THE WHOLESOMENESS OF GAMMA-IRRADIATED DIETS AS DETERMINED BY LONG-TERM ANIMAL FEEDING AND BREEDING STUDIES WITH ALBINO RATS

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A. INTRODUCTION

The lethal effect of gamma radiation on microorganisms offers considerable promise as a basis of a new method of processing perishable foods to increase their storage life. Gamma radiation induces some chemical changes in foods also. Whether such changes affect the wholesomeness of gamma-irradiated foods must be established before the process can be used. That some loss in vitamins occurs as a result of irradiation has already been indicated by preliminary studies.[1,2] It remains yet to be proven whether or not a diet in which the bulk of the intake is from irradiated food is otherwise nutritious and capable of supporting normal growth without any undesirable or toxic effect when used for long periods of time. At the time this study was initiated there seemed to be no information which would indicate the undesirability of irradiating the proteins, the principal food constituent responsible for growth, or the carbohydrates, usually the principal energy constituent of a diet. After consultation with representatives of the Food and Drug Administration of the U. S. Government, it was decided that this question should be investigated by a long-term feeding and breeding experiment using albino rats as the experimental animals. This study has been supported by Michigan Memorial-Phoenix Project No. 41.****

B. DIET

To determine the wholesomeness of irradiated food by means of an ani-

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****The Michigan Memorial-Phoenix Project is a memorial to the Michigan dead of World War II. The Regents of The University of Michigan voted to "create a War Memorial Center to explore the ways and means by which the potentialities of atomic energy may become a beneficent influence in the life of man, to be known as the Phoenix Project of The University of Michigan."

mal feeding and breeding experiment, it was decided that a diet would 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. This point was demonstrated with a medium for <u>Tetrahymena</u> <u>pyriformis</u> irradiated with a 4-megarep dose in the anhydrous state. The irradiated medium was unaffected by this dose of radiation,[1] whereas the irradiation of an aqueous solution of the medium resulted in a reduced rate of growth of the protozoa.[1]

The diet composition is given in Table I. The canned meat constituted 50% of the diet and was supplemented with casein, corn starch, and alpha cellulose. This portion, consisting of 88.4% of the diet, was given a sterilizing dose of radiation. After irradiation, the vegetable oil, the vitamin supplements, and the salt mixture were added.

TABLE I. DIET USED IN STUDIES WITH ALBINO RATS

Irradiated Constituents in Diets	Amount per Kg of Diet, g	mg
1. Swift's Beef for Babies	500	
2. Corn starch	281	
3. Casein	71	
4. Alpha cellulose	38	
	844	
Nonirradiated Supplements		
5. Corn oil	5.4	
6. Yeast, brewers'	11.28	
7. HMW[3] salt mixture	24	
8. Liver concentrate	11.28	
9. Cod-liver oil	10.6	
10. Chlorine chloride	1.8	
11. Inositol	0.744	
12. P-Aminobenzoic acid	0.31	
13. α-Tocopherol acetate	*	
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. Calcium pantothenate		36.00
21. Folic acid		0.53
22. Biotin		0.15

*Administered by dropper to rats individually.

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 certainty, the diet was irradiated with approximately 4 megarep.

C. SHORT-TERM STUDIES

As an aid in planning the long-term study, a number of short-term studies were made before commencing the long-term feeding and breeding experiment. Short-term studies using a radiation dose of 2 megarep indicated little effect of irradiation. When the radiation dose for complete diet was increased to 20 megarep, an appreciable loss of vitamins was indicated (see Fig. 1). Feeding a nonirradiated vitamin supplement was shown to correct the symptoms of apparent vitamin deficiency (see Fig. 2), but even with the addition of nonirradiated vitamin and fat supplements, growth was slightly under that of the animals on nonirradiated diet.

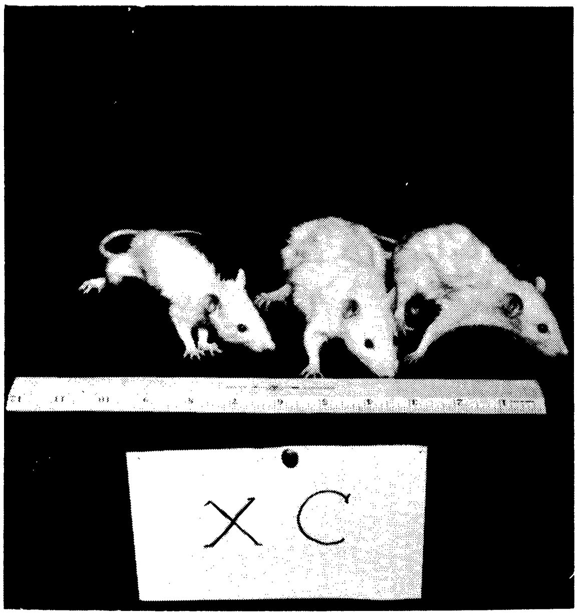


Fig. 1. Two males on left, female on right, showing effects of feeding a diet completely irradiated at 20-megarep dose.

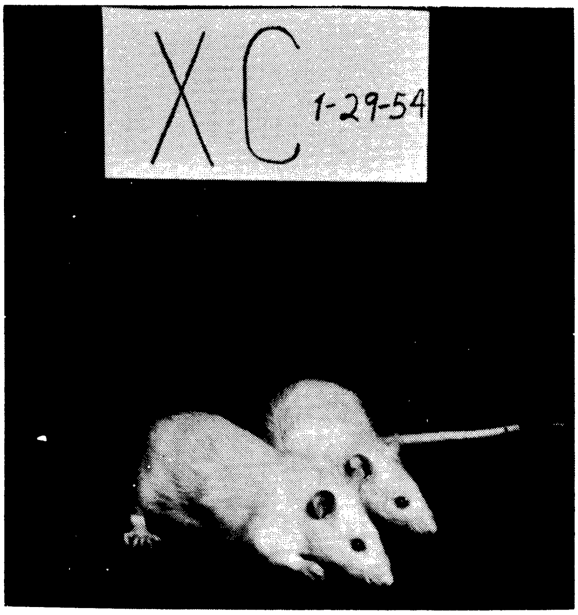


Fig. 2. Male (left) and female fed exclusively on completely irradiated diet (at 20 megarep) for 10 weeks, then fed the same diet supplemented with the water-soluble vitamin premix for 3 weeks. (Note: these animals are the same as the right two in Fig. 1 and show recovery from vitamin deficiency.)

Based on these data, the general conclusion is made that if the radiation dose is greater than 2 megarep, the growth, reproduction, and lactation performance of albino rats on the control diet are slightly superior to that of the animals on the irradiated diet, even though the diet is supplemented with non-irradiated vitamins. Pathological examinations of organs and tissues of animals used in these studies indicated no significant difference between those on the control and irradiated diets.

The short-term studies indicated that, as compared with changes in palatability and vitamin content, acute toxicity is an unimportant aspect of irradiated food, even at levels of irradiation much higher than would be encountered in future commercial radiopasteurization or sterilization with gamma radiation. Thus, any effects of radiation of the diet will be subtle and will require maintaining the animals throughout their lifetime and obtaining and raising several generations of offspring on the diet.

D. THE LONG-TERM EXPERIMENT

The plan of this experiment was to study growth, reproductive performance, hematology, and pathology of male and female albino rats fed both the irradiated and nonirradiated diets throughout their natural lives. The diet is described in Table I and was fed *ad libitum*. From the parent generation on each diet three successive generations of rats were to be obtained and raised on the same diet. The animals in each generation were to be bred twice and weanlings from the second breeding were to be selected to give the successive generation. Data on growth and reproductive performance would be obtained from these offspring.

The experiment began January 13, 1954, when the animals in 14 litters of Holtzman rats were divided to include 31 males and 31 females in each of two groups, one fed the irradiated and the other the nonirradiated diet. As of January 25, 1956, the 106th week of the experiment, there were seven males and nine females remaining on the nonirradiated diet and seven males and sixteen females remaining on the irradiated diet. The third-filial and final generations had been obtained and had reached maturity. The parent, first-, and second-filial generations were bred twice, as planned. Blood-cell data were obtained at two different times from the parent-generation rats and one from the first-filial-generation animals. Pathological observations of all parent animals were made weekly, and observations at autopsy together with histopathological reports were made of all animals which died or which were sacrificed. The results to date for this are described in the following section.

1. *Growth*.—Figure 3 shows the average body weights of all four generations of male and female rats on each diet. It also shows the approximate relationship of each of the generations with respect to week of experiment.

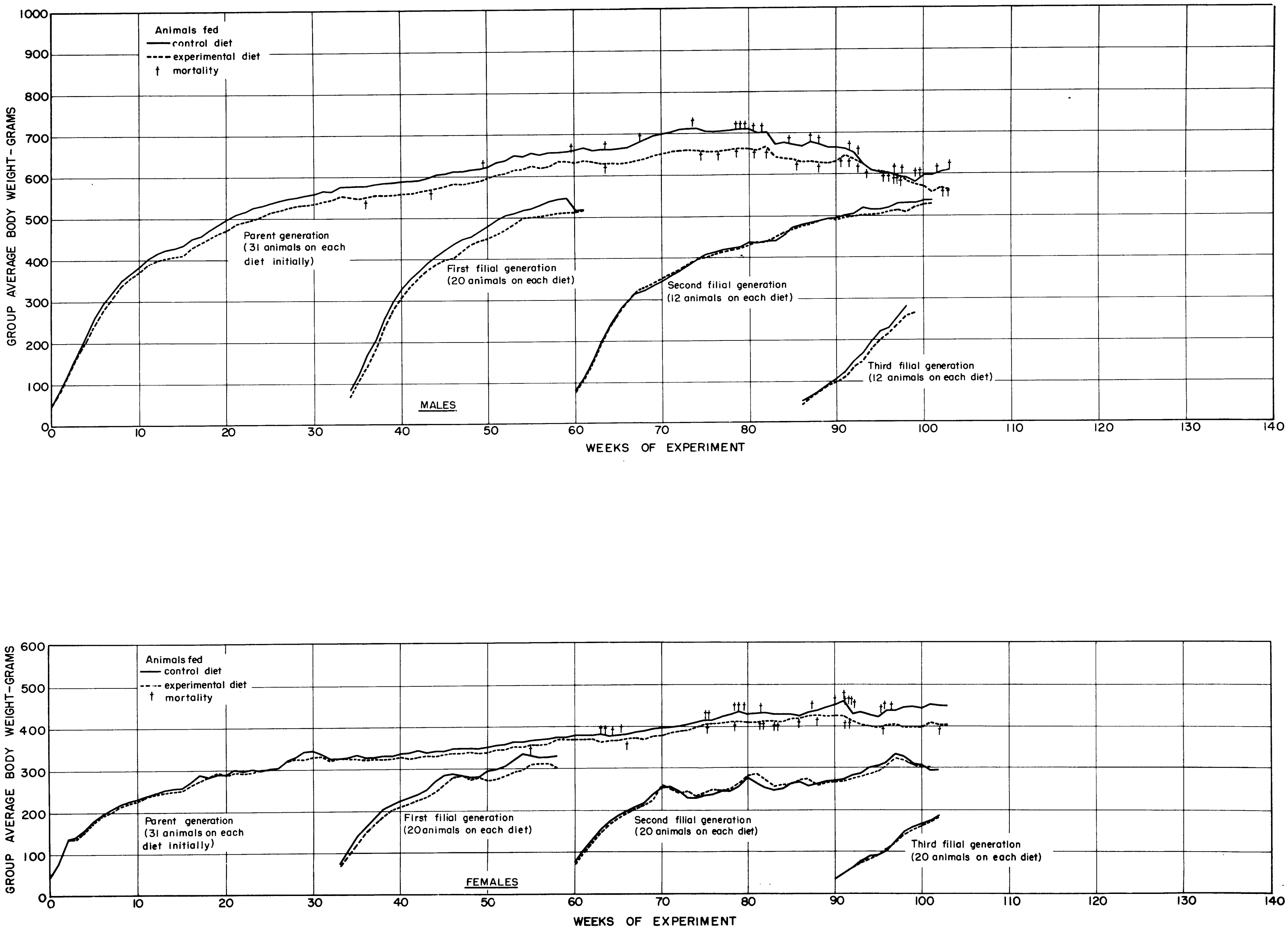


Fig. 3. Average body weights of rats fed 4-megarep irradiated and nonirradiated diet on long-term experiment.

Only the animals of the first-filial generation other than diseased animals have been sacrificed to date. The occurrence of deaths (natural or as a result of sacrifice) is indicated by a dagger symbol placed on each graph during the week in which it occurred. In the case of the parent-generation rats, comparisons of average body weight are significant only for the first sixty or seventy weeks, since the increasing mortality thereafter obscures the true effect of irradiation of the diet. Not shown on the growth curves for the second-filial generation is the fact that two males and three females have died in the control group, and one male and four females in the experimental group; no deaths occurred prior to the 87th week, and none since the 97th week of the experiment.

These data indicate that the animals fed the irradiated diet showed growth rates and average body weights which were slightly less than those of the animals fed the nonirradiated diet. The difference was less pronounced in the case of the females than in the case of the males. With the exception of the second-generation animals, the difference appeared to increase slightly with age, although the percentage difference probably was not increasing. During the period of rapid growth, the difference was the same from one generation to the next, except for the second-filial-generation males and first-filial-generation females.

Other laboratories, using rats of different strains and a variety of diets, have obtained growth rates and total body weights similar to the results reported here. However, certain diets high in raw-vegetable content have shown the reverse effect; that is, growth was slightly superior on the irradiated diet. This might be explained by a slight increase in digestibility of the irradiated raw food as compared to the nonirradiated raw food.

Other studies have shown that when only the protein portion of the diet was irradiated, a slight growth depression resulted and that this was reversible by adding certain nonirradiated amino acids, depending on the protein. This indicates that the effect of irradiation is the loss of nutrients as a result of the irradiation, rather than as a result of the production of any toxic substances.

2. Reproduction.—Table II presents the data from the first and second breedings of the parent-, first-filial-, and second-filial generation rats. The breeding practice was to mate one male with two females on the same diet for one week, and then to rotate the males. Parent-generation males were mated with one female each week. Females were considered sterile if they failed to become pregnant after six matings. Males were considered sterile if they were mated with at least one female which later became pregnant by another male. The time of conception was considered to be 21 days prior to birth of young. The females resorbing fetuses were those whose weight gains following mating indicated pregnancy but which failed to give birth to young. Litters of more than ten pups were reduced to ten to avoid the death of young owing to competition among pups for the mother's milk.

TABLE II. SUMMARY OF REPRODUCTION PERFORMANCE IN LONG-TERM EXPERIMENT

	1st Breeding of Parent Gener.		2nd Breeding of Parent Gener.		1st Breeding of 1st Filial Gener.		2nd Breeding of 1st Filial Gener.		1st Breeding of 2nd Filial Gener.		2nd Breeding of 2nd Filial Gener.	
	Cont. Group	Exp. Group	Cont. Group	Exp. Group	Cont. Group	Exp. Group	Cont. Group	Exp. Group	Cont. Group	Exp. Group	Cont. Group	Exp. Group
No. females bred	20	20	20	20	20	20	20	20	20	20	20	20 (7)
No. males used	20	20	20	20	12	12	12	12	10	10	10	10
No. females sterile (1)	2	5	2	6	2	1	3	2	1	0	0	0
No. males sterile (2)	8	9	10	5	4	3	3	3	0	2	2	2
No. males not proven (3)	2	3	2	2	0	0	2	2	0	0	0	0
No. females conceiving first week	8	3	5	4	6	5	10	12	12	12	10	8
No. females conceiving second week	1	3	3	4	6	6	3	4	6	4	5	6
No. females conceiving third week	3	4	4	3	3	7	3	0	1	2	3	1
No. females conceiving fourth week	0	0	3	2	0	0	1	0	0	1	1	1
No. females conceiving after fourth week	4	5	1	1	3	1	0	2	0	1	1	2
No. females resorbing fetuses	2	0	2	0	0	0	0	0	0	0	0	0
Total no. litters born	16	15	16	14	18	19	17	18	19	20	20	18
Total no. litters born dead	1	1	1	0	2	0	1	1	0	0	0	0
Total no. litters born alive, not surviving weaning	1	1	1	3	0	1	1	0 (6)	3	3	6	2
Total no. pups born	157	119	145	115	184	195	173	188	195	204	161	163
Total no. pups born dead	5	3	17	11	22	9	17	13	3	0	3	4
% pups born dead	3.18	2.52	11.72	9.56	11.95	4.62	9.83	6.91	1.5	0	1.87	2.45
No. pups born alive per female bred	7.6	5.8	6.4	5.2	8.1	8.3	7.8	8.8	9.6	10.2	7.9	8.8 (7)
Total no. pups born alive, not surviving weaning	25	21	30	23	17	24	21	14 (6)	64	76	88	59
Average no. pups born per litter	9.8	7.9	9.1	8.2	10.22	10.26	10.18	10.44	10.3	10.2	8.05	9.05
Average no. pups per litter at 5 days	8	6.6	6.1	5.6	8.4	9.3	8.5	9.2	8.7	8.4	7.1	7.5
Average no. pups per litter at 21 days	8	6.6	6.1	5.6	8.0	8.6	7.6	8.7 (6)	6.7	6.4	3.5	5.6
Total no. young disposed of (4)	0	0	0	0	0	0	5	7	3	7	0	0
Average weight of young at 21 days, grams	48.6	45.5	57.8	52.7	45.5	47.7	52.9	49.9 (6)	27.1	27.0	--- (8)	--- (8)
Total no. young reaching weaning (5)	127	95	98	81	145	162	135	161 (6)	128	128	70	100
Average no. young weaned per female bred	6.43	4.8	4.9	4.1	7.2	8.1	6.5	8.1 (6)	6.4	6.4	3.5	5.0
% pups born alive which survived 21 days	83.5	81.8	76.6	77.9	89.4	87.1	86.5	92.0	66.7	62.7	44.3	62.9

(1) Mated six times unsuccessfully.
(2) Mated unsuccessfully with at least one female which later became pregnant by another male.
(3) Mated only with pregnant or sterile females.
(4) For purposes of reducing litters to ten after birth.
(5) Does not allow for animals that were disposed that might have survived to weaning age.
(6) Data do not include those of pups from females conceiving after 4th week.
(7) Two females on experimental diet died during breeding. They were not counted in computing averages.
(8) The young were noticeably low on weight at weaning. Therefore, they were often left with their mothers after weaning date in an attempt to enable them to grow at the optimum rate; therefore, we do not have any accurate weaning-weight data on these animals.

An examination of the data reveals no consistent superiority of animals fed the nonirradiated diet over those fed the irradiated diet. With regard to female sterility, total number of pups born, average number of pups per litter at 21 days average weight of young at 21 days, and total number of young reaching weaning, the parent generation on the nonirradiated diet showed superior performance. However, the first- and second-filial-generation animals fed the irradiated diet were superior to the control animals in the above performance groups. It is believed that few of these comparisons of differences in performance show any statistical significance. Some comparisons, such as those of average weaning weights which usually are greater the smaller the litter, cannot be evaluated independently of other comparisons. It is concluded, however, that the irradiated diet fortified with vitamins is capable of supporting normal reproduction in the rat.

3. Efficiency of Food Utilization.—In an effort to utilize the mass of data on food intake, the comparative efficiency of food utilization by the control and the experimental animals, a calculation was made based on: (1) the record of diet fed each day, (2) the record of total refuse (diet removed from diet dishes each day), and (3) the average weights of control and experimental animals (computed weekly).

During the first 9 weeks the controls consumed an average of 3.89 grams per gram of weight gain as compared to 3.85 for the experimental animals. The average over the first 13 weeks was 5.13 for the controls as compared to 6.26 for the experimental animals. These findings were compared with those of a similar experiment conducted by Swift and Company, who reported a utilization of 3.16 for their controls and 3.28 for their experimental animals during the first 9 weeks following weaning.

4. Hematology.—Table III gives the results from the first hematological studies, made in July, 1954, on parent rats in the long-term experiment. The table lists the arithmetic average of tests on 27 individual animals. No differences were found among the arithmetic averages of each of the four groups for percent hemoglobin, percent hematocrit, percent reticulocytes, the white blood count, the eosinophil count, or the percent lymphocytes and polymorphonuclear leukocytes in the differential count. In addition to the studies reported in Table III, brilliant cresyl-blue films were examined for platelets and no marked differences were observed.

In March, 1955, a second series of the complete blood-cell counts was made on representatives of the parent generation of animals and was extended to 30 animals (7 from each of the two male groups, 8 from each of the two female groups). In addition to the above determinations, the differential count was extended to include basophils, monocytes, and the segmented form of polymorphonucleocytes.

The results of the second series of determinations, in terms of

TABLE III. AVERAGE OF RESULTS OF HEMATOLOGICAL STUDIES OF INDIVIDUAL PARENT RATS IN LONG-TERM EXPERIMENT*

Group	Hemoglobin, g/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.

arithmetic averages for each of the above tests for each of the four groups of animals, are given in Table IV. The range of values for each average value is the lowest and highest values for the group as a whole, rather than the average values for the individual ranges. The table reveals only trivial differences among the groups for every type of blood cell counted. The values for percent hemoglobin and percent hematocrit are the same as those shown in Table III; the white counts reported here are slightly lower, as are also the eosinophils and lymphocytes. The values for the polymorphonucleocytes, however, are higher than those found in July, 1954.

At the time the first-filial-generation rats were sacrificed, blood samples were taken by tail nicking, and the percent hematocrit, percent hemoglobin, and percent corpuscular hemoglobin were determined. No differences are found in any of the comparisons between control and experimental animals. The values for the females are practically identical; those for the males may not be significantly different, owing to smaller numbers of animals. From these data it may be concluded that the hemopoietic functions of second-generation rats are not affected by irradiation of the diet of both the second generation rats and their parents.

5. Gross Observations of Incidence of Disease.—Because of the increasing occurrence of various degrees of respiratory infection, tumors, and other diseases among the parent-generation animals during the second year of the experiment, a systematic check of each of these animals was made weekly, starting at the 62nd week of experiment. Every animal was examined individually for any gross external abnormalities, and an effort was made to detect internal tumors. The degree of respiratory infection, if present, was rated mild or slight if confined to sniffling and a rattly throat, moderate if the rattling in the chest could be felt, and severe if there was visible heaving of sides and loss of weight.

With these data, a weekly chart was prepared and is shown in graphical form in Fig. 4. The four groups of parent rats are shown in separate graphs. Each bar in each graph represents the status of the original 31 animals in each group on the particular week examined. The data are presented in this way because the status reported, except in the case of deaths or malignant tumors, varied; for example, animals with respiratory infections during one week might show none the next week. This form of presentation permitted a rapid means of accounting for all the animals originally on the experiment.

Among the males, disease occurred in nearly all the animals and consisted mostly of respiratory infections and unclassified diseases (infections, abscesses, weight loss, general listlessness, alopecia, etc.). The difference between the males on the control diet and the males on the irradiated diet, in regard to distribution of diseases, was as follows: two tumors and one death attributed to a tumor occurred in the control males as compared to five tumors

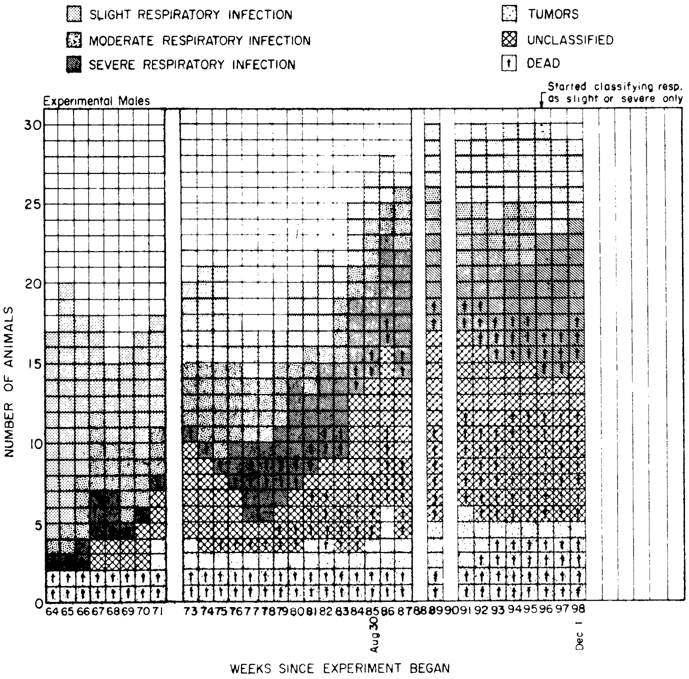
TABLE IV. BLOOD-CELL COUNTS ON REPRESENTATIVE PARENT RATS ON LONG-TERM EXPERIMENT, MARCH, 1955

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

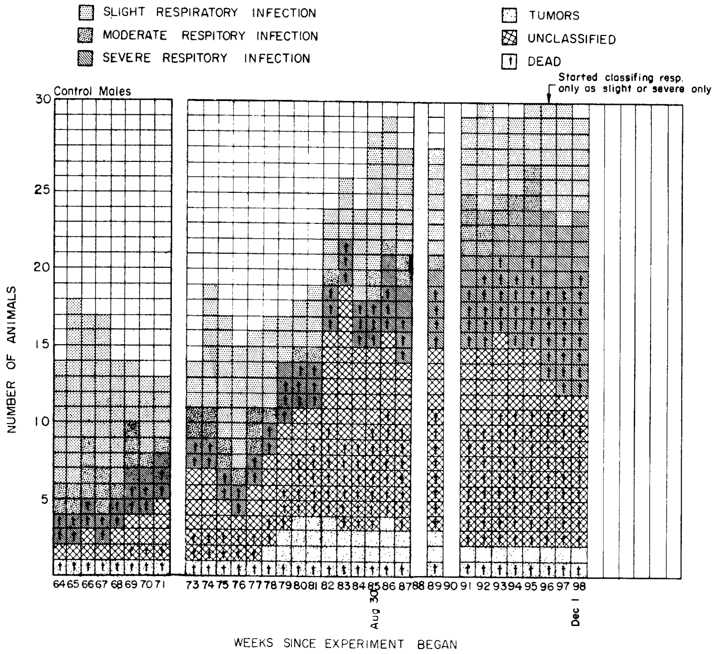
*Single number is average value for the animals in the group; the numbers in parentheses are the lowest and the highest values among the animals in the group.

**Data for 7 rather than 8 animals.

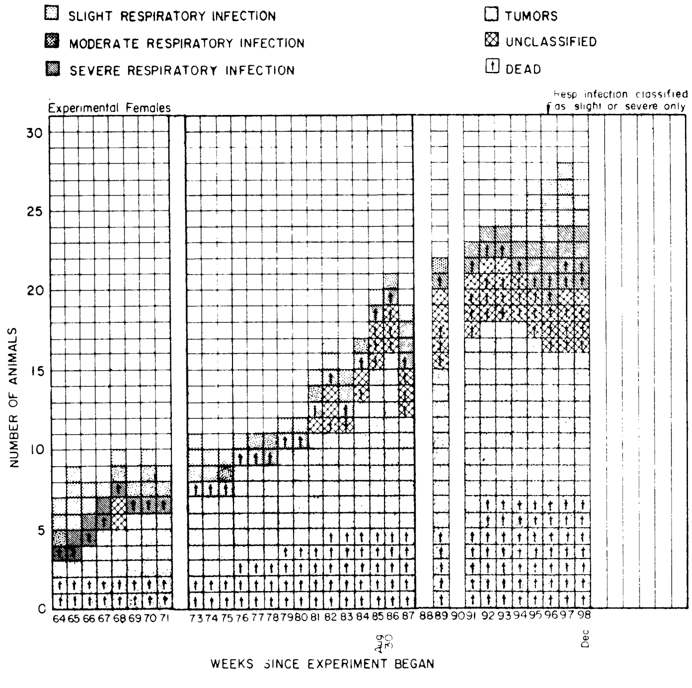
***Nonsegmented form of polymorphonucleocytes.



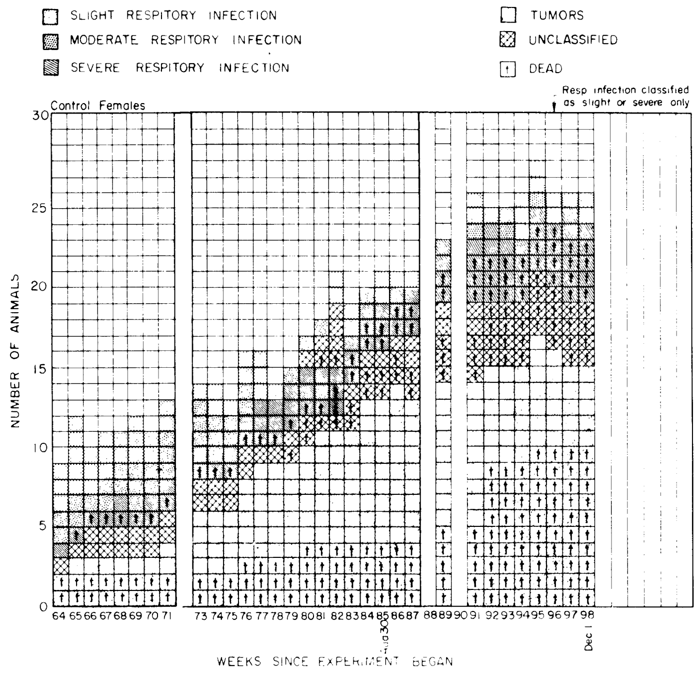
Experimental Males



Control Males



Experimental Females



Control Females

Fig. 4. Summary of weekly gross observations of incidence of disease of parent-generation rats on the long-term feeding and breeding experiment.

and four deaths attributed to tumors in the experimental males. The control males showed a greater incidence of unclassified disease, as exemplified by 14 cases of severe illness and 9 deaths as compared to 10 cases of severe illness and 6 deaths among the experimental males. The control males also showed a slightly larger death rate attributed to respiratory infection than did the experimental males, the ratio being five deaths for the controls to three deaths for the experimentals.

Among the females, those on the control diet showed a slightly larger incidence of disease than those on the irradiated diet, this increase being mostly in the greater number of cases of respiratory infection. Compared to the males, the females as a group showed a greater number of tumors but a much smaller number of cases of respiratory infection and unclassified disease. Figures 5 and 6 show an experimental and a control female with mammary tumors.

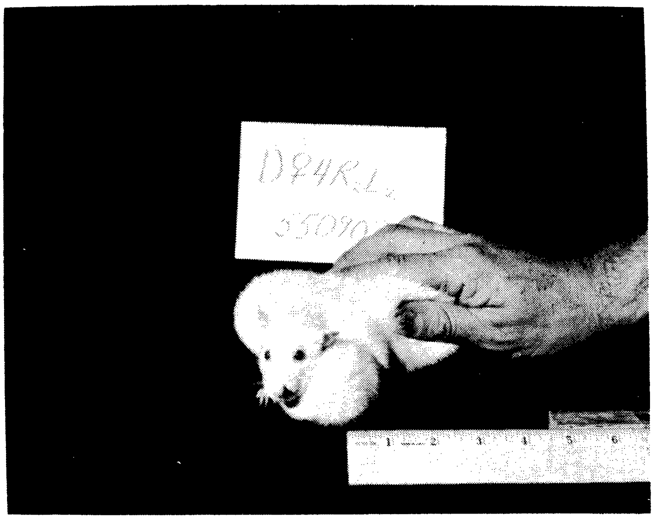


Fig. 5. Experimental female parent rat with two large mammary tumors prior to sacrifice and autopsy on September 7, 1955.

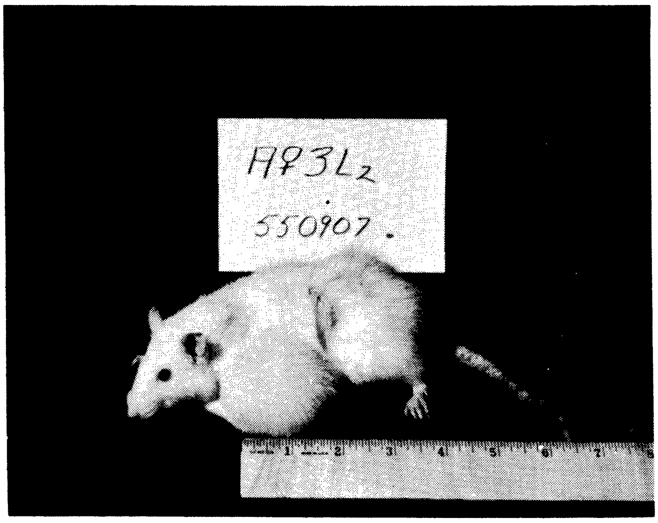


Fig. 6. Control female parent rat with large mammary tumor prior to sacrifice and autopsy on September 7, 1955.

Beginning at about 14 months, the incidence of tumors among the females began and then increased rather abruptly, there being 18 cases among the experimental females and 15 cases among the control females. The nature of this sudden increase would indicate that tumor formation may be related to a fundamental change which occurs normally in rats of this age. Most of the tumors were mammary tumors, and two cases were tumors of the uterus.

6. Pathology.—Insofar as possible, the parent animals were not sacrificed until moribund, so as to obtain data on longevity. The first animal to be sacrificed was an experimental male which developed a scrotal tumor which was detected for the first time on the 32nd week of the experiment. The animal had to

be sacrificed 17 days later. The heart, lungs, liver, spleen, kidney, adrenal gland, a section of the small intestine, and the testes were removed from the animal, and histopathological examinations were made of these organs in the Department of Pathology, The University of Michigan Medical School. Table V shows the report on this examination.

Since then, a total of 57 parent animals of the original 124 were sacrificed, or died, as of the 93rd week of the experiment, and the vital organs were removed and sent to the Department of Pathology for examination. These data are too extensive to be reported here, and, therefore, will be summarized.

During the first 68 weeks of the experiment, 6 parent animals were sacrificed or died as a result of the development of tumors (4 in the experimental animals and 3 in the controls) or severe respiratory infection (2 in the experimental animals and 3 in the controls). Tumors continued to develop thereafter and were the major reason for sacrificing the females in both groups. In the majority of such cases, the pathologist's report stated that each of these was an adenofibroma of the mammary gland, while one was, in addition, adenocarcinoma. One control female had an abscess on the neck, which may have started as an infection of the middle ear.

Respiratory infections continued to be a major cause for sacrificing the males in both groups. In general, the pathologist's report for these cases stated evidence of bronchitis and pneumonia in the lung, confirming the diagnoses. One control male died suddenly and autopsy revealed the lung tissue to be very congested with blood.

After the 68th week, parent animals began to be lost for reasons other than tumors and respiratory infections. The causes in such cases were varied and were listed as unclassified. A control female died suddenly without apparent cause. The port-mortem changes were extensive and obscured the pathological diagnosis. The pathologist's report showed that another control female died of inanition and still another of bronchiectasis. One experimental female bore a tumor, had a large hairball in its stomach, and had a severe respiratory infection when sacrificed. Ultimate death could have resulted from any one or a combination of these effects. Another experimental female with a hairball in the stomach was sacrificed. One control male which was sacrificed was reported to have had an inflammation of the parotid gland and a spindle cell sarcoma adjacent to the parotid gland. Another of this group was sacrificed because of severe weight loss; the pathologist's report was summarized as malnutrition.

One experimental male appeared to have died of a pyogenic infection and another was sacrificed because of a ventral abscess. However, with both groups of males the greatest cause of illness necessitating sacrifice between

TABLE V. HISTOPATHOLOGICAL REPORT $D4R_1$, 1832-LBG, ON EXPERIMENTAL MALE RAT WITH SCROTAL TUMOR

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 marrow. 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.

<u>Summary</u>: 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.

the 68th and 87th weeks was the development of hairballs. Of 23 animals lost during this period, 17 died with hairballs. Six were from control males, three from experimental males, three from control females, and five from experimental females. Also, two second-filial-generation rats died with hairballs in their stomachs. In some of the cases, the hairball completely filled the stomach and obstructed the passage of food. Figure 7 shows a hairball from an experimental female rat; the hairball has the shape of the stomach which it nearly filled. This rat also bore a tumor, a photograph of which is included in the figure. Figure 8 shows the same hairball sectioned so as to show that it was a nearly solid mass of hair. The two hairballs found upon autopsy of the second-filial-generation females were both from experimental females. The histopathological reports for many of these animals cite evidence of inanition. As the occurrence of hairballs in both groups of animals was essentially the same, it cannot be attributed to the irradiation of the diet.

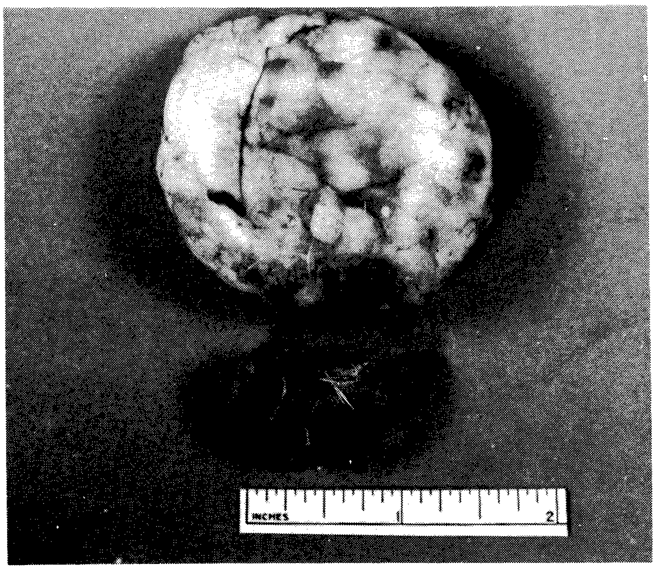


Fig. 7. View of tumor and hairball taken from an experimental female parent rat ($8R_1L_1$) on September 6, 1955. The hairball filled and took the shape of the stomach and is believed to be the immediate cause of death.

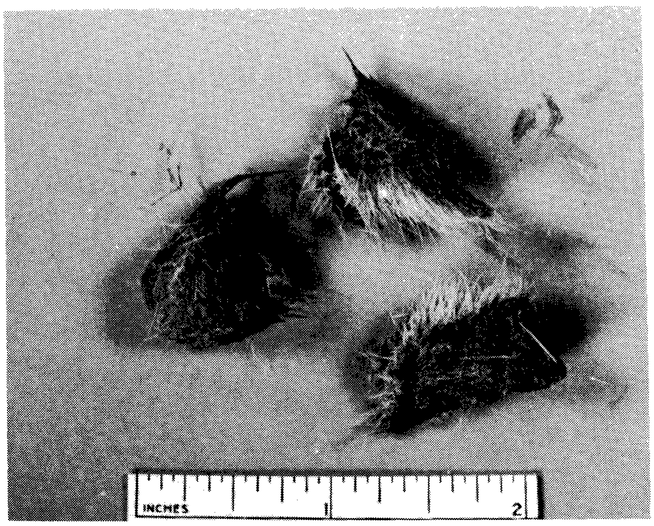


Fig. 8. The same hairball as shown in Fig. 7 has been sectioned to show that it was a solid mass of hair.

After the animals of the first-filial generation had given birth to their second litters of young and these had been weaned, the first-filial-generation parents were sacrificed and autopsied. Sections of the vital organs of each of the 40 females and 24 males were preserved. The tissues from three males and six females in each of the two groups were sent to the pathologist for examination. Because the animals were only about six months old when autopsied, and there had been no evidence during reproduction by gross examination or from blood-cell determinations that the experimental animals suffered from any abnormality, only this fraction of the total animals was examined pathologically. The reports turned out to be brief and repetitious and showed that none of the positive pathological indications predominated in any one group. Many of the observations made are directly attributable to the high fat content of the diet, viz., abundant quantities of lipids in the adrenal cortex, diffuse fatty infiltration in the liver, although no lipidosis was apparent. Congestion was noted in about half the samples of spleen from each of the four groups. The samples of kidney tissue appeared almost completely devoid of lipidosis in all four groups. Abundant mucin was noted in about half the samples of small intestine from each of the four groups. Most of the reports on the uterus were negative, while the observations on the testes were obscured by post-mortem change. Nearly all the lung-tissue samples, regardless of origin, showed patchy atelectasis, emphysema, and varying degrees of dilatation of the bronchi.

E. CONCLUSIONS

The results in short-term studies with rats indicate that, as compared with changes in palatability and vitamin content, acute toxicity appears to be an unimportant aspect of food irradiated at levels much higher than would be encountered in future commercial pasteurization or sterilization with gamma radiation.

The results of long-term feeding and breeding studies indicate no chronic or accumulative effects on rats of feeding a diet for nearly two years in which all but the vitamin and mineral supplements received 4 megarep of gamma radiation.

Animals fed the irradiated diets were slightly inferior in growth, reproduction, and lactation performance to those on the nonirradiated diets; however, these differences were small. No significant differences at all were observed between the two groups in the hematology studies, the pathology studies, or in the gross observations.

Thus, the conclusion is made that a diet consisting largely of food given a 4-megarep sterilizing dose of gamma radiation will be wholesome and capable of supporting the normal growth and reproduction of rats if this diet is supplemented with nonirradiated vitamins.

F. REFERENCES

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