diet removed from diet dishes each day), and (3) the average weights of control and experimental animals (computed weekly).

One procedure in the long-term experiment is the recording of the amount of diet placed daily in each cage. The total of these weights gives the total amount of diet fed to each group per day.

From this figure the weight of the corrected total amount of refuse found in the diet dishes the following day is subtracted to give a value for the total amount of diet consumed per day. Tests on dehydration of the diet over a 24-hour period under animal room conditions revealed that approximately 3/4 of the total weight remained after evaporation during a 24-hour period. On this basis a correction was made by multiplying the weight of the refuse by 4/3.

The total weight of diet consumed per week for all of the animals in each of the two groups was divided by the respective total weight gain, yielding the amount of diet consumed per gm of weight gained. The results are listed in Table 13.

These findings were compared with those of Swift and Company who reported a utilization of 3.16 for their controls and 3.28 for their experimental animal during the first 9 weeks following weaning.

6. Hematology. Table 14 gives the results from the hematological studies on parent rats in the long-term experiment. The table lists the arithmetic average of tests on 27 individual animals. The table indicates no significant difference between control animals and animals on the experimental diet. In addition to the studies reported in Table 14 brilliant cresyl blue films were examined for platelets and no marked differences were observed.

7. Histopathology. Figure 13 shows a rat which developed a lateral tumor at approximately 8 months of age. This growth first became apparent August 16, 1954. The rat was photographed 9 days later. The period of most rapid growth for this particular tumor seemed to be during the first 10 days after its detection. The tumor continued to grow steadily

TABLE 13

FOOD UTILIZATION BY RATS FOR THE FIRST 13 WEEKS IN THE 4-MEGAREP LONG-TERM EXPERIMENT

Date	Control, gm food/gm wt gain	Experimental, gm food/gm wt gain	Averages
1/20-1/26	1.62	1.61	
1/27-2/3	2.28	2.43	Mean for the 1st 9 weeks:—
2/4-2/10	2.40	2.52	
2/11-2/17	2.51	2.75	Control=3.89
2/18-2/24	4.30	4.05	Experimental=3.85
2/25-3/3	5.08	4.23	
3/4-3/10	5.03	4.81	Mean for the 1st 13 weeks:—
3/18-3/24	8.81	9.45	Control=5.13
3/25-3/31	7.29	6.34	Experimental=6.26
4/1-4/7	8.22	12.43	
4/8-4/14	11.04	16.11	

and 40 days after detection the infected rat appeared sickly and emaciated. The animal was sacrificed and an autopsy made. Table 15 shows the autopsy report for this animal.

Another tumor believed to be of this type was first observed in a male rat on the nonirradiated diet November 14, 1954. The rat was photographed on December 16, 1954, (see Fig. 14) and sacrificed on the following day. At the time of sacrifice the rat had dropped from a maximum weight of 613 gm to 586 gm. The tumor when removed weighed 66 gm. The autopsy report for this animal had not been received at the time of writing this report.

One rat on the irradiated diet developed a scrotal tumor which was detected for the first time August 23, 1954. This tumor developed so rapidly that on September 10, 1954, it seemed advisable to sacrifice the animal in order that the tumor might be removed before the rat died. Because of the rapidity with which this particular tumor grew and because of the haste with which the sacrifice had to be performed, no pictures of the animal were obtained. Figure 15 shows a photograph of the tumor after pathological examination. At the time of sacrifice the rat weighed 682 gm. The tumor when removed weighed 140 gm or approximately 21 percent of the total body weight. The heart, lungs, liver, spleen, kidney, adrenal gland, a section of the small intestine, and the testes were removed from the

TABLE 14

AVERAGE OF RESULTS OF HEMATOLOGICAL STUDIES ON INDIVIDUAL PARENT RATS IN 4-MEGAREP LONG-TERM EXPERIMENT*

Group	Hemoglobin, gm/100 cc	Hematocrit, %	Reticulocyte, %	White Blood Count, No./cu mm	Eosinophils, No./cu mm	Differential Count	
						Lymphocytes, %	Polymorphonuclear Leukocytes, %
Control Males	14.9	47.4	2.9	10,760	119	80.0	18.1
Control Females	14.5	45.5	3.0	9,550	120.0	81.0	17.3
Experimental Males	15.0	47.7	2.5	13,390	113.0	83.7	14.7
Experimental Females	14.8	45.9	1.7	11,260	128.0	81.7	16.3

*Five to seven animals were used for each test reported.

Fig. 13. Male Rat on Irradiated Diet Showing Development of Lateral Tumor at the Age of 8 Months.

Fig. 14. Male Rat on Control Diet Showing Development of Lateral Tumor at the Age of 12 Months.

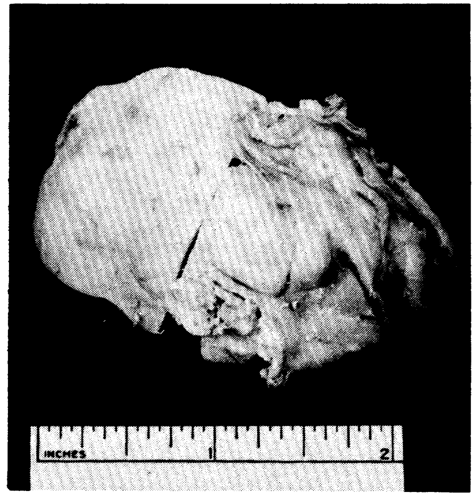

Fig. 15. Scrotal Tumor from Rat on Irradiated Diet (after Pathological Examination).

TABLE 15

HISTOPATHOLOGICAL REPORT ON MALE RAT WITH LATERAL TUMOR AND FED 4-MEGAREP IRRADIATED DIET

The neoplasm is an adenofibroma of the mammary gland. There is proliferation, in an orderly fashion, of ductal epithelium and an extensive periductal proliferation about the numerous ducts. The fibrous tissue is actively proliferating and is cellular. Neither element is pleomorphic enough to be considered malignant. The tumor may not represent a true neoplasm.

Tissue	Observation
Heart	Moderate acute passive congestion. No lipidosis.
Lung	Emphysema. Fat stain negative.
Spleen	Small foci of erythropoiesis.
Liver	Moderate lipidosis in hepatic cells and Kupffer cells. Rather diffuse distribution, but the center of lobules not involved.
Small Intestine	Negative.
Adrenal	Abundant cortical lipids.
Kidney	Thickening of basement membrane of tufts with ischemia. Occasional gray casts in tubules. Glomerulonephrosis. No lipidosis.
Vertebral Column	After decalcification, no evidence of avitaminosis-A, -C, or -D. Marrow and visible nerves normal.
Testes	Marked degerative changes in tubular epithelium. Aspermatogenesis. No mitotic figures present. Epithelium completely lost in many. Some tubules contain necrotic cells; others are calcified.

Summary: There is no satisfactory evidence that the lipidosis of the liver represents liver damage. The lipid is probably dietary lipid and its appearance is in response to the high fat diet. The renal changes indicate early reversible damage. The degenerative changes in the testes are definite and extensive. Adenofibroma of the mammary gland.

animal and histopathological examinations made of these organs. The complete report of the pathologist appears in Table 16.

8. Summary of Rodent Feeding and Breeding Studies to Date. Neither the pilot studies nor the results on the 4-megarep long-term feeding and breeding experiment have as yet indicated any acute toxicity to rats fed irradiated diets, receiving a dose of gamma radiation of up to 45 megarep. In the 4-megarep long-term experiment the animals on the control diet have shown a slightly superior growth rate as compared to the animals on the irradiated diet. In the long-term feeding experiment when the rats were 1 year old (49 weeks after weaning) 30 control males had an average weight of 617 gm as compared to 588 gm for the 29 males on the irradiated diet. There was less difference in weight between the females in the two groups. The 31 females on the control diet had an average weight of 346 gm at the age of 1 year as compared to 336 gm for the same number of females on the irradiated diet.

Pilot studies using a radiation dose of 2 megarep indicated little effect of irradiation but when the dose was increased to 20 megarep an appreciable loss of vitamins was indicated when the complete diet was irradiated. Feeding a nonirradiated vitamin supplement was shown to correct the symptoms of apparent vitamin deficiency.

With regard to reproduction and lactation performance, the albino rats on the control diet appeared to be slightly superior to the animals on the irradiated diet. Additional data are required to determine whether or not these results are significant.

The second generation of Bagg-strain albino mice on the 4-megarep experimental diet failed to reproduce, whereas the mixed, pigmented strain of mice on the same diet reproduced as did control groups for both strains. The number of animals involved was too small to be of statistical value; however, this pilot study indicates that Bagg-strain albino mice may be more sensitive to the effect of radiation on the experimental diet than are other animals used to date.

The experimental diet used in the 4-megarep experiment may be considered optimal, or nearly so, for growth, as evidenced by a growth rate of as high as 8 gm per day over the first 4 weeks after weaning in the animals on both the control and irradiated diet. This experimental diet is not as satisfactory as a commercial ration diet with regard to reproduction and lactation. Greater numbers of offspring born per litter and offspring weaned per litter have been observed with this strain of animals fed a commercial ration.

The experimental diet appears to introduce a certain degree of stress with regard to reproduction and lactation. However, the differences

TABLE 16

HISTOPATHOLOGICAL REPORT ON MALE RAT WITH SCROTAL TUMOR AND FED 4-MEGAREP IRRADIATED DIET

Tissue	Observation
Heart	No significant abnormality of the myocardium. No lipidosis.
Lung	Slight peribronchial lymphocytic infiltration, fat stain negative.
Spleen	A few lymphoid follicles remain but most of the spleen is replaced by tissue like bone morrow. Erythropoietic and granulopoietic cells and numerous megakaryocytes present. Myeloid metaplasia.
Small Intestine	Negative.
Liver	Acute passive congestion. Very slight lipidosis.
Kidney	Negative. No lipidosis.
Testes	No spermatozoa present. The seminiferous epithelium presents various stages of atrophy and necrosis. The pattern is variable in different areas. In the more nearly normal tubules spermatogenesis is abnormal. No mitotic figures present. There are bizarre cellular forms, some with large dark nuclei, and others with many nuclei. Interstitial cells are not increased in number.
Scrotal Tumor	A moderately well differentiated spindle cell supporting tissue neoplasm with many minute blood vessels. Angiofibrosarcoma. Tissue growing by expansion and invasion; no capsule can be identified. No metastases have been found. This is compatible with the lack of anaplasia of the neoplasm. Areas of necrosis are present within the tissue.

Summary: Well differentiated angiofibrosarcoma of the scrotum. Atrophy and necrosis of the seminiferous tubules with the production of bizarre multinucleated forms. Marked myeloid metaplasia of the spleen.

in performance observed between the control animals and the animals on the irradiated diet are comparatively small.

Histopathological examinations of animals used in the pilot study indicated no significant difference between those on the control and irradiated diets. In the 4-megarep long-term rat experiment 2 males out of 62 animals on the irradiated diet and 1 male out of 62 animals on the control diet have developed rapidly growing tumors. Further data are required to indicate whether or not the irradiation of the diet has a significant influence in the development of such tumors.

Indications to date are that as compared with changes in palatability and vitamin content, toxicity appears to be an unimportant aspect of food irradiation even at much higher levels than would be encountered in future commercial pasteurization or sterilization with gamma radiation.

II. CHICKEN FEEDING EXPERIMENT

A. Introduction

Discussions were held in 1953 in the Washington offices of the Food and Drug Administration regarding feeding experiments at the University of Michigan using irradiated food. Representatives of the Food and Drug Administration pointed out the advisability of using animals other than rodents in some of the experiments. The use of monkeys, dogs, and chickens was suggested. Chickens are the least expensive of these animals and data on chickens fed an irradiated diet would be a valuable supplement to similar information obtained on rats. A proposal to study the wholesomeness of a gamma-irradiated diet fed to chickens was submitted to the Office of the Surgeon General of the U.S. Army. This proposal was approved by Contract DA-49-007-MD-581 which established Project 2307 in the Engineering Research Institute of the University of Michigan. Authorization to commence the study was received in September, 1954. This first report has been prepared by L. E. Brownell, H. C. Eckstein, and C. H. Burns. Mr. Charles C. Guider, graduate in animal husbandry from Michigan State College with 5 years experience as field representative in poultry for the Ralston Purina Company, has acted as consultant regarding commercial practice in raising chickens.

The first few months of activity on Project 2307 have been spent on developing the overall plans for the experiment, completing plans for the laboratory to house the chickens, selecting a suitable diet, procuring and irradiating the diet, adding suitably trained personnel to the staff of the Fission Products Laboratory, procuring about 200 day-old chicks of a suitable strain, initiating the experimental feeding, and preparing the first report.

B. General Plan of the Experiment

The general objective of the experiment is to place one group of 100 day-old chicks of a well established breed and strain on an irradiated diet and to place a similar group on a control diet. Inasmuch as body weight is a sensitive measure of the nutritional adequacy and toxicity of a diet as well as of its acceptability, growth curves are expected to afford valuable information. Biweekly weighings at the same hour of the same days on an accurate scale will continue for the duration of the experiment.

Birds that die or are removed from the experiment (other than chicks lost during the first week) because of conditions such as severe chronic respiratory infection will be autopsied. The lung, heart, liver, spleen, kidney, a section of small intestine and possibly other glands will be preserved for histopathological examination. The birds that survive to the end of the experiment will undergo similar examination. If marked differences develop between the control and experimental groups, appropriate biochemical studies to determine the cause and nature of the changes can be undertaken in conjunction with investigations on the nature of radiation-induced changes in foodstuffs. Such studies would be in line with the recommendation of Lehman, et al.,[30] that organ function and enzyme activity determinations be made in experiments on appraisal of foods for toxicity. Data will be collected for both groups on: (1) growth of individual chickens, (2) egg production, (3) fertility based on incubation data, (4) observations on physical appearance, (5) pathological results, (6) food efficiency, (7) longevity, and (8) second-, third-, and fourth-generation studies.

This experiment will supplement the data obtained with rodents and will check the effect of an irradiated diet on a different species.

C. Description of Chicken Room

A one-story tile building adjoining the high-level radiation laboratory of the Fission Products Laboratory has been allocated for use in the chicken feeding experiment. Plans have been made to adapt this room to hold 120 cages for adult birds. A plan view and elevation view of the arrangement of the chicken room plus a description of the details are given in the Appendix. The room will be kept at 64°F during winter and as cool as possible without air-conditioning equipment during the summer. Adequate ventilation will be provided.

D. Diet

Selection of a diet was based upon experience obtained in rat studies. The diet used for feeding rats is quite expensive as a result of the use of 50% canned meat and crystalline vitamin supplement. As of January 1, 1955, the cost of materials for the experimental rat diet was approximately $1000 per month.

To minimize the cost of the diet and the cost of preparing the diet it was decided that a commercial ration should be used as the basic diet for chickens. Commercial rations for raising chickens have been studied, improved, and standardized by the companies producing mash for chickens; these feeds can be used to advantage in the present chicken feeding experiment. The decision was made to feed the chicks a wet mash so as to check the secondary effect of radiation on water which is present to a large percent in most foods for humans that might be irradiated. Tests showed that mixing the mash with an equal weight of water gave a mash with a good consistency; therefore, this amount of water presently is being used with a commercial starting mash (Purina "Startena"). This mash normally contains .0025% 3-nitro-4-hydroxyphenyl arsonic acid and antibiotic-supplement growth stimulants. These growth stimulants were considered undesirable because they are not found in a normal diet for humans and were excluded by obtaining a special lot of mash. Chicks will be kept on this starting mash for approximately 4-1/2 to 5 weeks until 2 lb of dry starting mash has been consumed per bird. This starting mash consists of the following ingredients: meat scrap, fish meal, soybean oil meal, ground yellow corn, dehydrated alfalfa meal, dried whole whey, ground oats, ground grain sorghums, wheat gray middlings, condensed fish solubles, vitamin A feeding oil, riboflavin supplement, calcium pantothenate, niacin, deactivated animal sterol, .75% low fluorine rock phosphate, 1.5% calcium carbonate, .5% iodized salt, and .02% manganese sulphate. These ingredients are blended by the producer in order to provide the following guaranteed analysis: protein, not less than 20.0%; fat, not less than 3.0%; fibre, not more than 5.0%; and n.f.e., not less than 50.0%.

During the first week the chicks in each group were given a supplement of about 5 lb per 100 chicks of cracked grain for chicks to prevent "pasting" of the vent which sometimes develops with newly hatched chicks placed on only a mash ration. These cracked grits are not well digested by baby chicks but act as a purge and aid in cleaning out the mucous in the intestinal tract of the newly hatched chick. The grits were not irradiated.

The mash is mixed in the Hobart mixer with an equal part of water and divided into two portions. Both portions are packaged in either polyethylene bags or No. 10 tin cans. One is used as a control and the other is irradiated in the high-level radiation "cave." The radiation dosage used is 3 megarep. This is the dosage which Dr. Bruce Morgan of the Quartermaster Corps considers may be required for commercial sterilization by gamma irradiation. After irradiation the wet mash is placed under refrigeration until feeding if it is to be used in the next few days. If it is to be stored for a longer period it is quick frozen in a "deep freezer" and kept frozen until shortly before use. Because irradiation destroys a large portion of the vitamins in the commercial mash, a vitamin supplement is added. This supplement supplies the vitamin allowance recommended by the National Research Council.[31] It is added to both the irradiated mash and control shortly before feeding. Table 17 gives the composition of the vitamin supplement fed to both groups of chicks. (Chicks do not require ascorbic acid.).

After the chicks are about 4-1/2 to 5 weeks old they will be placed on a commercial-growing mash such as Ralston Purina Growena. Approximately 16 to 18 lb of dry-growing mash per bird will be required to reach 10 percent laying in pullets. With 50 percent of the pullets laying, about 22 to 24 lb per day of dry-growing mash will be required per 100 birds. With 70 percent of the birds laying, about 26 to 28 lb per day of dry-growing mash will be required per 100 birds.

TABLE 17

VITAMIN SUPPLEMENT USED IN 3-MEGAREP CHICKEN FEEDING EXPERIMENT

Supplement	Weight per Kgm Dry Ration, gm	mgm
Cod-liver oil (2000 A, 400 D)	2	
Choline chloride	2	
i-Inositol	1	
p-Amino benzoic		500
Niacin		100
Calcium pentothenate		40
Riboflavin		12
Pyridoxine HCl		8
Thiamine HCl		6
Folic acid		5
Menadione (Vit K substitute)		0.5
Biotin		0.6
α-Tocopherol		0.3
Vitamin B_{12}*		.05

*As a 0.1% triturate in sodium chloride

E. Breed and Strain of Chicken

White Rocks and White Leghorns were both considered as suitable breeds. The White Rock is an all-purpose medium breed used both for meat production and egg production, whereas the White Leghorn is a light-weight breed used chiefly for egg production. The White Leghorn breed was selected because this breed requires 25 percent less floor space and less feed than the heavier White Rock breed and is considered to show more uniformity in growth. The Dryden Class B strain was selected because of high egg production and rapid growth for the Leghorn breed. Day-old chicks (190) of this strain were obtained from the Brewer Hatchery at Dundee, Michigan. The females in the Dryden Class B strain are individually pedigreed with a background of more than 25 consecutive years of pedigreed breeding. The females have trapnest records of 200 to 300 eggs per year and the dams of the males have records above 300 eggs per year.

F. Results During First Two Weeks

On December 20, 1954, 190 newly hatched chicks were obtained from the Brewer Hatchery at Dundee, Michigan. These chicks were sexed at the hatchery and showed 100 cockerels and 90 pullets. The chicks were wing-banded on December 21 (bands obtainable from the National Band and Tag Company) and were individually weighed. There were 49 males and 45 females in the control group and 48 males and 45 females in the group on the irradiated diet. Figure 16 shows the chicks being wing-banded by Dr. C. H. Burns and being placed on 3-mesh wire under a commercial brooder. The brooder temperature was kept at 95°F. for the first week and lowered 5°F per week thereafter.

The chicks were weighed twice weekly and the average weight was plotted as shown in Fig. 17. One cockerel died in transit from the hatchery and one cockerel on the irradiated diet and two cockerels on the control diet died the first week of the experiment. No differences in weight gain or general appearance have been observed during the first 2-week period of the experiment.

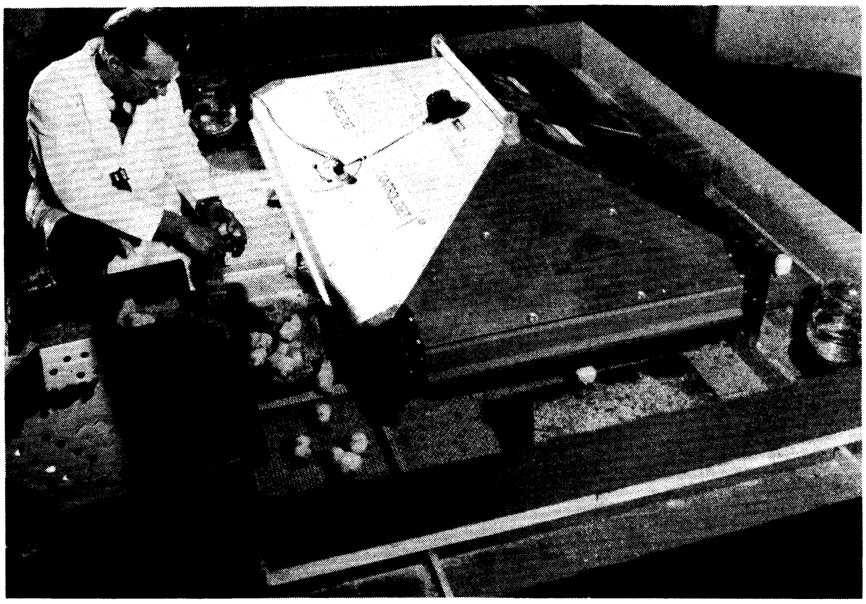

Fig. 16. View Showing Chick Brooder and Wing Banding of Day-Old Chicks.

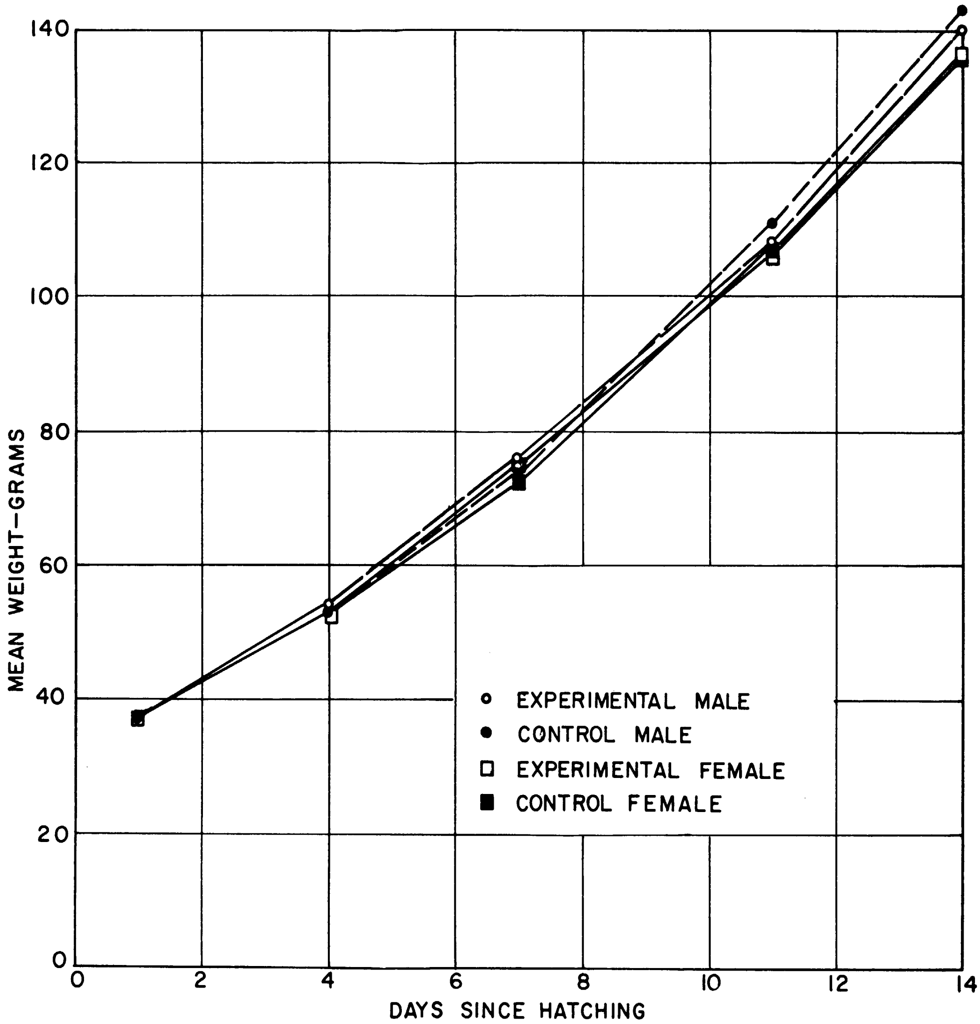

Fig. 17. Growth Curve for First 2 Weeks of Chicks in 3-Megarep Experiment.

APPENDIX

DESCRIPTION OF CHICKEN ROOM

The plan and elevation views of the chicken room are shown in Figs. 18 and 19. It is estimated that this building will accomodate about 120 adult chickens of a medium heavy breed, such as White Rocks, or 160 White Leghorns (based on 4 square feet of floor area per adult chicken of heavy breed or 3 square feet per chicken of light breed).

Optimum temperature for adult chickens was considered to be 64°F. Ventilating air will be drawn from the first floor of the high-level radiation laboratory which is maintained at approximately this temperature by thermostatic control and steam heat. This requires an increase in the area of steam-blast heaters on the first floor of the high-level radiation laboratory. Two ventilators, approximately 18 inches by 18 inches, are to be placed several feet apart in the common wall between the two buildings. These ventilators are to be of the one-way type so as not to permit the air from the animal feeding room to enter the high-level laboratory. Such ventilators are supplied by the Jamesway Equipment Company.

The roof is to contain two ventilated cupolas, approximately 18 inches in diameter and 18 inches high, with electric exhaust fans designed to remove sufficient air to prevent the temperature from dropping below 64°F in the winter and to remove about 2.0 gallons of water per hour as water vapor given off by the adult birds (based on 1.67 gallons of H_2O per 100 adult birds).

Individual cages will be used for the birds when they are 9 or 10 weeks of age, or older. These cages will be hung from the ceiling in batteries elevated 3 feet above the floor. Experience has shown that birds kept in such cages have only about 1/5 the yearly mortality of birds kept on the floor. This procedure also simplifies maintaining records on individual birds. The cages are being supplied by the Pockham Manufacturing Company, Decator, Alabama.

A water line is to be installed to each battery of cages to provide drinking water for the birds. The overflow from the water troughs is to be removed by a rubber hose (to minimize plumbing expense) and is to be collected in a galvanized steel pipe leading to the sewer. A sink is to be installed in one corner of the room for washing equipment in the chicken room.

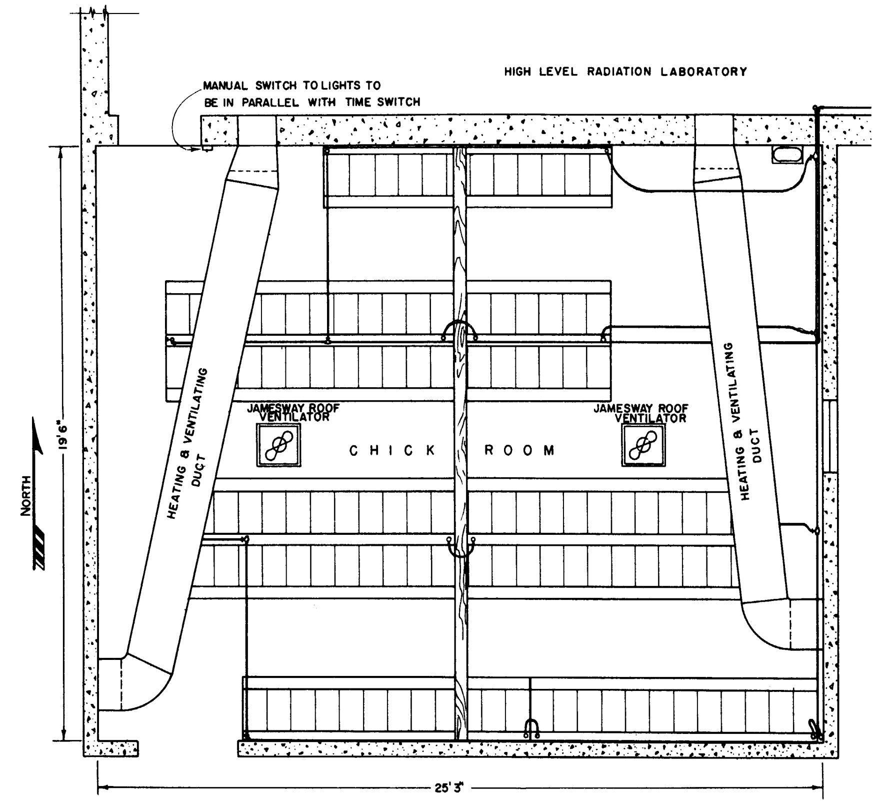

Fig. 18. Plan View of Chicken Room.

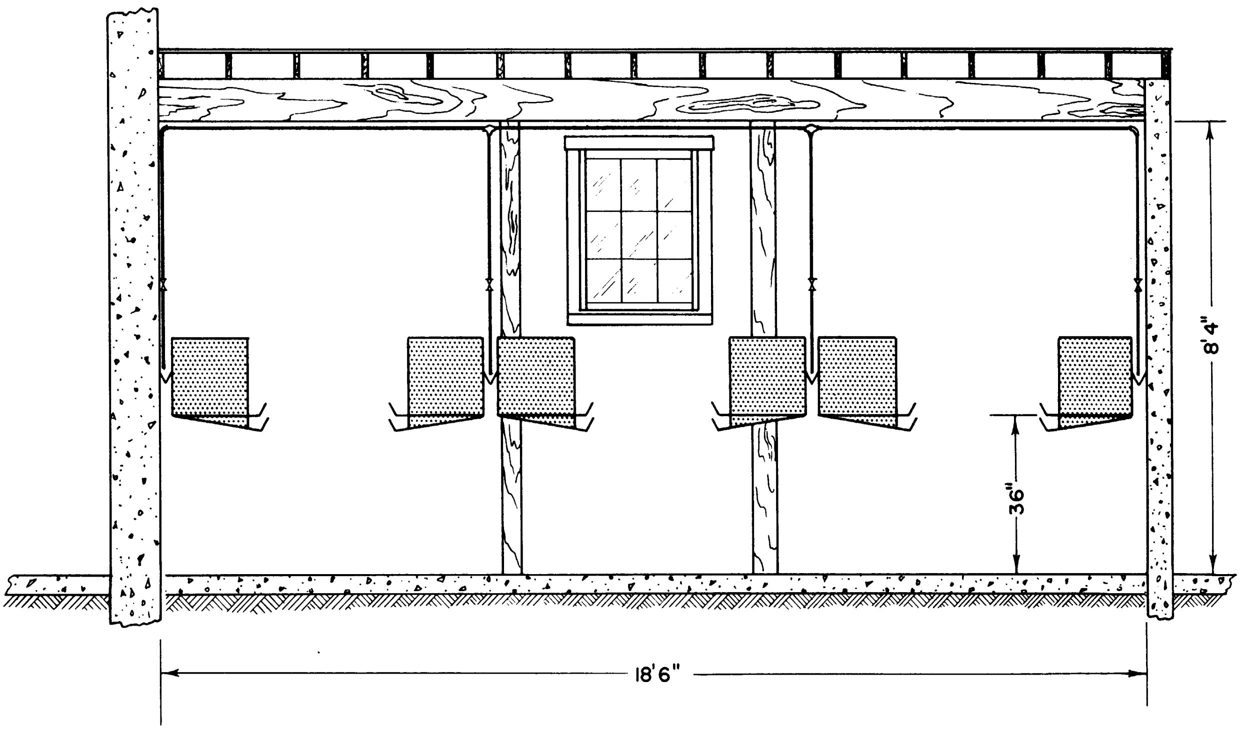

Fig. 19. Elevation View of Chicken Room.

Excessive light and white walls have been found to excite chickens, particularly Leghorns, and to cause cannibalism. The optimum lighting recommended by the Ralston Purina Company is one watt per 4 square feet of floor area. The walls of the chicken room will be painted green, as chickens have been found to show a preference for this color.

A doorway is to be located in the common wall between the radiation laboratory and the chicken room to provide easy access to the chicken room and to facilitate the transfer of irradiated chicken feed from the radiation cave to the chicken room.

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