

Columbia University
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NEW YORK 27, N. Y.
 DEPARTMENT OF CHEMISTRY
 HAVEMEYER HALL

September 27, 1955

Lt. Colonel Tyrone E. Huber
 Research and Development Division
 Office of the Surgeon General
 Department of the Army
 Washington 25, D. C.

Dear Colonel Huber:

Professor King has asked me to send you the enclosed copy of a manuscript entitled, "Nutritional and Biochemical Effects of Irradiation," by Becker, Kung, Barr, Pearson, and King, which has been accepted for publication in FOOD TECHNOLOGY. The manuscript is based upon the presentation made at the Institute of Food Technologists meeting in June 1955, with some minor changes suggested by the referee.

Very sincerely yours,


 Robert R. Becker
 Instructor in Chemistry

RRB EJS

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Drs. C. G. King & R. B. Becker MD-550
 Studies on Nutritional & Biochemical Effects of Radiation

NUTRITIONAL AND BIOCHEMICAL EFFECTS OF IRRADIATION*

R. R. Becker, H.-C. Kung, N. F. Barr, Constance S. Pearson, and
C. G. King

Department of Chemistry, Columbia University, New York, N. Y.

Introduction:

Experiments carried out in many laboratories have shown clearly that certain essential nutrients are destroyed to varying degrees when subjected to sterilizing doses of radiation (1, 2, 3, 8, 10). It has been amply demonstrated also that undesirable changes in flavor, odor, color, and texture may occur, but it is hoped that these difficulties may be overcome. It is of obvious importance, also, that the chemical changes produced by ionizing radiation do not lead to injurious, unwholesome, or carcinogenic compounds. Little information derived from long-term feeding experiments is available to give indications as to whether or not such changes occur. This paper presents some of the results of a long-term feeding experiment carried through three generations of experimental animals, as well as certain data regarding destruction of specific nutrients.

* Paper presented at the Annual Meeting of the Institute of Food Technologists, Columbus, Ohio, June 15, 1955.

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A large amount of information regarding reproduction and longevity has been accumulated over a period of years on continuing generations of albino rats of the Sherman strain fed a diet composed of ground whole wheat, whole milk powder, and salt. It is known, too, that this strain of experimental animals is susceptible to certain carcinogenic agents. The test diet was prepared by mixing 53 g. of irradiated butterfat (1.68×10^6 rep in a Co^{60} source), and 147 g. of skim milk powder, in substitution for 200 g. of whole milk powder, as used in Professor Sherman's experiments. The other dietary components were 1000 g. of ground whole wheat and 20 g. of salt. Both the control and experimental groups received vitamin A and D supplements.

When animals of the same litter reached sixty-three days of age, they were placed in breeding cages (one male per female). Pregnant females were separated to single cages until their offspring reached twenty-eight days of age; then the mother was put back in the original cage. In the first generation, six females each were used in the control and experimental groups. Thirty-two rats, sixteen males and sixteen females, of the second generation (matched offspring of the first generation) and the same number from the third generation were tested in the same way. The

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rats were weighed once a week. Other data were recorded on the basis of daily observations of all animals.

Growth data for the males of three generations are shown in Figures I, II, and III. The growth pattern was similar in all groups, and, although the control groups in the first and third generations showed slightly greater gains in weight, the differences were not significant.

Figure IV includes the growth curves for females of three generations up to the age of 70 days. It is evident that no significant difference was found in this respect, nor was there a significant difference in the average weight of young at 28 days, as shown in Table I. The slight differences in body weight of the young at 28 days were favorable to the control groups, however, in the three successive generations.

All females of the initial group (first generation) proved to be fertile. Forty-one litters, with 280 young, were delivered by the six control females, compared with twenty-eight litters and 193 young, by the six experimental females (Table I). This difference, thirteen litters and eighty-seven young, is statistically significant.

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Table I

Generation	I		II		III	
	control	irrad.	control	irrad.	control	irrad.
Diet						
Number of females mated	6	6	8	8	8	8
Number of litters born	41	28	47	39	40	33
Number of young born	280	193	307	293	269	239
Number of young reared*	239	151	264	262	215	215
Number of females reared*	131	87	135	142	109	108
Number of males reared*	108	64	129	120	106	107
Average body weight of young at 28 days	39	37	42	39	41	38

* Young rats were reared to 28 days of age, then either kept for experiment or sacrificed.

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The results obtained from the breeding records of the second and third generation rats were similar to those from the first generation, except that the differences between the control and experimental groups were less significant than in the first generation, as is shown in Tables I, II, and III. The breeding period of the second generation females was almost complete when the experiment was terminated. One female of this group on the experimental diet was sterile.

Sixty-two animals, those surviving at the end of the experiment, were autopsied and examined for pathological changes by Dr. Benjamin Berg of the Columbia College of Physicians and Surgeons. All the pathologic changes found were essentially the same in control and in experimental animals and they occurred with equal frequency in both groups. There was not a single instance of carcinogenesis, although this strain of animals is known to be susceptible to certain carcinogens. In view of the extensive experience of the pathologist who examined the tissues, the great variety of lipid constituents in butterfat, and the length of time during which the experiment was continued, we regard the clearly negative findings as being highly significant.

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Table II

Number of Litters per Female

Generation	Control		Experimental	
	mean	P.D.*	mean	P.D.*
I	6.8 ± 0.5		4.7 ± 0.6	
II	5.9 ± 0.7		4.9 ± 0.8	
III	5.0 ± 0.5		4.1 ± 0.5	

* Probable deviation of the mean.

Table III

Number of Young per Female

Generation	Control		Experimental	
	mean	P.D.*	mean	P.D.*
I	46.7 ± 4.2		32.2 ± 4.8	
II	38.4 ± 4.6		36.6 ± 5.4	
III	33.6 ± 4.5		29.9 ± 3.7	

* Probable deviation of the mean.

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The reasons for the slightly poorer performance of the animals on the partially irradiated diet are not clear. Work in other laboratories in addition to our own, has shown that vitamin E is radiation-sensitive, and others have reported that a deficiency developed in animals fed an irradiated diet. We are currently checking this point in feeding experiments similar to those just described, in which vitamin E is being given independently as a supplement. The results are not yet definite. The composition of the diet was such that one would not expect a deficiency of vitamin E to occur unless the residual peroxides in the irradiated butterfat, upon mixing, destroyed the tocopherols present in the ground wheat, which contains about 2 mg. of the vitamin / 100 g. Dr. Stanley Ames of Distillation Products Industries has kindly analyzed our irradiated butterfat, as well as wheat germ oil by both a chemical method and by bioassay. Chemical assays indicated a slight (apparent) increase in the tocopherol content of both products after irradiation. However, bioassays showed over 80 per cent destruction of the vitamin in butterfat, and about 15 per cent destruction in wheat germ oil. These data are summarized in Table IV.

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Table IV

Assay	<u>Wheat Germ Oil</u>			<u>Butterfat</u>		
	Untreated	Irradiated*	% Destruction	Untreated	Irradiated	% Destruction
Chemical, mg./g.						
(total tocopherol)	1.34	1.51	—	0.049	0.061	—
Bioassay, mg./g.						
(d- α -tocopherol)	0.92	0.79	14	0.031	0.005	82
Bioassay, I.U./g.						
(dl- α -tocopherol acetate)	1.06	0.92	13	0.036	0.006	83

* Dose: 1.2×10^6 rep.

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The formation of peroxides and other oxidative changes in fats have been demonstrated in several laboratories (4, 5, 6, 7). In connection with our feeding experiments, we have also determined peroxide values in several fats, including butterfat, which has been more extensively studied by Hannan (3, 5, 10). Typical data are summarized in Figure V. It is apparent that under these conditions, the amount of peroxide ($\mu\text{g.}/\text{g.}$) increases with increase in dosage (69,000 rep per hour). A slight increase in the acetyl value of irradiated butterfat was found (controls, 6.1, 6.1, 4.9; irradiated at 1.7×10^6 rep, 7.3, 7.3 and 6.1 respectively). Whether or not this slight change in apparent hydroxy acids would have an effect on our experimental animals is not known.

The radiation-induced destruction of essential nutrients and of enzymes in foods has been studied quite extensively (1, 2, 3, 8). The results of such studies have shown that the vitamins are destroyed in widely varying degrees, and that, in general, enzymes are relatively stable. Typical results of tests on milk are shown in Figure VI.

Vitamin A and ascorbic acid were highly sensitive to destruction within the time period required for sterilization. Carotenoids and riboflavin were moderately sensitive. The enzyme phosphatase, however,

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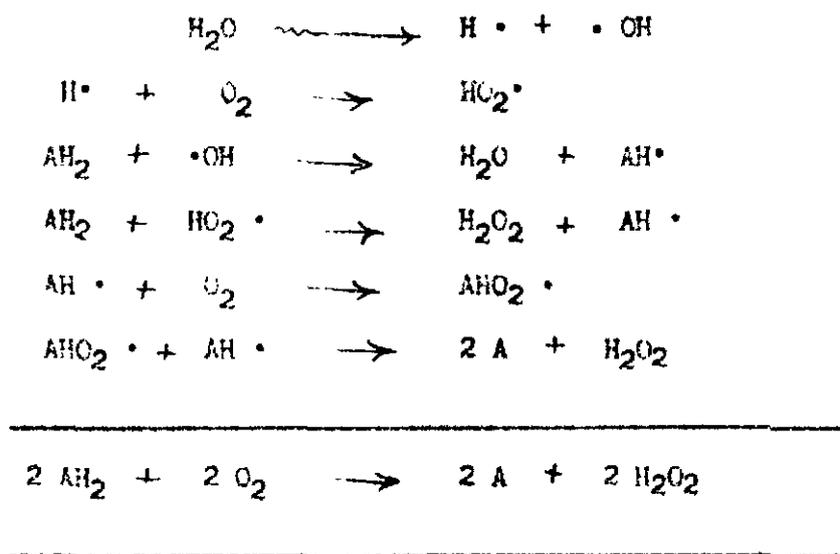
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which is very sensitive to heat inactivation, was only slightly changed during identical exposures. Similar results were obtained with raw whole milk, cream, margarine, and cheese. Extensive destruction of ascorbic acid by ionizing radiation has been reported also by Proctor and O'Heara (9).

We have studied in detail the oxidation of ascorbic acid to the dehydro form as an example of a class of compounds commonly used for the inhibition of oxidative changes that occur upon irradiation. In these studies, initial rate data at low dosages were obtained, and interpreted on the basis of the ferrous-ferric reaction under similar conditions (11). The stoichiometry of the reaction, including data on the amount of oxygen consumed, leads to a postulated mechanism as follows:



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The absence of chain utilization of oxygen is consistent with the known protective effect of ascorbic acid and other easily oxidizable substances, such as thiourea, tocopherol, and hydroquinone, on biological systems. These substances apparently exert their protective action by limiting extended chain utilization of oxygen.

Summary:

A long-term feeding experiment with albino rats, in which the butter-fat portion of the diet consisting of five sixths whole ground wheat and one sixth powdered whole milk, was irradiated from a Co⁶⁰ source (dose = 1.68×10^6 rep), has shown no evidence of carcinogenicity in three generations of animals. Growth rates of the test and control groups showed no significant differences, although slight differences tended to favor the control groups.

The reproductive performance of animals on the partially irradiated diet was below that of the control group, for reasons not yet clear. The difference for the first generation groups was higher than the later values (2nd and 3rd generations).

Oxidative changes in several fats similar to those reported by other laboratories have been found, including a marked rise in peroxides and

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a slight increase in acetyl values.

Nutrient loss was greatest in ascorbic acid and in the fat soluble constituents, vitamin A, carotenes and vitamin E. Enzyme inactivation tends to be very slight, relative to nutrient destruction or sterilizing dosage, when radiation is compared with heat effects. Hence a relatively mild combination of both may offer advantages over either, alone, in specific products.

Acknowledgment:

These investigations were supported by the U. S. Atomic Energy Commission Contract AT (30-1)-1186, Office of the Surgeon General Contract DA-49-007-MD-550, and The Nutrition Foundation, Inc., New York, N. Y.

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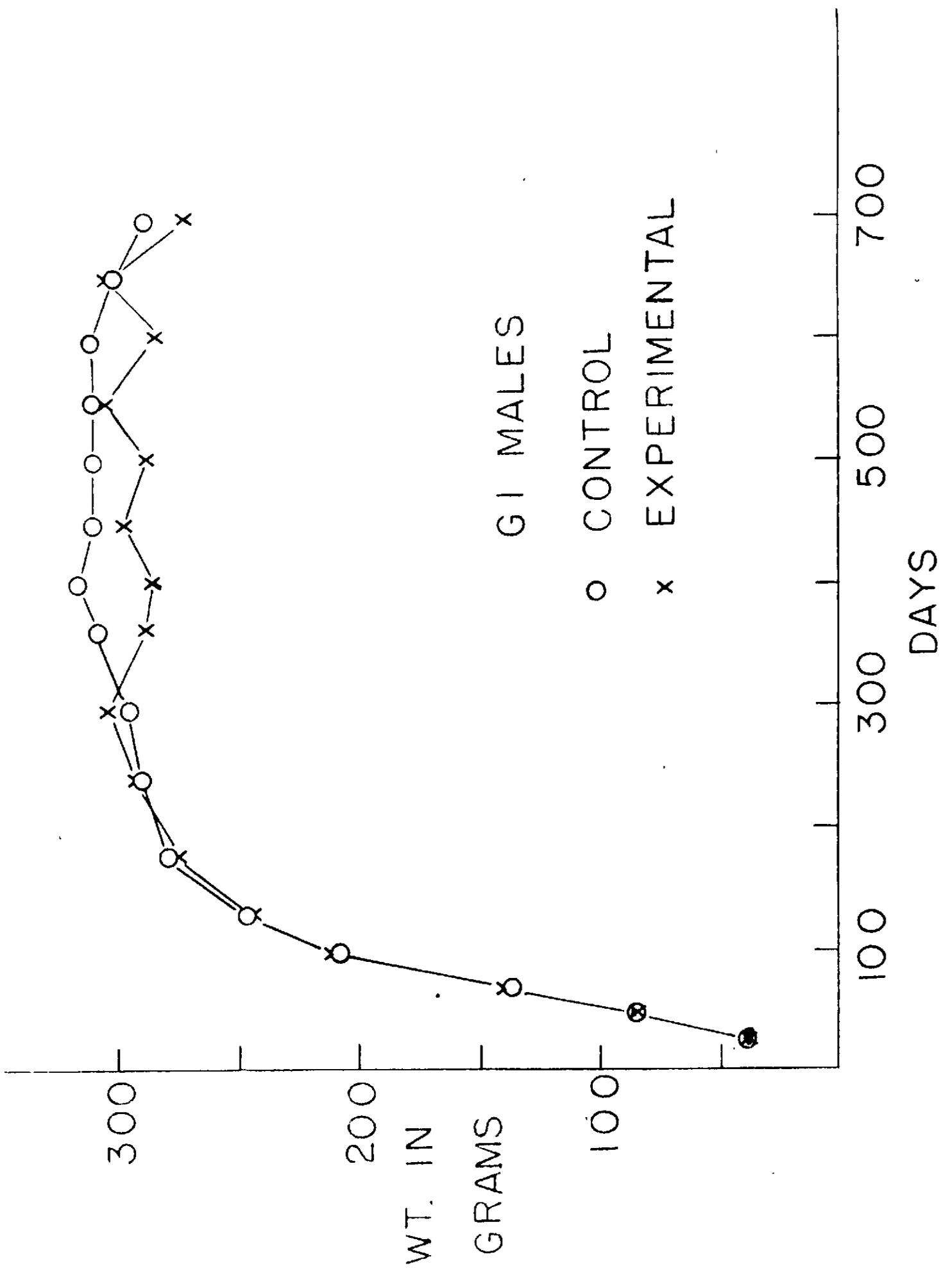


Figure I. First generation.

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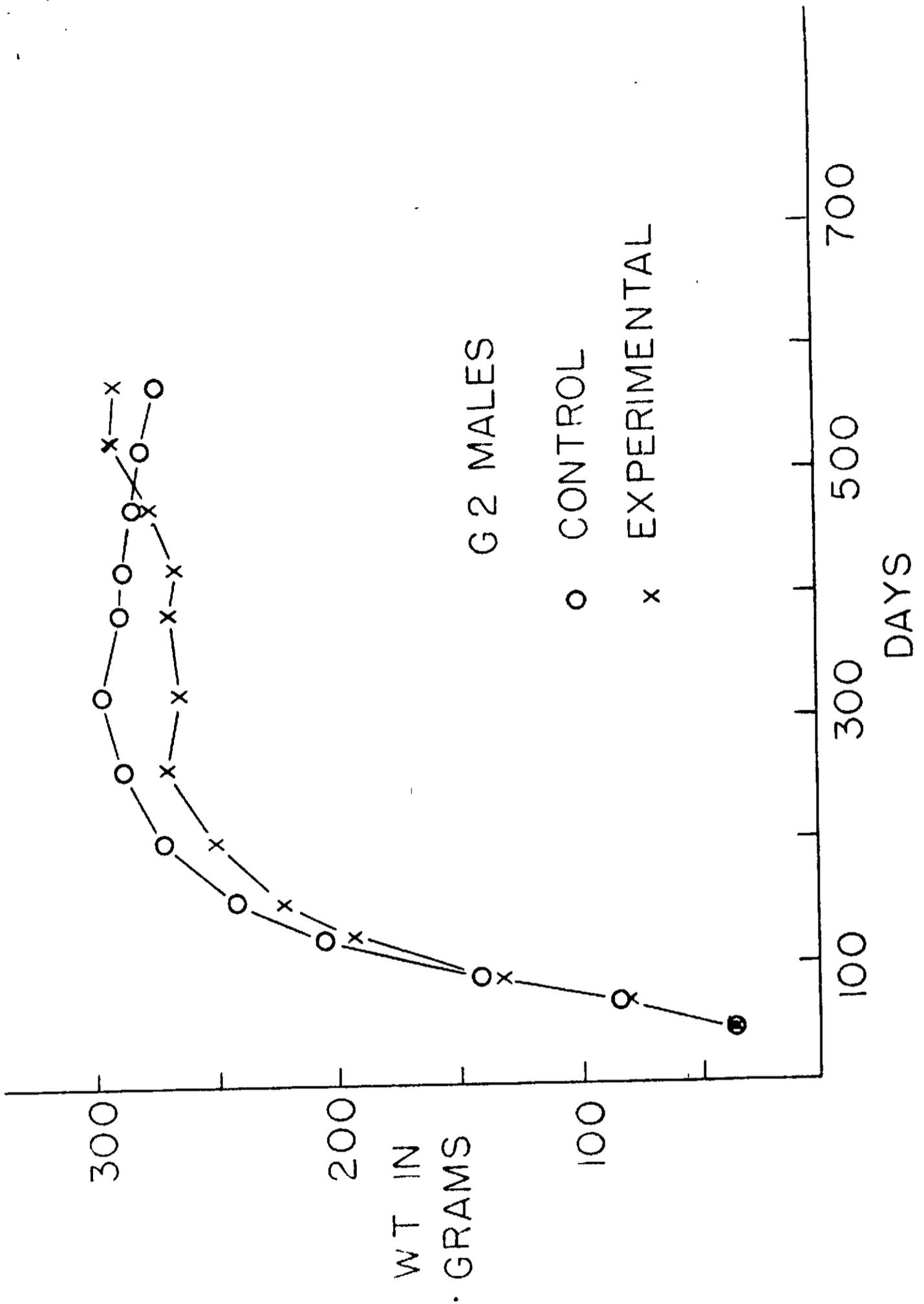


Figure II. Second generation.

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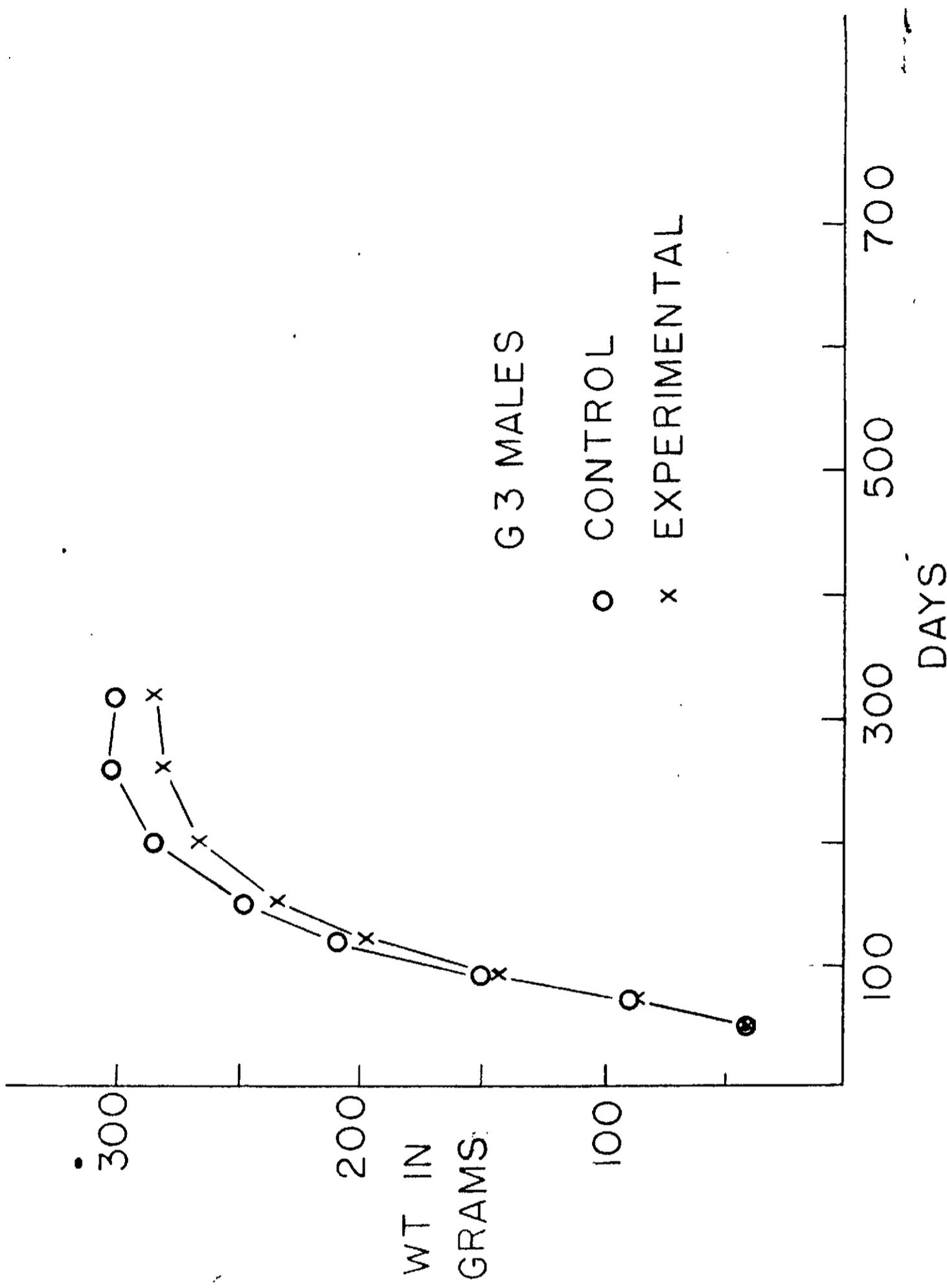


Figure III. Third generation.

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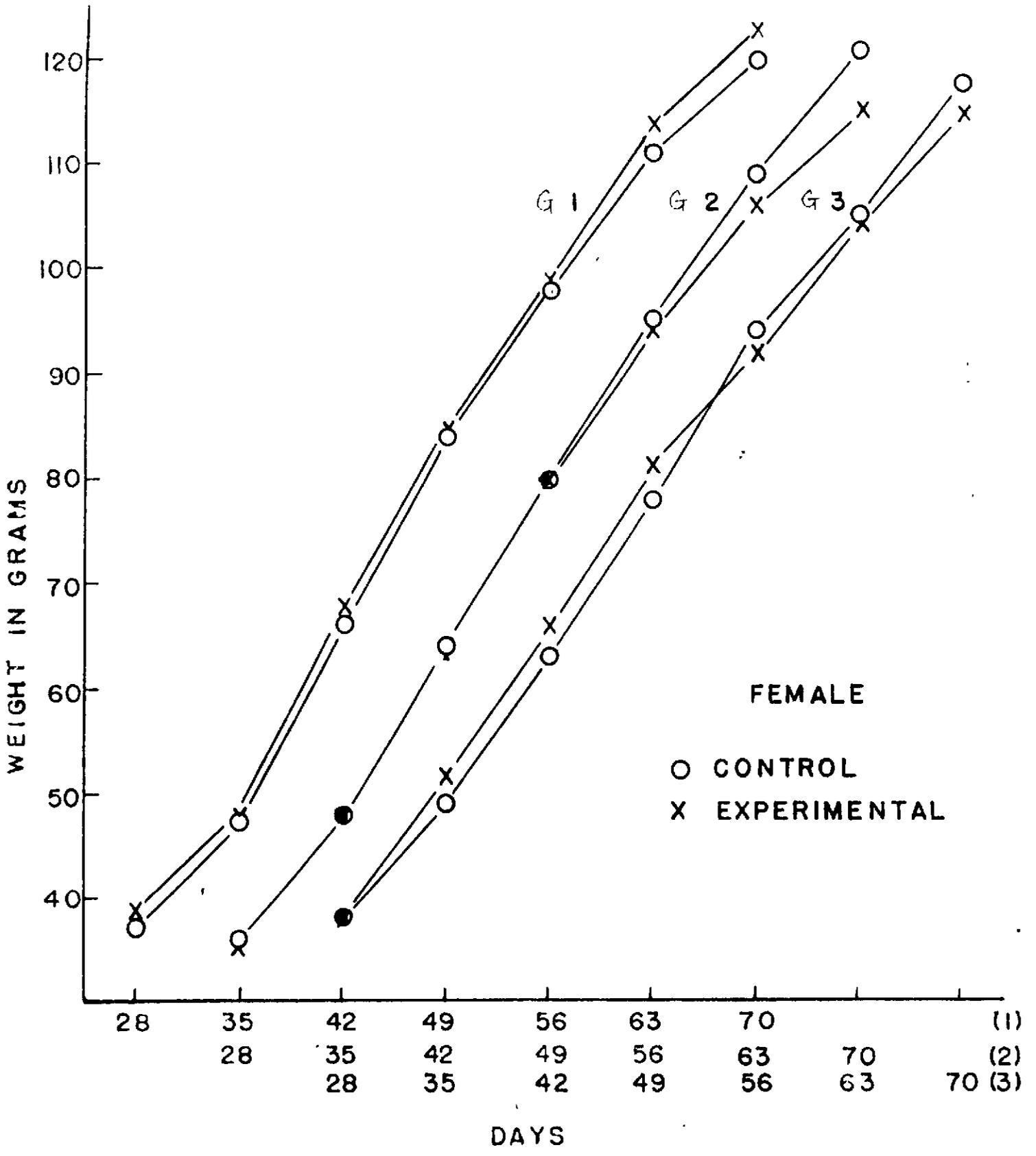


Figure IV. Growth of females.

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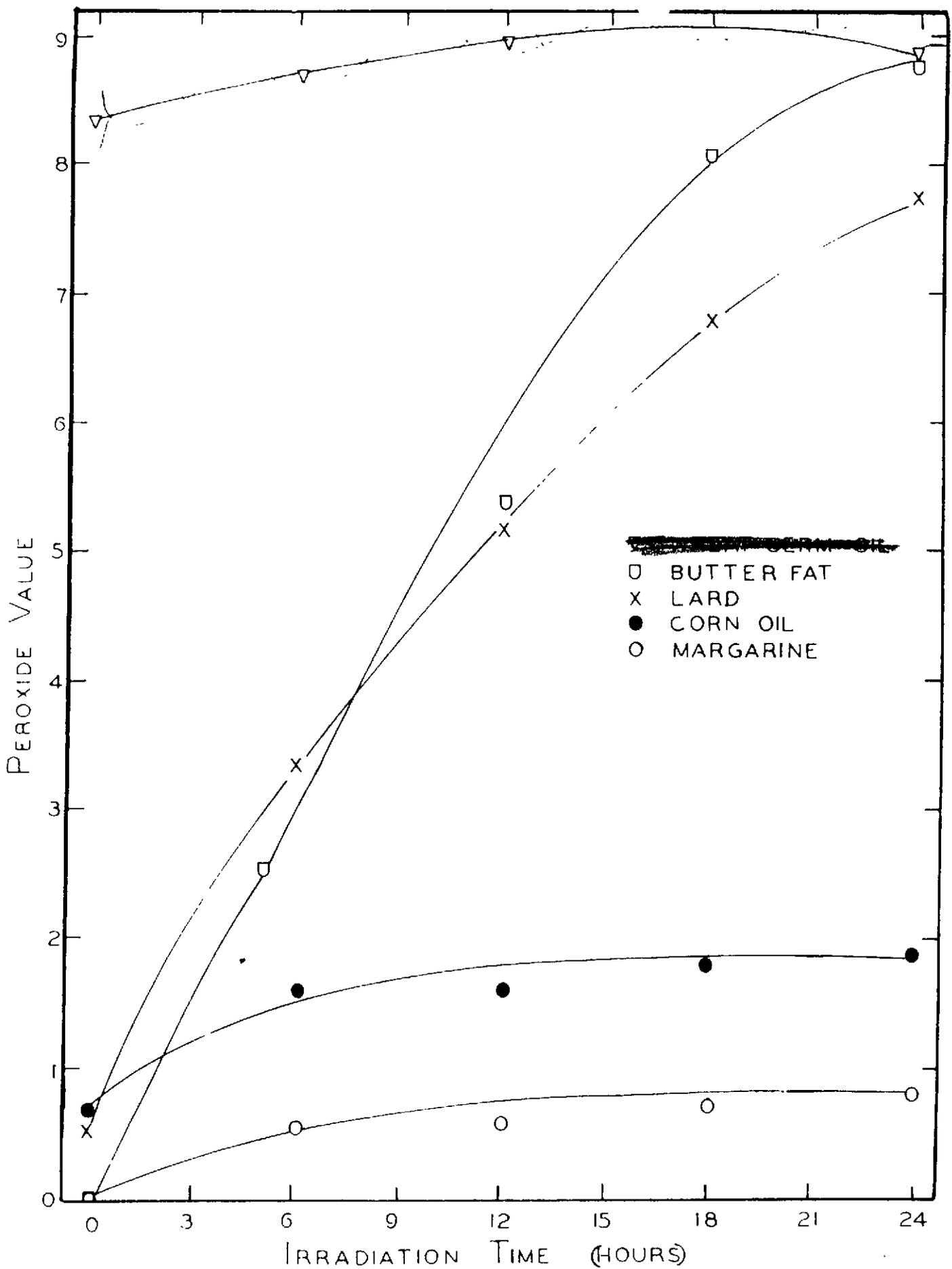


Fig. V. Peroxide value of irradiated fats
Effect of γ -rays. Peroxide production
in several fats.

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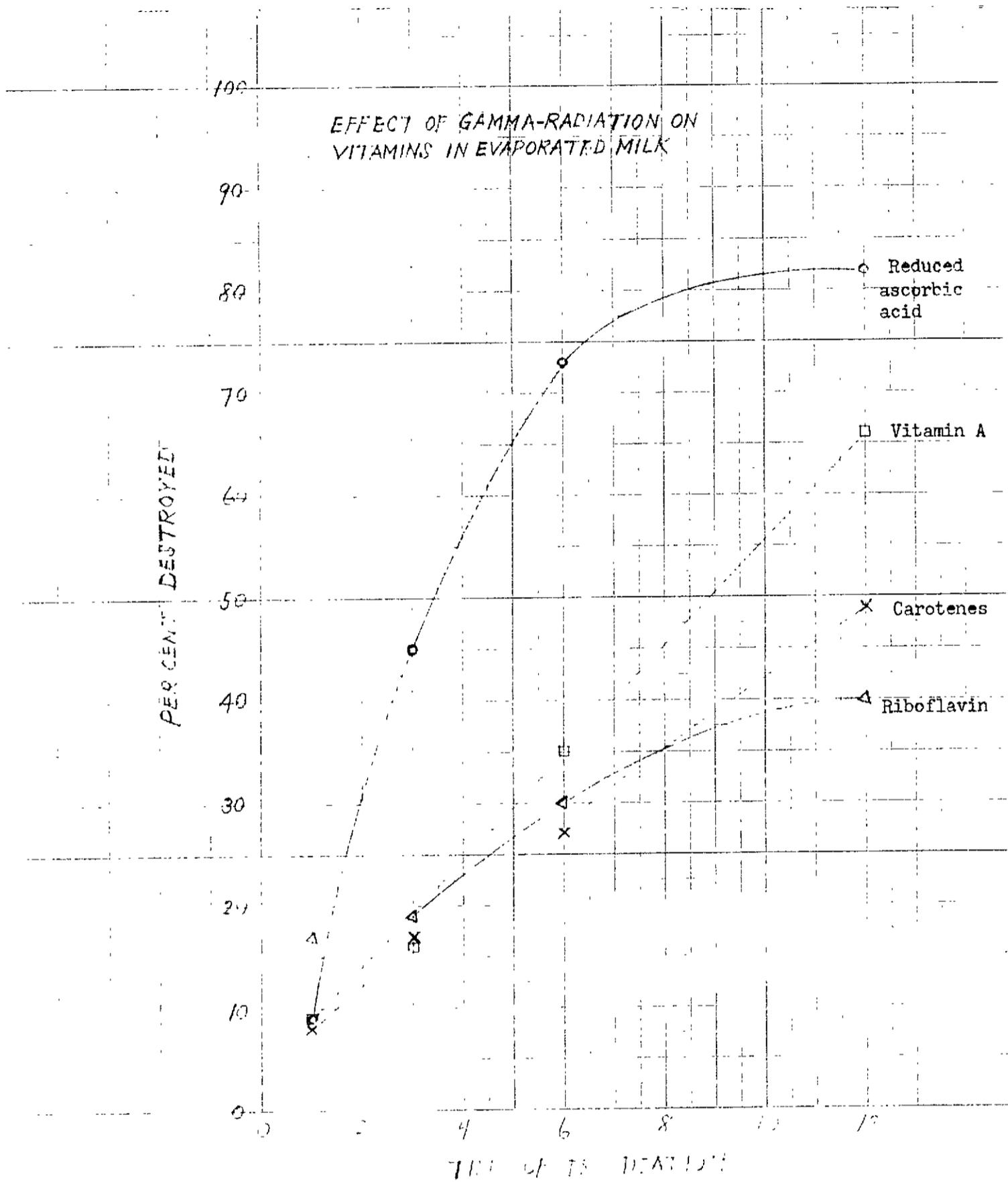


Figure VI. Effect of γ -rays on vitamins in evaporated milk. Dose = 80,000 rep/hr.

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