

THE APPLICATION OF HIGH-ENERGY ELECTRONS
TO SOME GRAIN-INFESTING PESTS

By

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A THESIS

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and Applied
Science in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Agricultural Engineering Department

Year 1958

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This study reports the results of work undertaken to determine the minimum lethal ionizing radiation requirements to kill the metamorphic forms of various species of grain-infesting pests. Previously published work has shown that radiation is capable of killing many species of insects; however, because all the forms of all the insects have been killed, with few exceptions, at the doses used, minimum requirements are not known.

Twelve grain-infesting pests, including a non-insect species, were treated with 1-Mev electrons at various doses to determine the lethality of the treatment to the metamorphic forms and to determine sterility effects on the adults. Order of death curves of survivors against time at near-100%-lethal doses, where obtained, verify the sigmoid shape of the curves; at sub-100%-lethal doses, plots of survivors against dose on log-normal coordinates represent the data. In general, graded resistance is exhibited, within a species, increasing with development stage of the insect from egg to adult; of the insect species, the three moths showed higher radiation resistance than the beetles. Pests covered by the study include: confused flour beetle, rice and granary weevils, sawtoothed grain beetle, cadelle, Angoumois grain moth, Indian-meal moth, Mediterranean flour moth, lesser grain borer, yellow meal worm, a mite and a psocid.

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ACKNOWLEDGMENTS

The author wishes to express particularly his appreciation to his major professor, Prof. D.E. Wiant, Agricultural Engineering Department, Michigan State University, for his advice, patience, and constant encouragement.

The invaluable cooperation of Prof. Ray Hutson, Head, Entomology Department, Michigan State University, in organizing the project and insuring that facilities were provided for conducting the tests is hereby gratefully acknowledged.

To Messrs Richard Schweiger, William Drew, Hari Agarwal, Ronald Hodges, and Arthur Wells, Entomology Department, the author expresses special thanks, particularly to those who worked with and suffered from the mites, for their help in collecting and rearing the extensive breeding stock required by these experiments.

The author also wishes to thank the other members of his committee, Dr. W.M. Carleton, Dr. Merle Esmay, Dr. D.J. Montgomery, and Dr. C.P. Wells.

To Dr. Arthur W. Farrall, Head, Agricultural Engineering Department, Michigan State University, the author is especially grateful for his interest and for the allocation of funds for this investigation.

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Final examination, April 10, 1958, 9:30 A.M., Agricultural Engineering
Conference Room

Dissertation: The Application of High-Energy Electrons to Some
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of making many books there is no end;
and much study is a weariness of the flesh.

- Ecclesiastes

TABLE OF CONTENTS

	Page
I. INTRODUCTION	1
II. OBJECTIVE	6
III. REVIEW OF LITERATURE	7
IV. EXPERIMENTAL	25
V. DATA	31
A. Mediterranean flour moth	35
B. Sawtoothed grain beetle	45
C. Indian-meal moth	53
D. Lesser grain borer	58
E. Granary weevil	66
F. Rice weevil	79
G. Angoumois grain moth	84
H. Yellow mealworm	95
I. Cadelle	97
J. Confused flour beetle	103
K. Mites	118
L. Book lice	122
VI . DISCUSSION AND CONCLUSIONS	124
APPENDICES	136
LIST OF REFERENCES	151

INTRODUCTION

"Dost thou not recall the picture of the farmer, when the tenth of his grain is levied? Worms have destroyed half of the wheat, and the hippopotami have eaten the rest; ... " (1)

Thus an Egyptian scribe of the second millenium B.C. begins the record of man's long and still continuing fight to save his grain from pests. The Sermon on the Mount contains the clear injunction against these pests, "Lay not up for yourselves treasures upon earth, where moth and rust doth corrupt," (2) Tobias Smollett (3) in the autobiographical novel Roderick Random remarks the quality of the biscuits on board the fighting ships of the 18th century: " ... bread from the same country [New England], every biscuit whereof, like a piece of clockwork, moved by its own internal impulse, occasioned by the myriads of insects that dwelt within it; ... " The most casual check of the world's literature reveals the problem.

How much progress has been made from the time of the Egyptian farmer who, 4000 years ago, might lose half his crop? Markovitch (4) concludes from a survey of Israeli storages: "Where proper control measures are not taken, the damage may frequently amount to 30%." "It is often stated that the losses

in stored grain caused by insect feeding amount to at least 5% of the annual production." (5) A frequently quoted measure of grain-pest damage is that of Cotton (6): "... this group of insects [stored-grain pests] exacts a yearly toll of at least \$300,000,000 in the United States alone." However, such estimates have been criticized. "Most of the currently available information on the magnitude of agricultural and other losses attributable to insects is at best a compilation of estimates which may or may not deserve the title of 'Educated Guesses'." (7)

An accurate measure of the extent of grain damage by insects becomes important only when the current economic feasibility of any particular plan for reducing the damage is under consideration. But if the above loss estimate of 5% of the total grain production (and this loss after the annual expenditure of \$300,000,000) is only approximately correct, the existence of a stored-grain problem must be admitted. Room for only 5% improvement over existing methods of control may seem like a small margin, particularly in view of the grain surpluses enjoyed by the United States; but losses to stored-grain insects are world-wide and in some areas of the world a 5% saving in grain could conceivably be the difference between adequate food and starvation; moreover, any new methods of control that are less wasteful of energy deserve consideration.

Who or what are these pests? For the most part they are insects of the orders Coleoptera and Lepidoptera. Cotton (op. cit. p. 5 et passim) discusses these insects generally on the basis of whether they are primary or secondary pests of the

stored grain; that is, whether the insect attacks and thrives in otherwise sound grain, or whether the insect lives best on milled grain, grain already damaged by primary pests, or grain out of condition. Of the primary insect pests of sound grain, "Most of the damage done by insects to grain in storage and shipments is brought about by four species. These are the granary weevil, the rice or black weevil, the lesser grain borer or Australian wheat weevil, and the Angoumois grain moth." (8)¹ All four species can attack sound grain. The ravages of the rice weevil are estimated to destroy 10% of the South's corn crop (Cotton, op. cit. p. 7). Two of these insects, the rice weevil and the Angoumois grain moth, can and do infest grain while in the field, from where they are carried to the storage bin, so that even an insect-tight bin would be no guarantee of insect-free grain. Of the secondary pests, the confused flour beetle " ... is undoubtedly the most abundant and injurious insect pest of flour mills in the United States. It is found in granaries, mills, warehouses, and wherever grain or grain products are stored." (8) Daniels (9) has shown, however, that the confused flour beetle and the rust-red flour beetle can live in sound grain. This fact would admit these species to the primary pest classification, although they are most famous for their activities in already damaged grain and grain products. These two species are called by Cotton (op. cit. p. 16) " ... the worst pests of flour mills and their presence in grain constitutes the chief source of infestation

¹Common names of insects, where extant, have been used throughout. Appendix A contains the scientific names and other data on the insects used in this study.

in flour mills." The relative frequencies of occurrence of these and other species can be inferred from various samplings of their habitats. Of 29 insect species found in peanuts stored in Alabama, the confused flour beetle and the Indian-meal moth were the most abundant beetle and moth respectively (10). Collections made in 11 feed and flour mills in the Buffalo, N.Y., area during the winter season 1954-55 contained 23 species of Coleoptera, the most abundant being the confused flour beetle, black carpet beetle, sawtoothed grain beetle, and the cadelle (11). In this latter study 2632 insects were found in the 27 pounds of material collected. Osmun (12), in a study of shelled corn in Indiana, found the sawtoothed grain beetle to be the most prevalent; the flat grain beetle, rust-red flour beetle, Indian-meal moth, and foreign grain beetle followed in the order given. In eastern Washington, thirteen samples out of 451 of first elevator-delivered wheat were found to contain 45 insects of the five species: granary weevil, rust-red flour beetle, sawtoothed grain beetle, Trogoderma simplex Jayne, and Laemophloeus pusillus Schon (13). The non-insect pest, the mite, is also a problem in stored grain. Krantz (14) found some 24 species of mites in 56 samples from 15 western New York state county farms.

A complete list of insects that have been found living in stored grain or stored grain products, if one existed, would be long indeed (cf. Cotton, op. cit.). A list of those insects which do by far the largest portion of the damage would be considerably smaller. Nor is the list by any means static, nor constant from one region of the country to another (15).

The most important species, together with a suggestion of their occurrence, have been mentioned.

The insects to be studied in this work were selected primarily on the basis of their economic importance. Therefore, the rice and granary weevils, the lesser grain borer, and Angoumois grain moth were included. To represent important secondary pests, the sawtoothed grain beetle and the confused flour beetle were added to the list. Although the Indian-meal moth is, perhaps, the second most important Lepidoptera, only meagre data on it was collected because of difficulties in rearing large numbers. Availability, family, and size considerations suggested the inclusion of the cadelle and yellow mealworm, but insufficient numbers prohibited extensive study of these two species. Several experiments on mites and book lice were made to attempt some comparison of order and class. The insects selected, then, do include those most important economically, as well as other pests representative of the grain-infesting spectrum.

OBJECTIVE

The objective of this work is to determine the amount of high-energy electron radiation required to deinfest grain of the most prevalent grain-infesting pests. For the purpose of the discussion, deinfestation is taken to mean the failure of the particular species to survive beyond the first generation. Since both Lepidoptera and Coleoptera have complete metamorphosis (that is, egg, larva, pupa, and adult forms), lethal treatment levels must be established for all four forms on the presumption that a surviving larva, for instance, is a potential parent of the second generation; the adult form must either be sterilized or killed. An infestation must be presumed, in the absence of contrary proof, to contain all four forms of the species, so that deinfestation will be assured only when the treatment level applied will kill all three immature forms and either sterilize or kill the adult.

REVIEW OF LITERATURE

The literature pertaining to stored-grain pests is vast; the literature pertaining to high-energy electron applications, though of more recent vintage, is large enough; the literature pertaining specifically to the application of high-energy electrons to grain-infesting pests is, by comparison, infinitesimal. But to restrict arbitrarily the discussion to the latter would necessarily omit important contributions to the problem of deinfesting grain with this type of radiation.

High-energy electrons belong to the class of phenomena known collectively as ionizing radiations, which includes the otherwise different X and gamma rays, alpha particles, protons, and other particles. In fact, it is this common method of energy exchange between the ionizing radiations and matter that initiates the effect of the radiations on biological materials. Ionization is not, by any means, the only way in which radiation energy is dissipated in matter, but it is believed to be the only means of biological importance (16).¹ The theory may be briefly summarized as follows. Since the molecules are bound together by the valence electrons of the constituent atoms, and since the molecule depends for its existence upon these definite binding energies, it follows

¹However, Bacq and Alexander (17) present a rather convincing argument for the importance of excitations.

that the removal of one or more of these electrons (precisely the definition of ionization) creates a situation conducive to change in that molecule. The biological manifestations of these changes are seen as various debilities, temporary or permanent, (burns and loss of hair, for example, in the case of exposed humans); mutations, at sub-lethal exposures, throughout the plant and animal kingdoms; and death, from known and unknown causes.

The X- and gamma-ray quanta or other ionizing radiations give up some or all their energy to the struck electron, and, if the added energy is sufficient, the molecule becomes ionized.¹

The struck electron (primary electron) can, in turn, knock other (secondary) electrons from surrounding molecules; the process is repeated until the individual free electron energies fall below the energy required to accomplish further ionization. The mechanism is similar in the case of irradiation of matter with high-energy electrons, except that the bombarding electrons are regarded as the primary electrons.

Since the effects of all these ionizing radiations on biological material are believed to be identical, literature reporting the exposure of the grain-infesting pests to any of these radiations has been included in the discussion. The production of mutations mentioned above, although one of the most important manifestations of exposure to ionizing radiations, and the one most thoroughly studied, has not been reviewed.

¹This oversimplified description purposely avoids distinction between photoelectric effect, Compton effect, and pair production.

The fact that ionizing radiations, in particular, X rays, are capable of causing damage to living organisms has been known almost from their discovery in 1895. The earliest reference of the application of X rays to insects found by Hunter (18) was an experiment purporting to show that house flies could sense this radiation (Axenfelt, Centralblatt für Physiologie, 1897).

Davey (19), working in the X-ray section of the Research Laboratory, General Electric Company where he apparently had access to more extensive X-ray source material than Hunter, reports the work of a French scientist in which treated silkworm larvae failed to attain normal size and the moth failed to emerge (Bordier, Le Radium, 2, p. 410, 1905).

The law of Bergonié and Tribondeau (20) was enunciated in 1906, although it was either not widely known or not accepted until later. The original paper gives no details of the experiments that led to the law, but only a brief summary of the results in the statement of the law itself: "Les rayons X agissent avec d'autant plus d'intensité sur les cellules que l'activité reproductrice de ces cellules est plus grande, que leur devenir karyokinétique est plus long, que leur morphologie et leurs fonctions sont moins définitivement fixées." (X rays act with all the more intensity on those cells in which the reproductive activity is greatest, in which the meiotic development is longest, and in which the form and function are less definitively fixed.)¹

In Hunter's own work (op. cit.) he exposed a number of

¹See Spear (21) for a discussion and interpretation of this law.

insects, including the rice weevil, Calandra oryzae (Sitophilus oryza), to various amounts of X-ray radiation and observed the treated adults for sterility. His results were entirely negative in the sense that the irradiated adults produced offspring in numbers comparable with his controls. Morgan and Runner (22) in a later, but similar, experiment with the cigarette beetle also obtained negative results. That these men failed to kill the insects or harm them in any observable way was surprising to them for they knew of the accidental sterilization of radiation workers and like effects. The simple explanation is that, even though exposures lasted for as long as one hour, they did not give the insects enough radiation. Hunter and Morgan and Runner can hardly be criticized since the X-ray equipment of 1912 is not to be compared in output with that of today. Treatment intensities and quantities were reported in terms of milliamperes-minutes of exposure at specified distances from the tube; tube voltages (varying up to 75,000 volts) and currents are usually given. There is no way to calculate the roentgen units commonly used today from such data, but rough comparisons might be made from similar results. Hunter's exposures lasted 10 to 20 seconds at distances of 14 to 20 inches from the tube; Morgan and Runner's highest treatment levels can be expressed as 120 milliamperes-minutes at 25 cm from the tube with an applied voltage of 75 kv.

Runner (23) performed experiments on all four metamorphic forms of the cigarette beetle. Various treatment levels were used, but the results obtained with equal amounts of radiation applied to the four forms (600 milliamperes-minutes at 19 cm from

the tube with an applied voltage of 65 kv.) are instructive.¹ Eggs of two different ages, four days vs. five days, showed no eclosion for the four-day old eggs compared with 38 out of 52 for the five-day old eggs. Of these 38 larvae, none reached the adult stage. None of the irradiated (at 600 milliamperes-minutes) larvae reached the adult stage, but growth was so retarded that some of the larvae lived for nearly six months (the normal life cycle of this beetle is 45 to 50 days; the larval period under Runner's experimental conditions was 40 days). Fifteen of 20 pupae became adults, but all these died within seven weeks without laying eggs. All the irradiated adults finally died; the eggs they laid were not viable. These data have suggested to some writers that a difference in sensitivity of the metamorphic forms exists, and, further, that resistance increases with age of a particular form. Runner's data certainly bear out this contention in the case of eggs; but since none of the treated forms succeeded in perpetuating the species, and in the absence of a criterion of resistance applicable to the four forms, other than the fraction of fertile adults surviving the treatment (in this case zero), the writer fails to see proof for the other forms.

By 1917 the output of X-ray tubes had been improved to the extent that, with reasonable exposure times, readily apparent effects were observed on treated insects. Davey (op. cit.) was successful in killing the adult of the confused flour beetle.

¹Compare with the highest treatment level used by Morgan and Runner, which gave negative results.

His paper is a thorough examination of the relationships between number killed, time of death, and treatment level. The treatment levels he used cover the range from those levels at which little or no lethal effect was observed to a level at which all the adults were dead at the end of the exposure. The work includes the results of seven preliminary experiments by which were eliminated such possible causes of death, other than radiation, as NO₂ generated by the apparatus, ionized air, handling of the insects, and X-ray irradiation of the food. In specifying the amount of radiation received by the insects, Davey has carefully recorded the target material, filter information, voltage and current wave forms, distance to the irradiated material, and exposure time. Final treatments are compared by giving the time-current product, root-mean-square tube voltage, and distance. For the purposes of analysis he divided his treatments into two groups: those for which the treatment was lethal to a fraction of the insects, and those for which all the insects died. The lowest treatment level at which lethal effects were clearly separable from natural mortality was 500 milliamperes-minutes at 25 cm from the tube with 50 kv. r.m.s. tube voltage. This treatment can be compared with that of Morgan and Runner (op. cit.) of 120 milliamperes-minutes at 25 cm with 75 kv. applied voltage [53 kv. r.m.s.?]. The conclusion that Morgan and Runner failed to give the insects enough radiation seems justified. Davey does not specify the current used, but other evidence in the paper suggests that it was constant in all the experiments and was between 50 and 100 milliamperes. His treatment times were,

therefore, in the range from 5 to 10 minutes up to 2-1/2 to 5 hours. Davey makes two observations about the order of death of the irradiated insects: first, for all those treatment levels in which the treatment was 100% lethal, that a plot of the percent survival (or percent dead) against time has the form of the integral of the normal error curve (that is, it is a straight line on probability paper); and, second, for the same levels, that a plot of the days of life for any particular fraction of survivors against the logarithm of the treatment in milliamperes-minutes gave straight lines for every survivor-fraction up to a threshold level at which level a second straight line expressed the relation. He concluded from this evidence and from the plotted evidence for the sub-100%-lethal treatment levels, which were not straight lines on probability paper, that for sufficiently large doses of radiation the data fitted the law that states that a "... change in response to an external stimulus is directly proportional to the change in the stimulus, but inversely proportional to the amount of the stimulus." He also concluded from the same evidence that the insects were affected in two ways.

The work of the forty-year period following Davey's experiments was largely devoted to studying the effects of X rays on embryonic and other immature and mature life forms in every phylum. Hardly any of this work was devoted merely to trying to kill the various forms of life, but was principally directed toward studying cell development and genetic properties. However, two studies that show what to expect when irradiating insects are pertinent.

Mavor (24) who worked with Davey, made a rather exhaustive study of the effect of X rays on the various metamorphic forms of the fruit fly Drosophila melanogaster. Using the criterion expressive of the two-dimensional nature of the lethal relationship, that is, percent survival at a time, Mavor showed that the dose required to kill 50% of the subjects in five days from the time of irradiation remained fairly constant from the egg to the beginning of the pupal stage, but that, after the first few days of the pupal stage, the required dose increased rapidly to 25 times the previous dose and remained at about that figure for the adult stage. From this evidence, and a knowledge of insect development, he concluded " ... the resistance of the pupae to X rays begins after all of their important organs have been laid down and that their resistance increases with the growth and differentiation of these organs." This conclusion is certainly in agreement with the law of Bergonié and Tribondeau, although the law was not cited by Mavor. A second observation was the higher susceptibility of males over females for both pupae and adults. Mavor's actual numbers (or rather LD_{50} 's¹) can be criticized since he had made no correction for the sometimes large natural mortality of the controls; however, such correction will not alter the basic finding. The LD_{50} values for eggs and larvae can be criticized because such small numbers of subjects (in the neighborhood of 20)

¹That dose lethal to 50% of the subjects. In view of the two-dimensional nature of lethality, the simple notation, LD_{50} , is inadequate since it does not specify the time (five days, for Mavor's data).

were used, and, to some extent uncontrolled rearing conditions.

Henshaw and Henshaw (25) examined, with the same objective as Mavor, a small segment of the life cycle of *Drosophila*, the egg stage. In addition to investigating the sensitivity as a function of age, they compared the results obtained from X rays with the results from alpha particles. The data are presented as the percent of eggs irradiated at various times from the moment of fertilization surviving two different, fixed X-ray doses. A survivor was determined by whether or not the egg eclosed. The results for the lower dose (of X rays) show a steady increase in the number of survivors throughout the period investigated; the higher dose shows a distinct peak in survival at the 3-1/2 hour point, a decline, and a final rise. The results for three different exposures to alpha particles (from a polonium source) show just the reverse action from the higher X-ray dose: an initially high resistance, a minimum at the 3-1/2 hour point, and a final increase. At first sight, this difference seems to indicate a definite difference in the effects of the two forms of radiation, but the authors point out that the difference can be entirely explained by the low penetrating power of the alpha particles and the position of the most active cells at the time of exposure. Initially, all activity is in the center of the egg beyond the range of the alpha particles, but, after the first few cell-division cycles, the most active cells move to the periphery of the by-now multicelled organism and, therefore, into the range of the alpha particles. The final increase in resistance represents the growth of the organism beyond one layer

of cells so that more and more of the cells move out of range. The sensitivity changes of the eggs exposed to X-radiation cannot be so explained since the loss of energy in the X-ray beam is negligible within the distance of such a small body. The overall increase in resistance can be explained by Bergonié and Tribondeau's law, if the aging of the insect is viewed as coincident with more definitive cell function.

About 1940 the development of electron accelerators of high output stimulated interest in the practical application of the biologically destructive effects of ionizing radiations. Since the advent of atomic piles, the pressing need to dispose of resultant wastes has prompted the suggestion that these wastes be used for food sterilization, grain deinfestation, and like applications. The agent of principal value in atomic pile wastes is gamma rays, which have effects like X rays on matter. The increase in the output of the electron accelerators is of the order of 10^3 - 10^4 times that of Davey's X-ray apparatus.¹

The entire field of radiation (in the sense used in this work) had become well enough understood that dose measurements were well standardized. Considerable faith in the identity of effects from the various sources of radiation can be seen in the definition of dose itself² which, fundamentally, is defined on the basis of number of ionizations produced rather than the source, kind, or

¹A very rough estimate based on comparable effects produced in like times and neglecting total output.

²See Appendix B for these standards and definitions.

energies of the ionizing radiations. Two properties in respect to which the ionizing radiations differ greatly, and, moreover, properties not always considered in evaluating effects, are the penetration and the ionizations per unit of path length. The proper interpretation of penetrating power is seen in the previous analysis of the difference in effect between X-ray and alpha-particle irradiation. The relative biological effectiveness is a way of accounting for differences in ionizations per unit of path length.

Four studies, (Youmans (26), Hassett and Jenkins (27), Baker (28), and Proctor, Lockhart, Goldblith, Grundy, Tripp, Karel, and Broglé (29)) characterize the work from about 1940 to the present time with these newer, more powerful sources of ionizing radiation.¹ Table 1 is a brief summary of their work showing the lowest dose used that was lethal to all subjects treated. As a matter of interest the lowest dose used was lethal to all the subjects in every case except where noted. The agents used by these men include X rays, gamma rays, and high-energy electrons. Youmans used a capacitron the output of which was 3-Mev electrons; Hassett and Jenkins used cobalt-60, a gamma-ray emitter; Baker used a Van de Graaff generator which produced a monoenergetic 2-Mev electron beam; Proctor et al. used 1.6-Mev X rays, cobalt-60 gamma rays, and 2.5-Mev electrons. Not all the insects treated by Youmans are included in the table, but only those species that at least one of the other three groups had irradiated. All the species treated by any of the others are included in the table.

¹The work of Cornwell, Crook, and Bull (30) came to the writer's attention too late to be included in this discussion.

TABLE 1

DOSE IN r OR rep, AS APPROPRIATE,
REQUIRED TO KILL ALL SUBJECTS

Species	Life form	Youmans	Hassett, Jenkins	Baker	Proctor, et al.
Flour beetle <u>T. confusum</u>	egg larva adult	310,000	16,000	50,000 ² 75,000 ³ 200,000 ³	25-50,000 ¹
Carpet beetle <u>A. piceus</u>	larva adult	310,000	16,000 64,000		
Bean weevil <u>A. obtectus</u>	larva adult	460,000 460,000		250,000 ⁴	
Cigarette beetle <u>L. serricornis</u>	adult all		16,000		25-50,000 ¹
Rice weevil <u>S. oryza</u>	adult		16,000		
Lesser grain borer <u>R. dominica</u>	adult all		16,000		25-50,000 ¹
Larder beetle <u>D. ater</u>	larva adult		16,000 64,000		
Granary weevil <u>S. granarius</u>	adult			75,000 ³	
Yellow mealworm <u>T. molitor</u>	all				25-50,000 ¹
Sawtoothed grain beetle <u>O. surinamensis</u>	all				25-50,000 ¹

¹Range of levels ultimately lethal to all forms with all three radiations.

²Live, but sterile, adults from larvae treated at 10,- 25,- and 50,000 rep, but larvae treated at 1,000 rep fertile.

³Lower doses used, but subjects counted only up to one week. At the lower doses, the adults were sterile, except for those at 1,000 rep.

⁴One beetle survived this treatment, but all treated at 10,000 and 100,000 rep killed.

From one point of view, the information derived from these experiments is useless, because there is, for instance, no way to compare the results among reporters on the confused flour beetle. If all of Hassett and Jenkins' beetles died at exposures of 16,000 rep, then, assuming that all reporters worked with comparable insect populations, one can conclude that every population irradiated at a higher dose is certainly going to die. Also, since all the subjects died at the lowest doses used, the least dose required to deinfest a product of a particular species is still a mystery. The table does reflect the thinking of these men about the expected ability of insects to survive irradiation. The supplementary information obtained from the experiments, and the observations of the reporters are valuable and will be discussed at some length.

There are at least two phenomena to be looked for in treatments of this kind: the two-dimensional aspect of the order of death, and differential resistance depending on the age of the insect calculated from the time of egg fertilization.

Youmans irradiated larvae of the potato tuberworm of three different ages at the same dose and found that the greatest kill (at some unspecified time) was achieved in the case of the oldest larvae; however, he points out that the oldest larvae happened to be at a depth in the potato at which the ionization was a maximum, and that the younger larvae were at points of lesser ionization. He also noted the varying delay time before the death of all subjects in two groups of codling moth larvae irradiated at different doses.

The Hassett and Jenkins method of obtaining data was to expose comparable numbers of insects (about 100) to six doses

increasing by about a factor of two (range: 16,- to 322,000 r). Periodic counts were made to determine the number of survivors at any given time. Treatments were delivered at such a rate that treatment was over, in the case of the highest doses used, in about 100 minutes. As a result, their data are precisely the sort required to analyze the order of death in the same manner as Davey. Unfortunately, in half the experiments the control (unirradiated) subjects died at nearly the same rate as the treated subjects. However, in certain of the remaining cases, the order of death followed the form found by Davey. An attempt at comparison of the six species was made by comparing mortality rates at a particular dose; however, no correction was made for the mortality of the control, so that the radiation death rate was confounded in the total death rate.

Baker was the first of these reporters to observe the effect on reproductive ability of the insects. Although the table may not bring out the fact clearly enough, he used some treatment levels comparable with those of Hassett and Jenkins, but he failed to extend his counts beyond one week, after which time the flour beetles would soon have died, according to Hassett and Jenkins' findings. Confused flour beetle larvae and adults irradiated at 1,000 rep survived the treatment and were, moreover, fertile. Death rates, which may prove to be a good means of comparing species resistance, can be obtained from some of his data.

The work of Proctor et al. is the most extensive of the four studies cited. All four forms of five species were exposed to various doses of three kinds of radiation. Some highlights of the

results are here quoted. As expected, "... gamma, cathode, and X rays were equally effective when applied in doses such that the same amounts of ionizing energy were absorbed by the samples." Only small differences were found "... in the radiosensitivities of the several insect species and in the radiosensitivities of their metamorphic forms ...". This conclusion was apparently based on plots of mean survival times (time of survival of 50% of the subjects) against dose. The one graph shown comparing four species of adult forms shows equal slopes, but the intercepts vary by as much as three days, whether this difference is to be considered small is debatable. The technique of comparison is similar to Mavor's (op. cit.). Dependence of death rate upon dose is noted. Several observations on sterility are noted; in particular, adult lesser grain borers treated at 25,000 rep laid about 1/5 as many eggs as the controls; of these eggs, but three eclosed and the larvae died within 24 hours. The survival curves shown exhibit the sigmoid shape found by Davey.

One other study by Nelson and Mattern (31) reported in part the results of electron irradiation of the rice weevil (Dennis and Soderholm, Special Report A-123: Stored Product Insects Section, USDA¹). "Dosages of 20,000 to 30,000 rep were fatal after one week to adult rice weevils irradiated separately from the wheat. For immediate mortality of adults, 100,000 rep treatments were required. Exposures of 10,000 rep prevented eggs of the rice weevil from developing in infested wheat, but did not control other

¹This report is not available for distribution (personal communication from R. Latta, Head, Stored-Products Insects Section, USDA, AMS, Beltsville, Maryland).

stages. The equipment was operated at 800 kv. peak." Whether the forms irradiated at 10,000 rep died after one week cannot be known from the meagre information available.

There are a number of lessons to be learned from the experiments reviewed. It seems safe to conclude that any insect (or living thing) can be killed by ionizing radiations provided enough is given, since the nature of ionization is primarily destructive. There exists some level of irradiation that will ultimately be lethal to the organism, although there is a period of delay before the death of the organism. Practically all the experiments reviewed exhibit this feature of death from ionizing radiations. The author has observed this phenomenon in his own experiments in which insects that were ultimately to die from the treatment apparently remained just as vigorous after treatment as before for over a week before their deaths. In spite of the absence of any direct means of communication with the insects, it seems fair to assume that they undergo some illness manifested as loss of appetite, or generally decreased activity. Baker and Proctor et al. both reported the loss of reproductive activity that appears as decreased numbers of eclosed eggs, death of the larvae that do eclose, and loss of fertility altogether. The sigmoid shape of the death curves and the dependence of death rate on dose that follows an exponential law are revealed wherever sufficient data are presented. Differences in the sensitivity to ionizing radiations among the metamorphic forms within a species are to be anticipated. Proctor et al. report only small differences; Mavor, and Henshaw and Henshaw found differences of over a factor

of ten. However, detailed comparisons are not possible without some common method of analysis of the results. That species differences exist is hinted at by the data, but, again, without a common method of comparison, differences, even though they may exist, cannot be analyzed. In many cases the insects irradiated all died no matter what treatment level was given, so that half the picture of resistance to these ionizing radiations is lost because of the absence of data on sub-100%-lethal levels. Mavor found differences in resistance between the sexes for both pupae and adults of the fruit fly; no other reporter found or investigated sex differences. Some evidence is available on the effect of dose rate. Davey (32) found that when confused flour beetles were treated over an extended period with a total dose previously demonstrated to be lethal that the order of death changed dramatically. The minimum dose necessary to kill all beetles was 500 milliamperes-minutes at 25 cm from the tube. Daily doses were given to five groups of nearly 1000 insects each. The respective daily doses given these groups were 1/80, 1/40, 1/20, 1/10, and 1/5 of the dose which delivered all at once would have been lethal to the beetles. A sample result was that of the group given daily doses of 1/10 lethal dose. At the end of 10 days, or by the time an all-at-once lethal dose had been delivered, the survivor percent was 88; at 20 days, 72% survivors; and at 40 days (four times the lethal dose), 45% survivors. A preliminary experiment of Hassett and Jenkins (op. cit.) in which a source of much lower power was used gave some unusual results with the confused flour beetle. Mortalities (at an unspecified time) were

80%, 90%, and 100% from treatments of 110,000, 140,000 and 196,000 rep, respectively. The source used (tantalum-182) had an output of 30,000 r/day, and, therefore, a required treatment time of 6.5 days for the highest level. These results may be compared with those for 16,000-rep treatments delivered in five minutes, which were lethal to all the adults. Dose rate, at least within certain limits, is, from this evidence, a critical factor. Runner found that in the case of cigarette beetle larvae, the life cycle was slowed, or extended; certain of Mavor's data suggest a similar effect on eggs. Grosch (33) noted a similar effect on the wasp Bracon hebetor Say. The life of the wasp was prolonged, under starvation conditions, by "massive" doses of radiation (10^5 rep) compared to normal (that is, permissive feeding) conditions.

Finally, because of the complex life cycle of the insects, there are numerous possibilities that can be explored; for example, is there a dose which will, when applied to eggs, permit some fraction to reach the adult stage, but yet give sterile adults, and is there some dose which will, when applied to larvae at a critical time, result in death only in the pupal stage? Proctor et al. found such phenomena. If such thresholds exist, they may lie in a small band, representative of individual insect differences, about some mean threshold expressive of inability for that form irradiated to perpetuate the species. No attempt was made in this study to examine all these possibilities; the criterion of death of first generation forms being equivalent to deinfestation was applied throughout, but care was taken to note, where observed, the details of deinfestation.

EXPERIMENTAL

The experimental procedures used in the following tests were of the simplest. All irradiations were performed with a General Electric 1-Mev electron beam accelerator, which is described elsewhere (34, 35). The actual techniques of exposure varied, and are significant because different degrees of uniformity of dose throughout the sample are associated with each method of exposure. The method of dose measurement, electron field characteristics, techniques of exposure, and the associated maximum and minimum doses delivered to the samples are discussed in Appendix C.

Since ionization varies markedly according to depth in the sample, some sacrifice of uniformity of dose, in addition to variation across the top of the sample, necessarily exists. The ideal condition for sample irradiation would be uniform dose throughout the sample, but 1-Mev electrons are almost completely stopped by 4.5 mm of water. A second condition of ideal sample thickness would be one in which the thickness is such that the entrance dose is equal to the exit dose. To achieve this ideal, the sample must be restricted to a thickness of about 3 mm (for material of 1 gm/cm^3 density). With such thickness restriction, the minimum dose received by the sample (aside from variations across the top of the sample), would be the calculated dose, and the maximum dose about 116% of the calculated dose. In practice,

the possibility of doses much lower than the stated dose existed, especially in those samples in which the insects were placed in flour, oatmeal, and corn. Flour and oatmeal samples were measured on a weight basis and then shaken or lightly smoothed to make the top surface level with the bottom of the container. In practice, small mounds of material, of unknown height, were invariably present, so that the ideal condition of uniform depth was only approximated. Oatmeal is uncooperative and neither flows nor tamps well so that the oatmeal samples suffer from the same depth defects as flour. Corn, on the other hand, is simply too thick, even in the smallest dimension, to satisfy the ideal sample depth condition. In the case of wheat, the only other grain or grain product used, there are fewer problems. Wheat flows easily, and a one-kernel layer is closer to the ideal thickness.

Except for about ten tests performed toward the end of the study, all the test subjects were kept in the insect-rearing room of the Entomology Department, the same room in which the insects were raised. Since every species has its peculiar optimum temperature and relative humidity environment, and since several species were being tested at any particular time, it was impossible to keep all species in their optimum environment. However, all these species do rather well at typical rearing room temperatures and humidities, say 78°F and 30-50% R.H. During the first few tests, the summer and fall of 1955, the insects were raised in the prevailing rearing room environment without benefit of controlled temperature and humidity. Shortly thereafter, a humidifier was installed and the humidity of the

room was kept at 50% R.H. This improved condition was readily apparent in the increased vigor of the confused flour beetle breeding stock. However, occasionally the relative humidity controller would fail in such a way as to keep adding moisture to the room, and occasionally, the humidifier would be off altogether. Moreover, no records were kept of temperature and relative humidity, so that the efficacy of the environment can be inferred only from the unirradiated insect samples which, with one exception, were handled in the same way and at the same time as all the irradiated subjects. All tests not carried out at the Entomology Department were carried out in an incubator cabinet at the Agricultural Engineering Department; the temperature in this incubator was maintained at 80°F and the relative humidity at about 60%.

The food in which to rear and treat the subjects was selected on the basis of the insect's food preference or the food in which it lived best under rearing room conditions. One partial exception is the case of the Angoumois grain moth which, in all later experiments, was raised on a soft wheat variety to avoid the dose complication of too thick a substance, corn. Since the average number of Angoumois offspring from untreated pairs living in wheat exceeded that of reported averages, one can make the assumption that no obvious ill effects resulted from the change of food, other than the smaller size characteristic of these moths when raised in wheat.

Every effort was made to handle the subjects as gently as possible. The adult beetles are hardy, but, occasionally, a lesser grain borer, for example, which had to be pried out of its

wheat-kernel home to see if it were dead, was crushed in the process. Such accidents are noted, and the counts adjusted accordingly, where appropriate. Sifting was the most common method used to separate the subjects from their food so they could be counted; in every instance the controls were similarly treated and no case of death from sifting was observed. With the exception of the adult moths, all adults and larvae were handled when necessary with a soft, small, watercolor brush; this handling was required when counting and transferring the subjects from sieve to rearing container and when poking to see if they were dead. The adult moths were handled with tweezers with resultant damage to the wings; but unless the body of the insect was accidentally squeezed in the process (primarily in mating couple transfer), the insects were used as test subjects and regarded as unaffected.

The criterion of death was the lack of motion of any kind observable with the naked eye. This criterion is somewhat unsatisfactory because 1) some of the insects are great fakers and will remain motionless for 10 minutes or more when disturbed; in such cases, patience and hunch became the final criteria; the rice and granary weevils, although fakers, have a characteristic posture when faking that is not their attitude in death, 2) no account is made of moribund subjects, even though such information might be valuable, and, 3) the test is difficult to apply to some of the larvae which seem to freeze when exposed to light; in very uncertain cases of this kind the larvae were left in a known position in the rearing dish and their position at the next count noted.

The samples of food and subjects were irradiated in 8.9-cm (small) or 13.9-cm (large) diameter Petrie dishes, or spread on 15-cm wide aluminum trays. In cases where the Petrie dishes were also the dishes in which the insects were raised, no problem of post-irradiation contamination existed. When trays were used, the samples were placed in clean glassware after irradiation to avoid the possible presence of untreated eggs in the original sample containers. The trays which were used several times in any particular test were wiped clean after each exposure. A possibility existed, when making counts, of transferring debris, which might contain eggs, from sample to sample, either from the sieve or from the large bowls into which the food was sifted. Two precautions were taken to reduce errors attributable to chance contamination: the sieve was cleaned with a 1-inch paint brush between counts and the bowl was wiped clean with a cloth; samples receiving the highest dose were always counted first when the possibility of egg transfer existed, so that eggs treated at a lower dose, or eggs from adults treated at a lower dose, could not be transferred to a sample treated at a higher dose. Another serious problem of contamination is the presence of an unnoticed adult in larvae or egg tests, because, to anticipate, doses at which eggs are killed will not invariably render the adult sterile, which adult, in turn, might lay eggs in the sample after treatment. Careful observation of the samples is the only way to minimize such contamination. The insect most difficult in this respect is the lesser grain borer which frequently bores into a kernel and is entirely hidden from view; the only clue to its presence is the

entrance hole and a somewhat darker color of the kernel - the dark body of the insect seen through the remaining outside layer of the kernel.

The insects were kept, during the observations in glass-covered Petrie dishes, cloth-covered Mason jars, or Petrie dishes covered with Saran Wrap. In spite of these precautions, some cases of post-irradiation contamination were encountered, particularly with mites. These cases were noted.

Additional remarks pertinent only to particular tests are discussed under the data for that test.

DATA

It is not within the scope of this work to discover the order of death of insects from irradiation; however, if predictions are to be made of deinfestation doses, such predictions must be made on the basis of these and similar studies, and without some scheme whereby the laboratory data can be translated into action in the field, these experiments would devolve into a mere collection of tables. If death by irradiation were a simple all-or-nothing phenomenon in which all insects lived when irradiated below a characteristic dose, and all died above that dose, then no special extrapolation or complicated interpretation of results would be required. The facts presented in the review of literature are sufficient to reveal that death by irradiation is not so simple. What the literature does not reveal is the exact order of death of insects by irradiation. Moreover, there is a natural desire to compare species and metamorphic forms within a species with respect to ability to survive irradiation. Without a standard of comparison that has reference to the mechanism of death, or at least the order of death, comparisons have little meaning, or, at best, reflect the whims of the reporter.

There are two separate aspects to the order of death that can be presented: the first arises from the delayed nature of the action itself, in which changes that are initiated during irradiation are not manifested by death until long after the treatment is

applied (long compared to the time required for treatment); the second aspect is the change with dose in the number of insects that ultimately survive, or succumb to, the treatment. The former aspect can be most usefully presented as a plot of survivors against time on probability paper. This practice has been followed in this work. An order of death of this kind is thought to be characteristic of the death of multicellular organisms, or, more precisely, multicellular organisms in which the death of more than one cell is required to produce death of the organism. This form of survival curve was found by Davey (19); its probable significance is discussed at some length by Rahn (36). The second aspect is the one upon which depends a correct assignment of a deinfestation dose. In the interpretation of the results of all these data, the experimenter is deeply involved with that field of investigations called bioassay, and, therefore, involved with all its attendant difficulties and uncertainties. Now all the data in this study are of the all-or-nothing, or quantal, kind; either the insect is dead or it is not; either an adult appeared or it did not; therefore, the data are binomially distributed, and from there it is only a step to the normal distribution. It has been found fruitful to analyze data of this nature, such as the killing of insects with insecticides, by plotting the percent of survivors against the logarithm of the concentration, or dose, from which plots inferences can be drawn as to recommended applications and comparative resistances. In any case, to quote Finney (37), "The validity and appropriateness of the logarithmic transformation

... are not dependent on the truth or falsity of any hypothesis ... ; use of the log concentration ... requires no more justification than that it introduces a simplification into the analysis." Use of log-normal plots of the data have been used in this work whenever such plot seemed to bring order to the data.

Because the number of tests is rather large, partial analysis and discussion of the individual tests are included under the data from each test. General conclusions, such as recommendations and comparisons between species, have been reserved for a separate section.

One correction to percent survival has been made wherever the necessary data to make the correction were available, that is, the correction for the natural mortality of the subjects. The formula for correction, sometimes called Abbott's formula, (Finney, op. cit.) assumes that the subjects can die from natural causes, a fraction, C; or from the treatment, a fraction, P; but not from both. The observed fraction dead, P', is a combination of the two causes of death: $P' = C + P(1 - C)$, from which the desired fraction, $P = (P' - C)/(1 - C)$, is calculated.

The insect data are presented, for want of a more systematic scheme, in alphabetical order by species and in order of metamorphic form, beginning with the egg, within a species. The two pests for which the species are not definitely known are discussed at the end of the identified species sections. The test numbers, T_i , used to identify the tests briefly, were assigned chronologically as a particular test was begun. The assumption is made that under rearing room conditions the time of year is of no importance in

the interpretation of the results of the tests; therefore, elapsed times only are given. All the tests performed are included, whether the data were usable or not. Brief descriptions of the tests include: the initial number of subjects (where known); the kind and amount of food in which they were treated; the number of treatment levels (in which, for convenience, the control is counted as a zero level); the method of treatment, whether stationary or by conveyor; and the container size and kind.

Ephestia kuhniella, the Mediterranean flour moth

T-26: Eight samples of flour in which adults had oviposited for four days were placed in small, uncovered Petrie dishes, and irradiated at four doses immediately after egg laying ceased. Periodic counts of the number of adults emerging in each sample were made beginning 43 days after irradiation and ending 81 days after irradiation. Table 2 gives the number of adults that emerged in each sample during the stated period.

TABLE 2
NUMBER OF ADULT MEDITERRANEAN
FLOUR MOTHS EMERGING FROM
IRRADIATED EGGS

	Dose (rep)			
	0	4,000	8,000	12,000
	2	4	0	1
	3	0	2	3
Total	5	4	2	4

A statistical analysis of the emergence shows no significant difference among samples.¹ The expected egg-adult period for this species is 53 days. In one of the samples treated at 12,000 rep, there were small larvae present on the 81st day after irradiation, in spite of the emergence of only one adult in that sample. Secondary infestation is, therefore, strongly suspected. These

¹The following convention is observed throughout the work: significant difference means significant at the 5% level of confidence, and highly significant means significant at the 1% level of confidence.

samples were nearly impossible to examine because of the dense, ubiquitous webbing spun by the larvae. In fact, this insect is, or was, a problem primarily because of this webbing, which clogged bolting mills and other machinery. The data show that 12,000 rep is insufficient to prevent some of the eggs from eclosing.

T-21: Five 20-larvae samples in 10 grams of flour were placed in small, polyethylene-covered Petrie dishes, and irradiated at five doses by conveyor method. The samples were examined 67 days after irradiation; the expected egg-adult period for the species is 52 days. The control and 2,000-rep samples contained second generation adults and larvae; the 4,000-rep and 6,000-rep samples contained 12 and 9 adults, respectively; no second generation larvae were noted. The 8,000-rep sample was examined in greater detail and found to contain 4 dead adults, 8 dead larvae, 5 dead pupae, and four live larvae (a total of 21 subjects). These samples were not examined more often because of an assumed risk of damage in up-rooting the tightly packed cultures.

T-67: Sixteen 3-larvae samples, eight (24 larvae) from each of two pairs of adults, were placed in 10-12 grams of flour in small, polyethylene-covered Petrie dishes, and irradiated at four doses by conveyor method. The purpose of the small sample size was to permit periodic examination. The moth pairs mated three days apart; the larvae were all irradiated on the same day at about the 0.6-completed point of the larval period. Figure I gives the number of survivors, whether larvae or emerged adults, as a function of time from irradiation. Since the progeny of the two

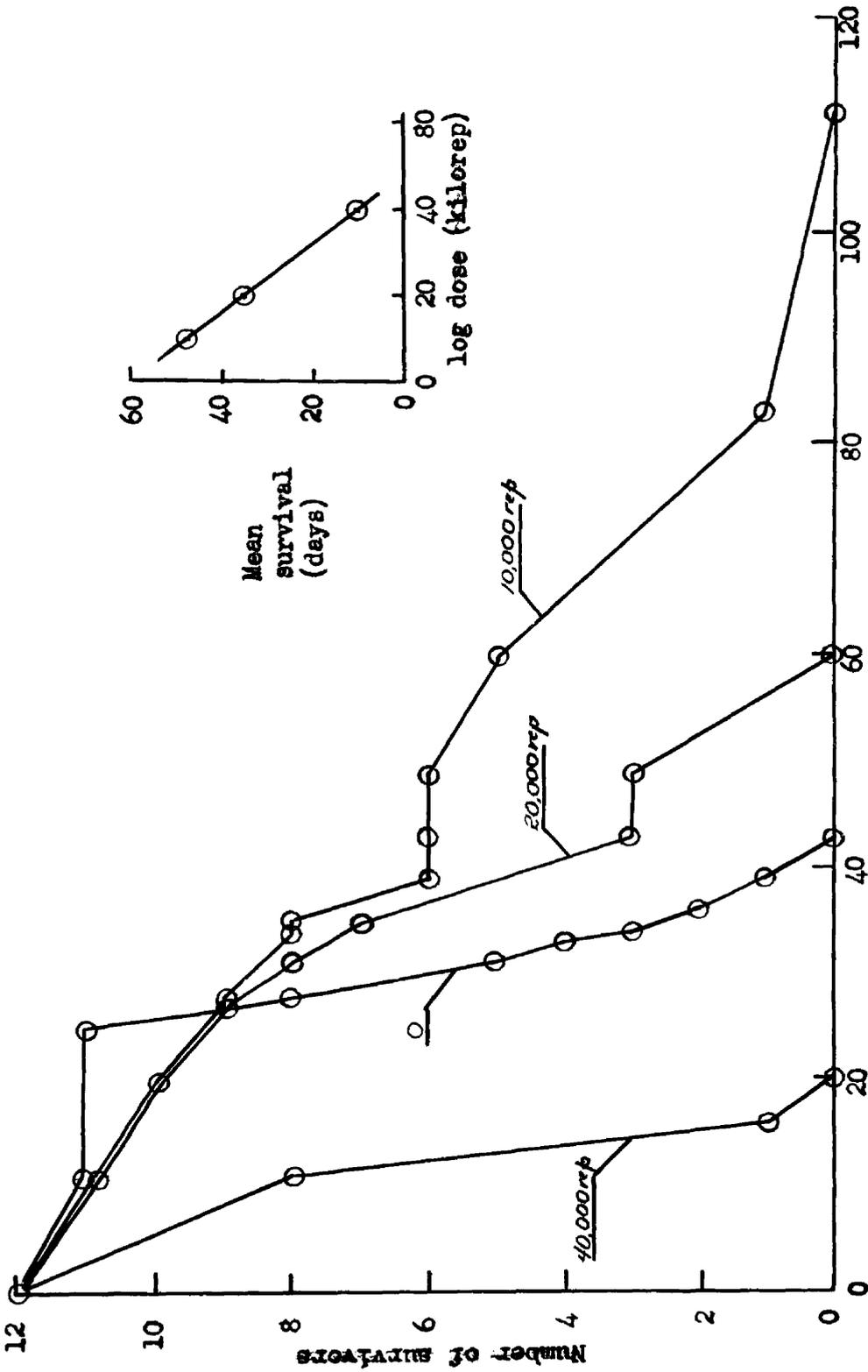


Fig. 1. Days survival after irradiation of Mediterranean flour moth larvae.

pairs seemed to show no appreciable difference in response at any dose, the figure gives the combined results for both families. The falling of the curve for the unirradiated subjects represents the death of the adults that emerged, lived a normal life span, and died. Of the irradiated subjects, only two (both irradiated at 10,000 rep) became adults; both of these adults were from the same parents. No other larvae reached the pupal stage, except, of course, the controls, of which 11 became adults. Whether the two moths that were irradiated in the larval stage were fertile is not known. They had no opportunity to mate, since they happened to be in different dishes. That these moths will breed in Petrie dishes was proved by the presence of second generation larvae in one of the control dishes. The life of the larvae irradiated at 10,000 rep and 20,000 rep was remarkably prolonged. These long lived larvae showed little disposition to move about and were of subnormal size for larvae of their age.

The results of this test suggest that the four larvae found in the 8,000-rep sample in the previous test (T-21) at the end of 67 days from irradiation may simply have been the survivors of larvae the lives of which were prolonged by irradiation. Figure II is a log-normal plot of the percent survivors from both larva tests (T-21 and T-67). The extension of the curve beyond the plotted points (Fig. II) can, in part, be justified by the result at 2,000 rep where the survival was high, but known precisely, and by two points at 20,000 rep and 40,000 rep which, though they can not be plotted, have statistical weight. The LD_{50} lies at about 5,000 rep; the point at which 99.9% would be killed lies at about 35,000 rep.

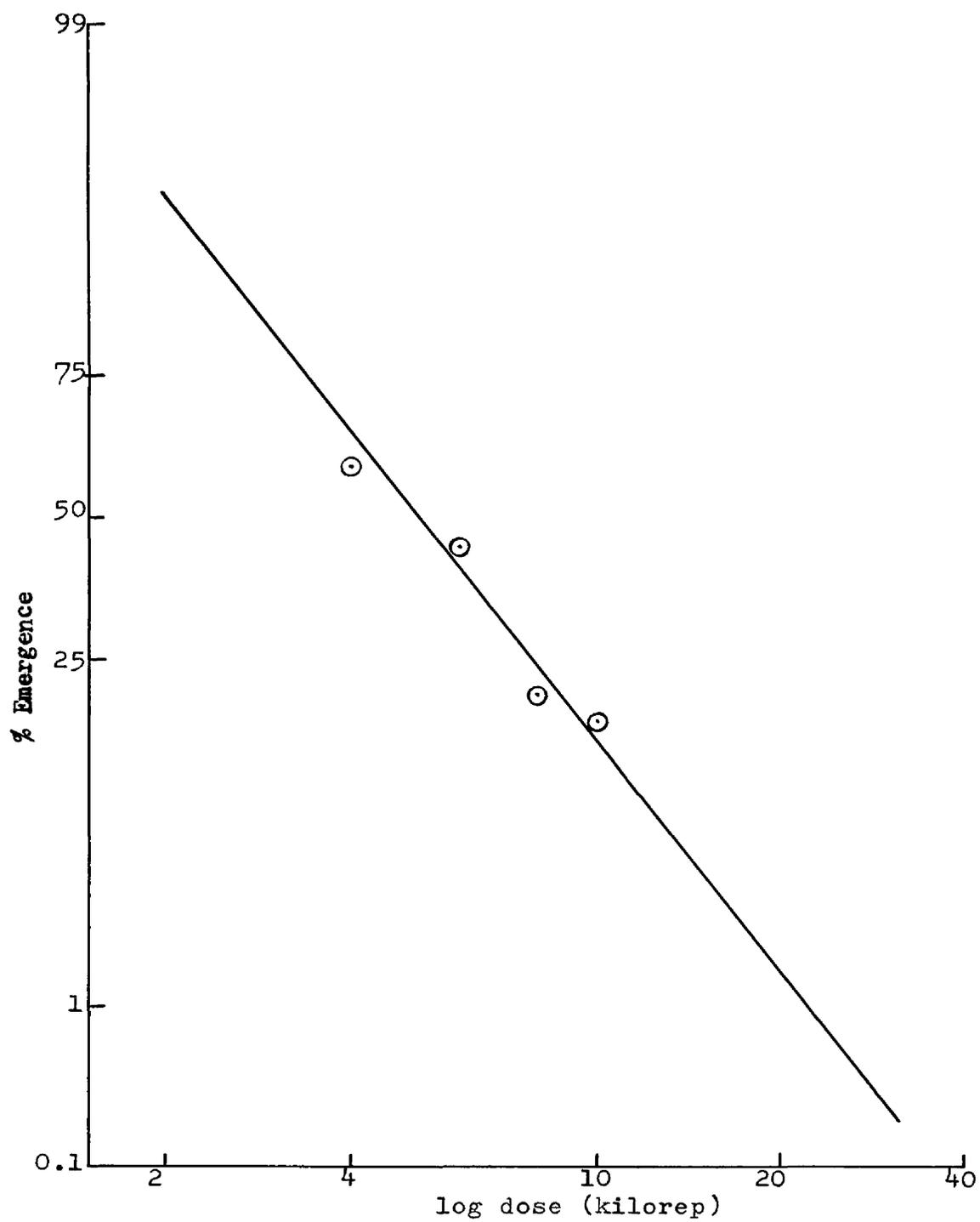


Fig. II. Emergence of irradiated Mediterranean flour moth larvae.

T-22: Five 20-pupae samples in no flour were placed in small, uncovered Petrie dishes and irradiated at five doses by conveyor method. Counts were made of the number of adults that emerged. Table 3 gives the total number of adults that emerged in each sample. Since no flour was available to the adults that emerged, and since the colonies were found to be infested with mites, the test was ended after all the adults had emerged. Therefore, no information was obtained on the fertility of the adults that did emerge.

TABLE 3
EMERGENCE OF IRRADIATED
MEDITERRANEAN FLOUR MOTH PUPAE

Dose (rep)	Number adults	Corrected % survival	Average days to emerge
0	13	100	7
2,000	8	63	5
4,000	10	77	5
6,000	6	46	6
8,000	5	38	8

T-71: Twenty-four pupae, 12 from each of two pairs of adults (the same two pairs in test T-67) were placed in some flour in small, uncovered Petrie dishes, one or two pupae to a dish, and irradiated at three doses by conveyor method. Periodic observations were made to determine the time of emergence and the time of death of the emerged adults. Table 4 gives the time from irradiation to emergence and the length of life of the adults; a missing entry indicates that no adult emerged; the two families are indicated by A and B.

TABLE 4
 TIME FROM IRRADIATION TO EMERGENCE
 AND SUBSEQUENT ADULT LIFE OF IRRADIATED
 MEDITERRANEAN FLOUR MOTH PUPAE

Dose (rep)	Family	Days to emerge		Days adult life	
		A	B	A	B
0		1.5	7.4	10.9	15.2
		4.6	6.4	10.0	13.0
		3.4	7.4	12.0	15.2
		4.6	8.5	10.9	14.1
	Mean	<u>3.5</u>	<u>7.4</u>	<u>11.0</u>	<u>14.4</u>
10,000		0.0	4.6	9.5	12.9
		1.5	-	9.8	-
		1.5	-	7.0	-
		3.4	-	9.0	-
	Mean	<u>1.6</u>	<u>4.6</u>	<u>8.8</u>	<u>12.9</u>
20,000		3.4	3.9	9.0	9.1
		5.5	8.0	6.0	5.0
		-	-	-	-
		-	-	-	-
	Mean	<u>4.4</u>	<u>6.0</u>	<u>7.5</u>	<u>7.0</u>

This experiment was naively designed with a view to comparing families and doses in a two-way classification, but the experiment was also designed to kill the subjects, and was, happily, successful, but with a large number of missing values. A number of comparisons can be made from these data. A striking result is the smaller variation within families compared to between families. An analysis of variance of the time to emerge for the controls shows the difference to be highly significant; however, this difference can be explained by the fact that the two sets of parents mated 2.8 days apart. With this correction to the mean time to emergence, the difference is not significant. Four other analyses were made. The adult length of life difference between families for the control samples is highly significant. A longer life

of pupae irradiated at 10,000 rep is suggested by the data, but the analysis of variance shows no significant difference. Two comparisons were made between families. In family A the pupae irradiated at 10,000 rep and 20,000 rep have statistically shorter lives than the controls, and in family B the 20,000-rep pupae have shorter lives than the controls.

The data do not show whether the irradiated pupae became fertile adults, but the fact that 20,000 rep reduced emergence by only 50% suggests that higher doses will be required to prevent appreciable emergence. The combined results of the two pupa tests are not in good agreement. However, failure to pupate may not be a good criterion by which to test radiation damage. Since the delayed effect of radiation is one of its prominent characteristics, it is conceivable that the pupa period is too short for radiation effects to become apparent; moreover, this moth has a short life span, and dying from radiation effects may look very like dying a natural death, particularly at lower doses.

T-60: Twenty-four mating pairs of moths were placed in about 10 grams of flour in small, polyethylene-covered Petrie dishes and irradiated at four doses by conveyor method. The pairs and the flour were then transferred to half-pint jars and the samples periodically observed for length of life of the moths and fertility. Table 5 gives the length of life in days after irradiation; fertile pairs are indicated by asterisks in the table, one each for the male and female. An analysis of variance of the data shows no significant difference among doses. The low fertility of the controls is evidently characteristic of this species (38).

The samples in which viable eggs were laid were kept until some of the second generation adults emerged.

TABLE 5
MORTALITY AND FERTILITY OF IRRADIATED
MEDITERRANEAN FLOUR MOTH PAIRS

Dose (rep)	Days of life after irradiation			
	0	2,000	10,000	50,000
	4.8	2.5	5.7*	5.0
	10.9	5.7	5.7*	6.5
	6.9*	8.0*	5.0	1.2
	15.8*	8.6*	5.0	3.0
	1.9	2.8*	8.0	3.8
	3.8	3.0*	8.0	6.9
	3.0	1.9	6.4	1.9
	3.0	4.1	7.0	2.2
	0.9	3.1*	9.2	5.5
	5.1	7.5*	11.1	6.0
	11.0*	3.1*	4.0	2.3
	<u>6.1*</u>	<u>4.5*</u>	<u>5.8</u>	<u>2.3</u>
Mean	6.1	4.6	6.7	3.9

*member of fertile pair

T-70: Thirty mating pairs of adults were collected, handled, and treated in the same manner as in the previous adult test, T-60, except that care was taken to collect only pairs that had emerged within 24 hours before irradiation. Data of the same kind as in the previous test were collected; the mean life spans in days after irradiation for doses of 0, 5,000, 10,000 and 20,000 rep were 9.4, 8.4, 9.8, and 9.6, respectively. An analysis of variance showed no significant difference among doses in length of life. The average life was, however, four days longer in the second test. Perhaps, on the average, the moths selected for irradiation in the first adult test were four days older at the time of irradiation.

Table 6 gives the number of fertile pairs at each dose, tests T-60 and T-70 results combined.

TABLE 6
FERTILITY OF IRRADIATED
MEDITERRANEAN FLOUR MOTH PAIRS

Dose (rep)	Fraction of fertile pairs	Corrected percent
0	7/14	100
2,000	4/6	130
5,000	4/8	100
10,000	5/13	80
20,000	0/7	0
50,000	0/6	0

Twenty thousand rep appears to be sufficient to sterilize the adults of this species, although at that dose there is no significant reduction in the length of life of the insect. The adults of this species are not destructive in themselves; they function only as egg producers and soon die so that inability to kill the adults with radiation is of no importance with respect to deinfestation.

The fact that doses of 10,000 rep and 20,000 rep produced significant reductions in the length of life of the pupa but not in the adults seems unusual. However, the pupa test was performed with insects from only two pairs of adults and the variation in length of life for adults from a particular pair was very small (a maximum of three days) compared to the variation among adults irradiated (a maximum of 15 days). The adults irradiated were taken from a breeding stock jar containing hundreds of moths and can be presumed to have come from many different parents.

Oryzaephilus surinamensis, the sawtoothed grain beetle

T-8: Seven 10-gram samples of flour in which 153 adults oviposited for three days were placed in small, uncovered Petrie dishes and irradiated at five doses. The samples were examined 46 days later; no adults, larvae, or pupae were found in any of the samples. The expected egg-adult period for this species is 27 days.

T-20: Five 24-gram samples of flour in which adults oviposited for three days were spread on trays to a depth of 3 mm or less and irradiated at five doses by conveyor method. The irradiated samples were placed in half-pint jars and examined 77 days later. Table 7 gives the results of the test.

TABLE 7

RESULTS OF IRRADIATION OF
SAWTOOTHED GRAIN BEETLE EGGS

Dose (rep)	Remarks
0	a thriving colony; about 60 live adults; many larvae
2,000	one dead adult
4,000	no sign of life
6,000	no sign of life
8,000	no sign of life

T-29: Six 20-larvae samples in 10 grams of oatmeal were placed in small, uncovered Petrie dishes and irradiated at six doses by conveyor method. Table 8 gives the number of adults in each sample on the stated number of days after irradiation. The corrected percents given in the table are based on the number of adults that emerged whether alive or not when counted.

TABLE 8
EMERGENCE OF IRRADIATED SAWTOOTHED
GRAIN BEETLE LARVAE
(NUMBER OF ADULTS)

Dose (rep)	Days after irradiation			Corrected percent emergence
	20	42	59	
0	10	-	12	100
2,000	14 ₁	-	13	117
4,000	3 ¹	2	2	25
6,000	0	0	0	0
8,000	0	0	0	0
10,000	0	0	0	0

¹one of which was dead

T-66: Five 15-pupae samples were placed on 10 grams of flour in small, polyethylene-covered Petrie dishes and irradiated at five doses by conveyor method. Periodic examinations of the samples were made to determine the time of emergence, the length of life after emergence, and the fertility of the irradiated pupae that became adults. Table 9 gives the number that emerged and the number that died within 10 days after emergence. The percent survival values in the table are based on the criterion that adults living 18 days after irradiation would live a normal life span. These still-living adults were removed from the samples at the end of 18 days after irradiation and the samples were kept for 52 days after irradiation. At that time only the unirradiated culture contained any second generation.

Figure III shows the emergence and survival as a function of time from the time of irradiation. The radiation does not seem to have any effect on the time at which the adults emerge; moreover, those adults which are about to die do so at about the same time (about 10 days after irradiation) at all doses.

TABLE 9
EMERGENCE AND SURVIVAL OF IRRADIATED
SAWTOOTHED GRAIN BEETLE PUPAE

Dose (rep)	Number emerged	Percent emerged	Number died ¹	Percent survival
0	15	100	0	100
2,000	13	87	1	80
4,000	8	53	3	33
8,000	4	27	4	0
16,000	3	20	3	0

¹within 10 days of emergence

T-31: Six samples of about 50 adults each were placed in otherwise empty Petrie dishes, covered with Saran Wrap, and irradiated at six doses by conveyor method. Periodic counts were made to determine the order of death and the number of survivors. These data are given in Table 10.

TABLE 10
SURVIVAL OF IRRADIATED SAWTOOTHED GRAIN
BEETLE ADULTS (NUMBER OF ADULTS)

Dose (rep)	Days after irradiation									Percent survival
	0	4	7	8	9	10	12	17	59	
0	50	50	50	50	50	-	-	50	TNTC ¹	100
2,000	47	46	45	45	45	-	-	44	TNTC	92
4,000	47	46	46	46	46	46	-	46	100 ²	98
6,000	52	52	43	39 ³	35	32	32	32	50 ²	62
8,000	49	49	43	29 ³	16	13	10	10	9	23
10,000	49	49	27	19	11	9	7	7	6	14

¹too numerous to count

²approximate number

³five adults escaped

The survival percents in Table 10 are based on the number of live adults 17 days after irradiation. The data show that the irradiated adults were fertile through doses of 6,000 rep and

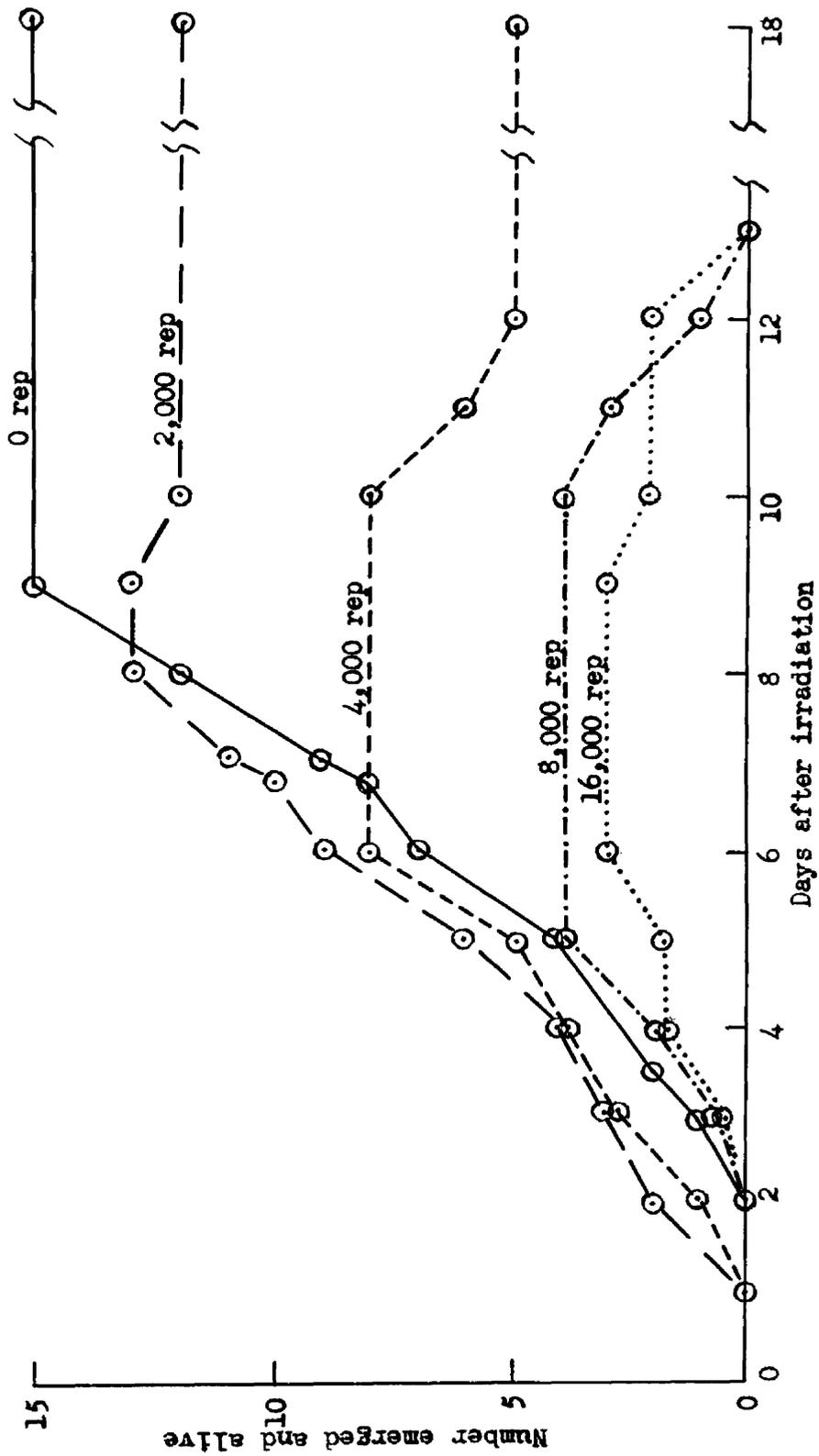


Fig. III. Emergence of irradiated sawtoothed grain beetle pupae.

sterile at 8,000 rep and above. Five adults escaped from the 8,000-rep sample and were never found. The percent survival at that dose is based on the assumption that the escaped beetles would have died in the same proportion as those that remained.

T-41: Six samples of about 50 adults each were placed in a few grams of oatmeal in small, Saran Wrap-covered Petrie dishes and irradiated at three doses by conveyor method, two replications at each dose. Table 11 gives the results of the periodic sample counts.

TABLE 11

NUMBER OF SAWTOOTHED GRAIN BEETLE
ADULTS SURVIVING IRRADIATION

Dose (rep)	Days after irradiation									Percent survival
	0	2	4	5	6	8	11	38		
0	50	50	-	-	-	-	-	-	-	100
0	47	46	-	-	-	-	-	-		
9,000	50	48	47	46	41	35	26	17	31	
9,000	43	43	42	42	37	29	16	12		
14,000	-	43	43	43	32	14	2	0	0	
14,000	38	38	36	33	28	12	2	0		

These samples were held for 94 days after irradiation. At that time both the control samples had vigorous colonies, but there were no signs of second generation in any of the irradiated samples.

The data from these two adult tests are consistent. Figure IV is a log-normal plot of survivors, data taken from T-31 and T-41. The LD_{50} lies at about 7,000 rep; the dose that might be expected to kill about 99.9% is 20,000 rep. Figures V and VI show the order of death of the irradiated adults. If the insects that die, die according to a normal error curve, then the survival plots at

sub-lethal doses will not be straight lines on probability paper. The sub-100%-lethal dose survival curves are plotted on rectangular coordinates (Fig. V); the sigmoid shape of the curves is evident. The survival data for the 100%-lethal dose are plotted on probability coordinates (Fig. VI).

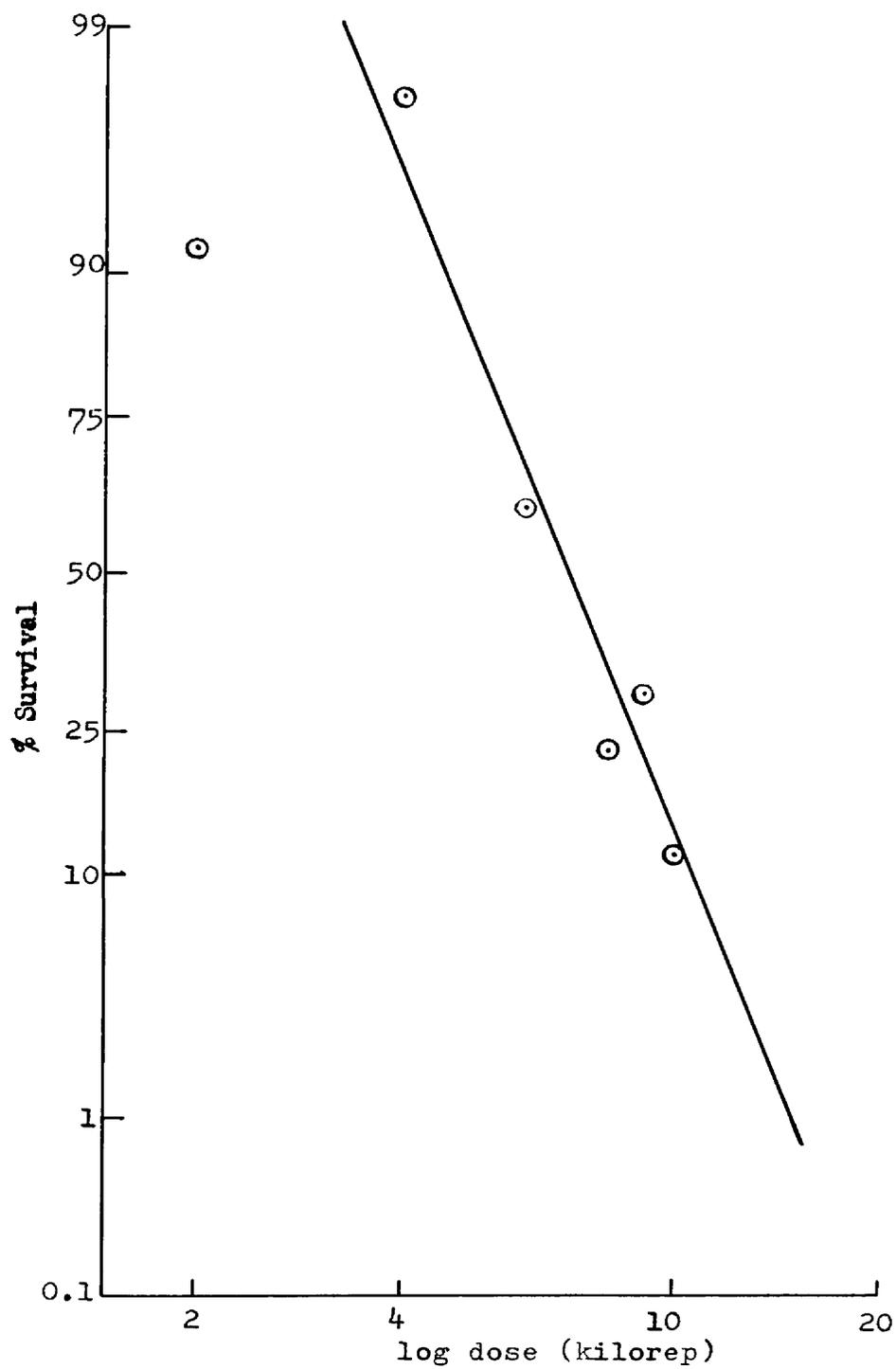


Fig. IV. Survival of irradiated sawtoothed grain beetle adults.

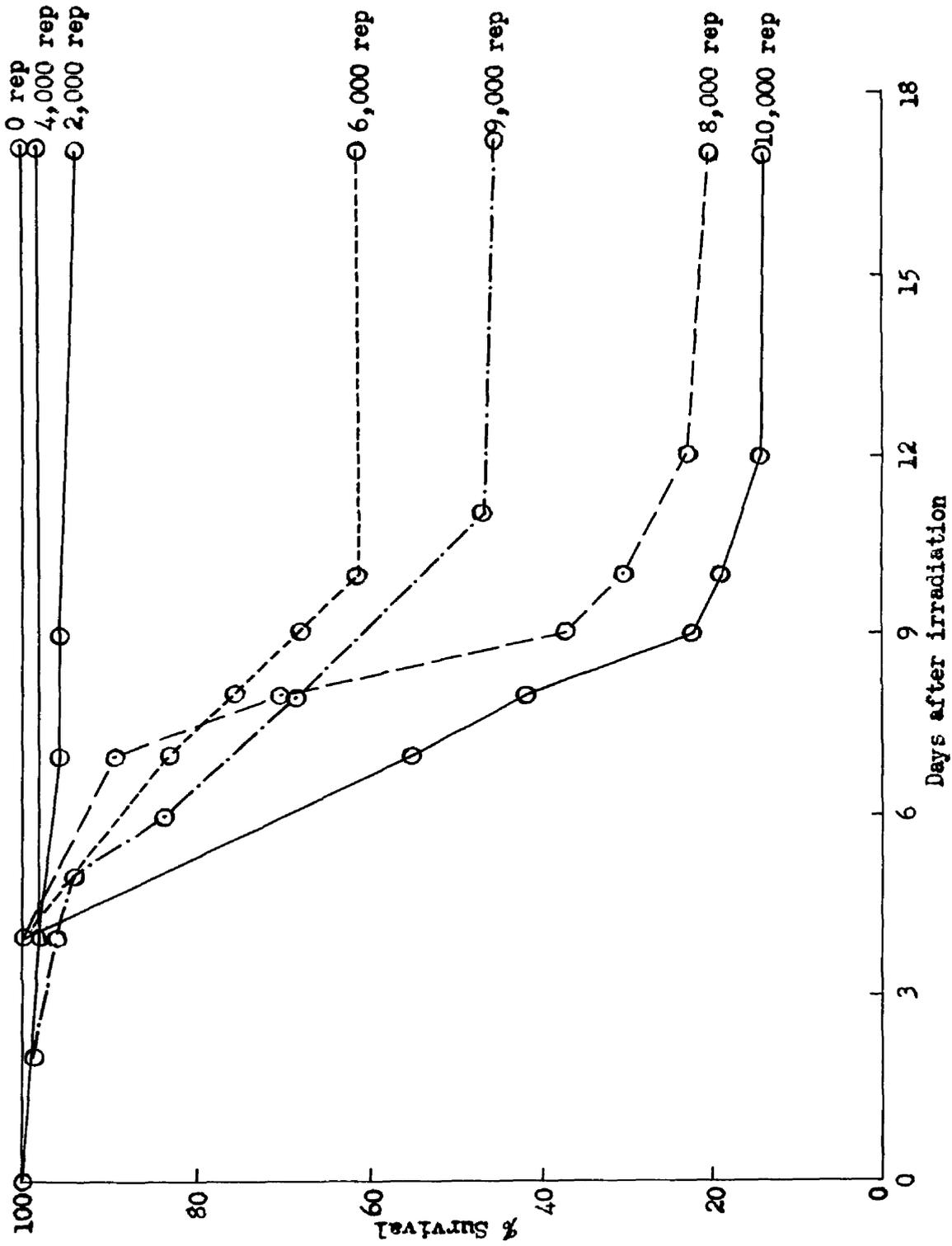


Fig. V. Order of death of irradiated sawtoothed grain beetle adults.
(Sub-100%-lethal levels)

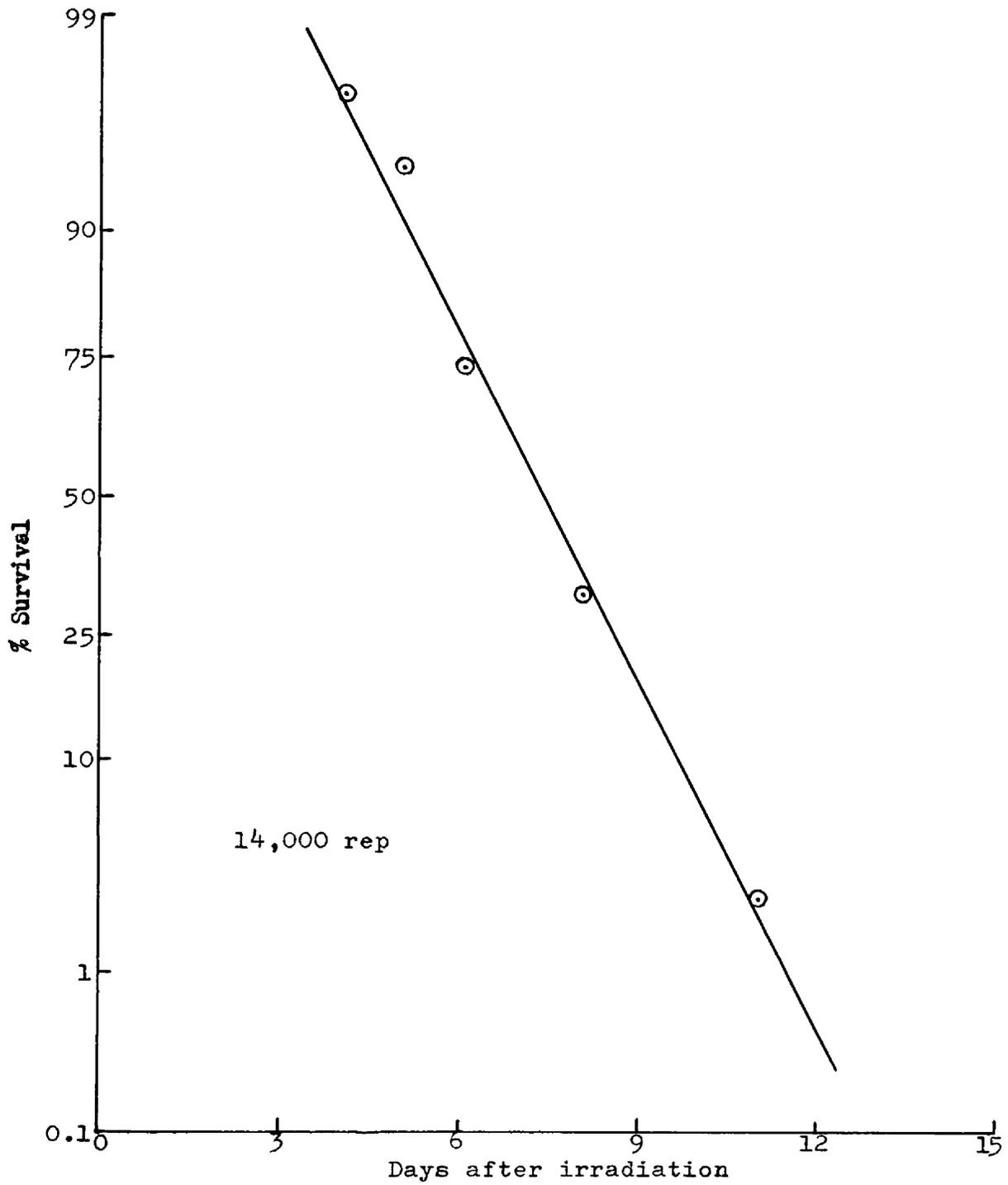


Fig. VI. Order of death of irradiated sawtoothed grain beetle adults (near 100%-lethal level).

Plodia interpunctella, the Indian-meal moth

T-55: Six 50-gram samples of whole wheat flour in which the adult moths had oviposited for three days were spread on trays, covered with polyethylene, and irradiated at three doses by conveyor method. These samples were discarded in a great clean-up of mite infestation. They were discarded before adults appeared in the samples.

T-32: Six 20-larvae samples were placed in 10 grams of whole wheat flour, covered with cellophane, and irradiated at six doses by conveyor method. The samples were examined periodically to determine the number of adults that emerged. Table 12 gives the emergence pattern.

TABLE 12

EMERGENCE OF IRRADIATED INDIAN-MEAL
MOTH LARVAE (NUMBER OF ADULTS)

Dose (rep)	Days after irradiation							Percent emergence
	13	16	20	22	24	34	51	
0	6	6	8	9	13	14	14	100
5,000	3	6	7	8	9	9	9	64
8,000	0	0	0	0	1	1	1	7
12,000	0	0	0	0	0	0	2	14
20,000	0	0	0	0	0	0	2	14
30,000	0	0	0	0	0	0	1	7

All the adults that emerged in the control and 5,000-rep samples were dead by the 34th day. The one adult that emerged in the 8,000-rep sample was dead by the 24th day. The four adults in the 12,000-rep and 20,000-rep samples were alive when counted. The one adult in the 30,000-rep sample was dead when counted. There were still live larvae in all of the irradiated samples even on

the 34th day after irradiation. This phenomenon is doubtless similar to the instance already noted of the Mediterranean flour moth larvae living far beyond their expected life span. The expected larva-to-adult period for this species is 44 days, and the larvae selected for this test, although of mixed, unknown ages, were fairly large; therefore, it seems safe to conclude that the time to pupation was prolonged by the radiation.

T-42: Six 20-larvae samples were placed in 10 grams of flour in small Petrie dishes, covered with polyethylene, and irradiated at three doses by conveyor method, two replicates at each dose. The samples were first examined 14 days after irradiation; nineteen adults had emerged in the two control samples, but none in the irradiated samples. By the 39th day after irradiation there were live adults in the control and 100,000-*rep* samples (the highest dose). There was considerable evidence of secondary infestation and the samples were all discarded. The experiment was a failure; nevertheless, it seems reasonable to conclude that the radiation retarded the larvae even though it had so far (by the 14th day) failed to kill them.

T-47: Six 20-larvae samples were prepared and irradiated in a manner similar to that of the previous larva test, T-42, except that different doses were delivered. The samples were examined 40 days after irradiation; the results are given in Table 13. The expected larva-to-adult period is about 40 days; the larvae used in the test were all medium to large size when irradiated. The results of the control suggest that the 40 day wait before

counting was long enough for most of the larvae to pupate and emerge. The fact that less than 20 subjects were counted is explained by the habit of these larvae of spinning a web wherever they go, and the fact that dead larvae dry up thus becoming very small and difficult to find. Incontrovertable evidence of secondary infestation is established by the 100,000-rep sample results. If only the small larvae represent secondary infestation, then a dose somewhere between 50,000 and 75,000 rep should prevent most of the larvae from becoming adults. Except for the presence of a live adult at 200,000 rep, these data are consistent with that conclusion; this species would have to be unusually resistant to radiation to make survival at 200,000 rep likely.

TABLE 13

OBSERVATIONS 40 DAYS AFTER IRRADIATION OF
20-LARVAE SAMPLES OF INDIAN-MEAL MOTH

Dose (rep)	Remarks
0	13 dead adults; 5 large larvae; numerous small larvae.
25,000	No adults; 15 dead larvae; one pupa; numerous small larvae.
50,000	No adults; 14 dead larvae; two large larvae; numerous small larvae.
75,000	No adults; 15 dead larvae; sawtoothed grain beetle adults; numerous small larvae.
100,000	No adults; 15 dead larvae; over 5 medium-sized larvae; one mite.
200,000	One live adult; 8 dead larvae; numerous small to medium larvae.

T-43: Six 15-pupae samples were placed in otherwise empty, small Petrie dishes, covered with polyethylene, and irradiated at six doses. The samples were examined 14 days after irradiation; no

emergence was observed in any sample. The expected pupal period for this species is about one week. Perhaps the handling of the pupae prevented emergence. From the time of this test on, moth pupae were obtained by placing larvae in the final test dishes and irradiating the samples when the larvae pupated.

T-48: Six 30-larvae samples in about 10 grams of flour were kept until most of the larvae had pupated. These samples were then irradiated by conveyor method at six doses. The samples were examined 42 days after irradiation with the following results: in the control sample were found 13 dead adults, 9 dead larvae, a few pupae, and one small larva; no adults were found in any of the other samples (doses ranged from 25,000 to 200,000 rep).

Rhyzopertha dominica, the lesser grain borer

T-63: Thirty approximately 15-gram samples of wheat in which adults oviposited for three days were placed in large Petrie dishes, covered with polyethylene, and irradiated, three samples at a time, by conveyor method. These samples were obtained by pouring a large sample of infested wheat over a Boerner sampler (a device that divides a sample into two equal parts) the required number of times. After irradiation the samples were placed in clean, half-pint jars. Table 14 gives the results of counts made about 50 days after irradiation.

TABLE 14

NUMBER OF ADULTS EMERGING FROM
 REPLICATE SAMPLES OF IRRADIATED
 LESSER GRAIN BORER EGGS

	Dose (rep)				
	0	2,000	4,000	8,000	16,000
	129	16	2	3 ²	2 ¹
	85	9	2	1	2 ²
	78	3	0	0	0
	62	0	0	0	0
	61	0	0	0	0
	<u>38</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
Total	453	28	4	4	4

¹ one of which was dead

² two of which were dead

The presence of live adults at doses of 4,000 rep and above must be viewed in the light of two facts of lesser grain borer behavior. First, the eggs are laid loosely in the grain; therefore, a minimum of handling is required to avoid losing eggs. Second, the lesser grain borer, true to its name, bores into the kernel

and stays until the food supply is exhausted; therefore, the adult is very difficult to see without practice and without examining nearly every kernel (cf. T-69 below). The probability of having left some adults in any particular sample is high, and the fact that at 8,000 and 16,000 rep five of the eight adults were dead, whereas no dead adults were found in any other samples, indicates they may have been in the samples during irradiation.

T-69: Thirty approximately 15-gram samples of wheat from the same infested stock used in the previous egg test, T-63, were prepared and treated in a similar manner. The counts, made 48 days after egg laying ceased and 35 days after irradiation, of the number of adults that emerged in each sample are given in Table 15.

TABLE 15
NUMBER OF ADULTS EMERGING FROM
REPLICATE SAMPLES OF IRRADIATED LESSER
GRAIN BORER LARVAE

	Dose (rep)				
	0	4,000	8,000	16,000	32,000
	183	2 ¹	0	0 ²	0
	168	1	0	0	0
	154	0	0	0	0
	150	0	0	0	0
	130	0	0	0	0
	125	0	0	0	0
Total	910	3	0	0	0

¹one a pupa, apparently alive

²infestation of book lice

Two points to be explained are: why is the average number of adults higher than in the previous test, T-63, when the wheat of this test came from the same infested source, and why are no adults

present at 8,000 rep and higher, as in the egg test? After the egg ecloses, the larva bores into the kernel from which it cannot be dislodged by handling. These samples were divided just prior to irradiation so that only one pass over the Boerner sampler was made while the insects were still in the egg stage, whereas, in the previous test, six passes over the sampler were made while the insects were still in the egg stage and, therefore, about half of the eggs were lost. Before and after these samples were irradiated, the grain was carefully inspected and any adults present were removed (an average of two to three a sample).

T-65: Six 30-adult samples of lesser grain borers were placed in otherwise empty, small Petrie dishes, covered with polyethylene, and irradiated at six doses by conveyor method. The insects were placed immediately in half-pint jars containing 35 grams of wheat. The six samples were counted every third day for 30 days. After each count, the dead adults were removed and the remaining adults placed in new 35-gram wheat portions until the 21st day after irradiation. After the 21st day they were simply returned, after counting, to the same jar. Table 16 gives the number of survivors at each count. Figure VII is a log-normal plot of the corrected percent survivors against dose. The percents were calculated on the assumption that accidentally killed adults would have lived. The jars in which these irradiated adults were allowed to lay eggs for successive three-day periods were kept to determine the fertility of the irradiated adults. Table 17 gives the number of second generation adults produced by the irradiated adults.

TABLE 16
ORDER OF DEATH OF IRRADIATED LESSER
GRAIN BORER ADULTS (NUMBER OF SURVIVORS)

Day	Dose (rep)					
	0	2,000	4,000	8,000	16,000	32,000
0	30	30	30	30	30	30
3	28	27	30	30	30	30
6	28	26	30	30	30	30
9	28	26	30	30	30	30
12	28	26	30 ¹	30	30	23
15	27	25 ¹	29 ¹	25	24	15
18	25	23 ¹	27	21	19	6
21	25	21 ¹	27	19	12	3
24	- ¹	-	27	19	12	2
27	24 ¹	21	26	17	10	2
30	-	-	-	16	7	2
38-62	23	21	19	11	3	1
Percent survival	100	96	79	46	12	4

¹one killed by accident

TABLE 17
NUMBER OF SECOND GENERATION ADULTS PRODUCED IN
SUCCESSIVE INTERVALS OF EGG LAYING
BY IRRADIATED ADULTS

Dose (rep)	Days after irradiation						
	0-3	3-6	6-9	9-12	12-15	15-18	18-61
0	215	271	227	244	233	224	103
2,000	27	104	60	19	6	0	0
4,000	2	25	4	0	0	0	0
8,000	0	0	0	0	0	0	0
16,000	0	0	0	0	0	0	0
32,000	0	0	0	0	0	0	0

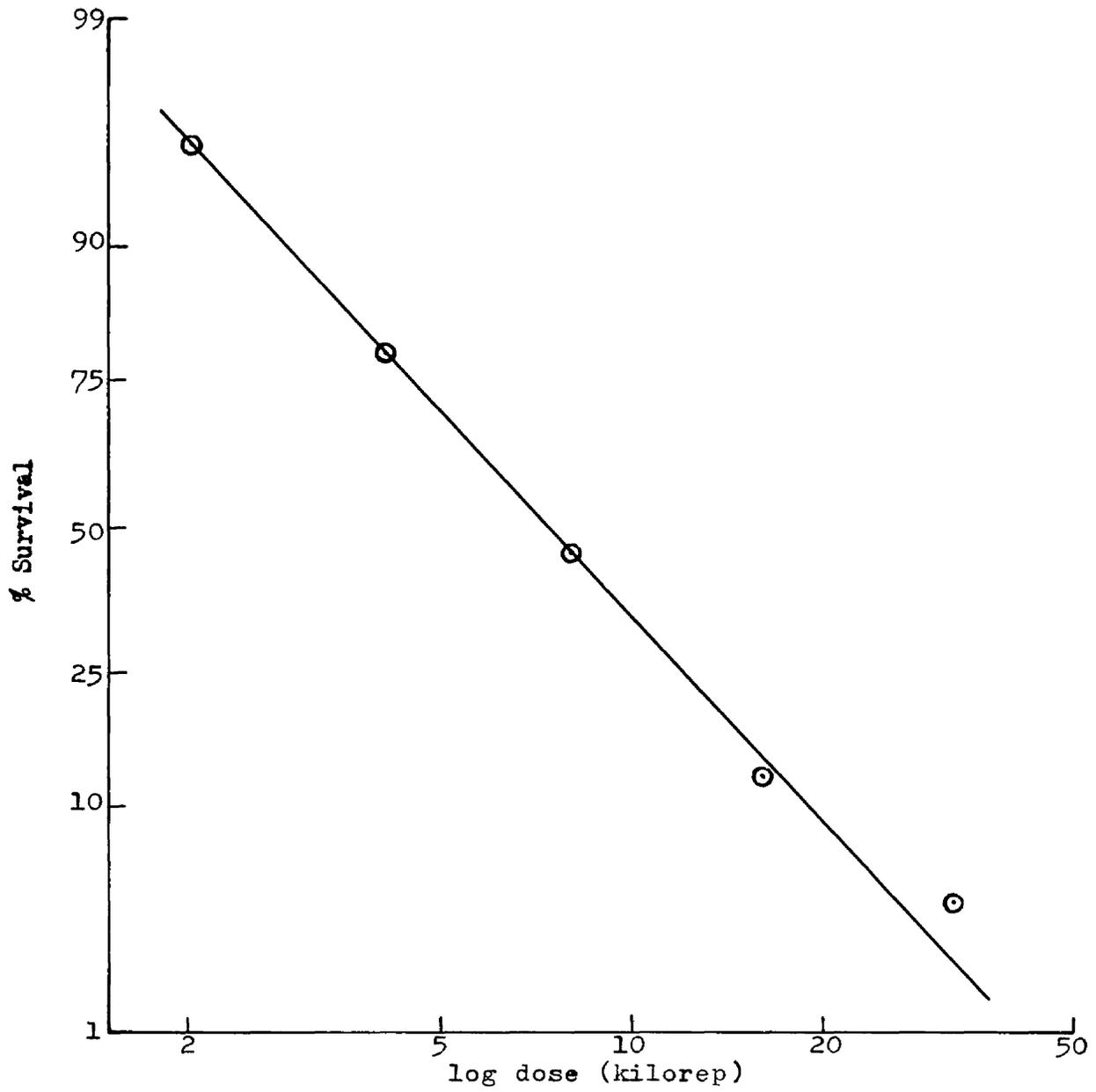


Fig. VII. Survival of irradiated lesser grain borer adults.

A strict comparison of the number of offspring from the irradiated adults with the number from the controls should be made on the basis of equal numbers of adults, or, preferably, equal numbers of females. Figures VIIIa and VIIIb are attempts to make such a comparison for those irradiated adults that did produce some offspring. Figure VIIIa is a plot against time of the number of second generation adults produced in each three-day period per living adult (the number of living adults during any three-day period is the average of the number living at the beginning and end of the period). Figure VIIIb is a plot against time of the ratio of second generation adults for each living adult during the three-day period to the corresponding number in the control sample.

There is a sharp increase in the number of second generation adults in the second three-day period for all doses at which immediate sterility was not achieved. This phenomenon can be considered as an abnormal decrease in fertility (or egg laying) during the first three-day period, a manifestation of a shock to the system. The shock was handling and irradiation after a relatively long period of quiet. If the above explanation is correct, it might be possible to eliminate the sharp rise by subjecting the insects to a few periodic handlings before irradiation.¹

The remarkable result is the existence of fertility, at the lower doses, immediately after irradiation, which is lost as time goes by. Can the explanation be that eggs already formed at the time of irradiation and the sperm stored in the female are both

¹Suggested by W.C. Nicholas, M.D. (personal communication).

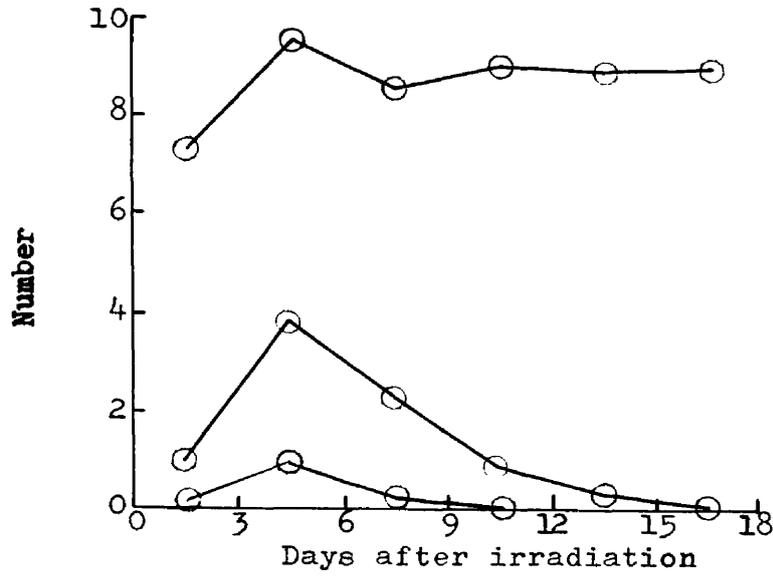


Fig. VIIIa. Number of 2nd generation adults per first generation adult produced in successive three-day periods.

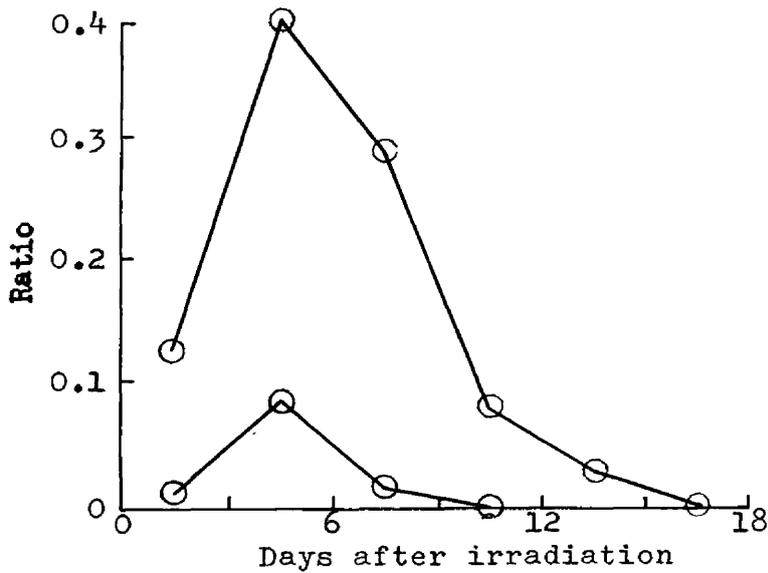


Fig. VIIIb. Ratio, to control, of 2nd generation adults per irradiated 1st generation adult produced in successive three-day periods.

slightly more resistant to radiation than the egg- and sperm-producing organs of the female and male, respectively, and that the decrease in fertility is simply the exhausting of these already formed eggs?

The large decrease in the number of second generation adults produced by the controls in the 18-61 day period can be attributed to Malthusian misery, 35 grams of wheat is not enough to sustain 24 adults and their potential progeny. At the time the control sample was counted, 53 days after irradiation, there was scarcely a whole kernel remaining.

Sitophilus granarius, the granary weevil

T-1: Fifty-four 11-gram samples of wheat in which adults had oviposited for three days were irradiated in small, uncovered Petrie dishes at six doses by stationary method. The samples were examined 56 days later; no adults were found in any sample.

T-6: Twenty 17-gram samples of wheat in which adults had oviposited for three days were irradiated in small, uncovered Petrie dishes at six doses by stationary method. The samples were counted every other day, beginning at the 52nd day after irradiation, until the 64th day. One final count was made 77 days after irradiation. Table 18 gives the total number of adults that had emerged in the replicate samples by the 64th day after irradiation.

TABLE 18

EMERGENCE OF ADULT GRANARY WEEVILS
FROM REPLICATE SAMPLES OF IRRADIATED EGGS
(NUMBER OF ADULTS)

	Dose (rep)					
	0	1,000	1,500	2,000	4,000	7,000
	10	9	5	2	0	0
	5	0	4	1	0	0
	0	0	0	0	0	0
	0					
	0					
Total	15	9	9	3	0	0

Two more adults had emerged by the 77th day; however, this period is longer than the expected egg-to-adult period (about 35 days) and the possibility that these adults marked the beginning of a second generation (for they were both in samples already

containing adults) must be admitted. At the beginning of the counting period just half of the control adults had emerged, but all but three of the irradiated adults had emerged. The numbers are, of course, too small to permit conclusions; nevertheless, if the trend is indicative, it represents a phenomenon the reverse of the expected delay or prolongation of the developmental periods. An analysis of variance of the data shows no significant difference in emergence among doses.

T-18: Five 130-gram samples of wheat in which adults had oviposited for three days were spread on trays to a depth of one kernel and irradiated at five doses by conveyor method. The samples were examined 77 days after irradiation; no adults were found in any sample.

T-30: Twelve 10-gram samples of wheat in which adults had oviposited for three days were placed in small Petrie dishes and irradiated at four doses by conveyor method, three replicates at each dose. The samples were examined 59 and 73 days after irradiation, at which time all the samples were found to be heavily infested with Indian-meal moth. The counts in the control samples on the 59th day were 7, 7, and 4; by the 77th day one of these beetles had died; no adult beetles were found in any of the irradiated samples (2,000, 4,000, and 8,000 rep). The analysis of variance shows a significant difference between the control and irradiated samples.

T-72: Eight 25-gram samples of wheat, obtained by dividing on a Boerner sampler, in which adults had oviposited for 3-3/4 days, were placed in large Petrie dishes and irradiated by conveyor method two samples at a time at four doses, two replicates at each

dose. The results of counts made every third or fourth day after the emerging adults were first seen are given in Table 19. The first time beetles were observed in any of the samples was on the 35th day after irradiation, the last previous observation at which none were seen was the 31st day after irradiation. The data are presented as live/dead adults; if the virgule is missing in a tabular entry, then the dead beetles were included in the total count. Dead beetles were removed after each count.

TABLE 19
EMERGENCE PATTERN OF ADULTS FROM IRRADIATED
GRANARY WEEVIL EGGS (LIVE/DEAD ADULTS)

Dose (rep)	Days after irradiation						Percent of control
	36	39	42	45	49	52-63	
0	95	186	215	223	219/6	219/6	100
0	62	181	210	217	219/1	219/1	
2,000	0	3/0	9/0	11/1	7/6	6/8	6.07
2,000	1/0	3/0	6/1	8/1	7/3	7/6	
4,000	0	0	1/0	1/0	1/0	1/0	0.45
4,000	0	0	0	0	0/1	0/1	
8,000	0	0	0	0	0	0	0.0
8,000	0	0	0	0	0	0	

The data suggest that there is a delay in the time to emerge for the irradiated subjects. The average time to emergence for the controls is before the 39th day; for the 2,000-rep beetles, about 42 days; and for the two adults at 4,000 rep, the 45th day.

T-14: Twenty-eight 17-gram samples of wheat in which about 250 adults had oviposited for three days were placed in small Petrie dishes and irradiated, 21 days after egg laying ceased, at five doses by conveyor method. Counts of the number of emerged adults were made beginning the 33rd day after egg laying. Only one

adult had emerged at the beginning of the count. Table 20 gives the number of adults that had emerged in the replicate samples by the 63rd day after irradiation.

TABLE 20
NUMBER OF GRANARY WEEVIL
ADULTS EMERGING FROM REPLICATE SAMPLES
OF IRRADIATED LARVAE

	Dose (rep)				
	0	2,000	3,000	5,000	7,000
	12	5	2	1	0
	11	4	1	0	0
	10	4	1	0	0
	9	2	0	0	0
	8	0	0	0	0
	7				
	6				
	2				
Total	65	15	4	1	0
Mean	8.1	3.0	0.8	0.2	0.0
%	100	37	10	2.5	0.0

The average number of days to emergence, after egg laying, was about 50 days. An analysis of variance of the days to emergence (complete data not given) shows no significant difference among doses. If the expected development periods are adjusted proportionally to the observed egg-to-adult period, the calculation shows that the larvae were irradiated at the 0.45-completed point of the larval stage. An analysis of variance of the emergence figures shows the difference between controls and irradiated samples to be highly significant. There is no significant difference among irradiated samples. Shortly after a steady state of the population was reached, over half of the adults died. A sudden increase in warmroom temperature may have been responsible for these deaths and the emergence failures in some other concurrent tests.

T-54: Twelve 10-gram samples of wheat were prepared and treated at four doses, three replicates at each dose, in a manner similar to that of the previous test, T-14. The irradiation was performed just seven days after egg laying ceased, or, if the development periods of test T-14 hold, just after the eggs had eclosed. The samples were examined 59 and 73 days after egg laying ceased. Table 21 gives the number of adults that had emerged by the 59th day after irradiation. There were a few more adults in the control samples by the 77th day, but these were assumed to be second generation. Every sample was heavily infested with Indian-meal moths.

TABLE 21

NUMBER OF ADULT GRANARY WEEVILS
EMERGED IN REPLICATE SAMPLES
FROM IRRADIATED LARVAE

	Dose (rep)			
	0	3,000	5,000	7,000
21	5	1	0	0
17	3	0	0	0
10	2	0	0	0
Total	48	10	1	0
%	100	21	2	0

Although the samples of this experiment were prepared in the same manner as in test T-51 (an egg test), the total number of emerged adults was considerably greater, 48 compared with only 18 in the egg test. The larvae of this test were irradiated at a much earlier stage in their development than in the previous larva test, T-14, but the data do not suggest any difference in resistance between the two stages (cf. Tables 20 and 21). An analysis of variance of the data shows a highly significant difference between

the control and irradiated samples, but no significant difference among irradiated samples.

T-74: Eight 25-gram samples, obtained by dividing on a Boerner sampler, in which adults had oviposited for 3-3/4 days were placed in large, uncovered Petrie dishes and irradiated 10 days after egg laying ceased at four doses, two replicates at each dose. This 200-gram wheat sample was the other half of a 400-gram sample used for the egg test, T-72. The data are presented as live/dead adults in Table 22. If the time for the egg-to-adult period is taken from the middle of the egg laying period to the time when the first adults were seen in the control samples, and if the observed-time to expected-time ratio is used to adjust proportionally the lengths of the metamorphic stages, then the calculation shows that these larvae were irradiated at about the 0.27-completed point of the larval stage.

TABLE 22
EMERGENCE PATTERN OF ADULTS FROM IRRADIATED
GRANARY WEEVIL LARVAE (LIVE/DEAD ADULTS)

Dose (rep)	Days after irradiation						Percent of control
	36	39	42	45	49	52-63	
0	32	130	185	203	206/1	206/5	100
0	35	125	178	200	202/3	203/6	
3,000	0	8	28/0	60/2	61/13	52/33	42.6
3,000	0	8	39/0	64/6	69/8	61/33	
6,000	0	0	0	2	1/1	1/2	1.91
6,000	0	0	0	4	3/2	2/3	
9,000	0	0	0	1	1/0	0/1	0.48
9,000	0	0	0	0	0	0	

As in the case of the egg test, T-72, a delay in the time to emergence is suggested by the results. The mean time to emerge for the controls is about 40 days; the mean time for the larvae irradiated at 3,000 rep is about 43 days; the mean for the 6,000-rep samples is 45 days.

Figure IX is a log-normal plot of all the results from the egg and larva tests. The positions of the two lines drawn through the plotted points were weighted, visually, in favor of the data from tests T-72 and T-74, for which the results were best. Figure IX brings out clearly the difference in resistance between the two metamorphic forms. The LD_{50} for eggs lies at about 1,300 rep, the LD_{50} for larvae at about 2,200 rep. Doses that would prevent 99.99% of irradiated eggs and larvae from becoming adults would be 5,600 rep and 11,000 rep, respectively. No account is taken of any possible change of larva resistance with age. The slopes of these curves are approximately the same. The significance of the slope of plots such as these is not known to the writer.

T-3: Six samples of about 150 adults each were placed in small, otherwise empty Petrie dishes, covered with Saran Wrap, and irradiated at six doses by stationary method. These adults were placed in uninfested wheat immediately after irradiation, allowed to remain for three days and then removed to a second set of jars. The first set of jars was examined 69 days after irradiation; there were no second generation adults in any of the jars. The second set of jars was examined 37 days after irradiation. About 90% of the adults in each sample, including the control, were dead. Because the mortality of the control was so high, the entire set was discarded.

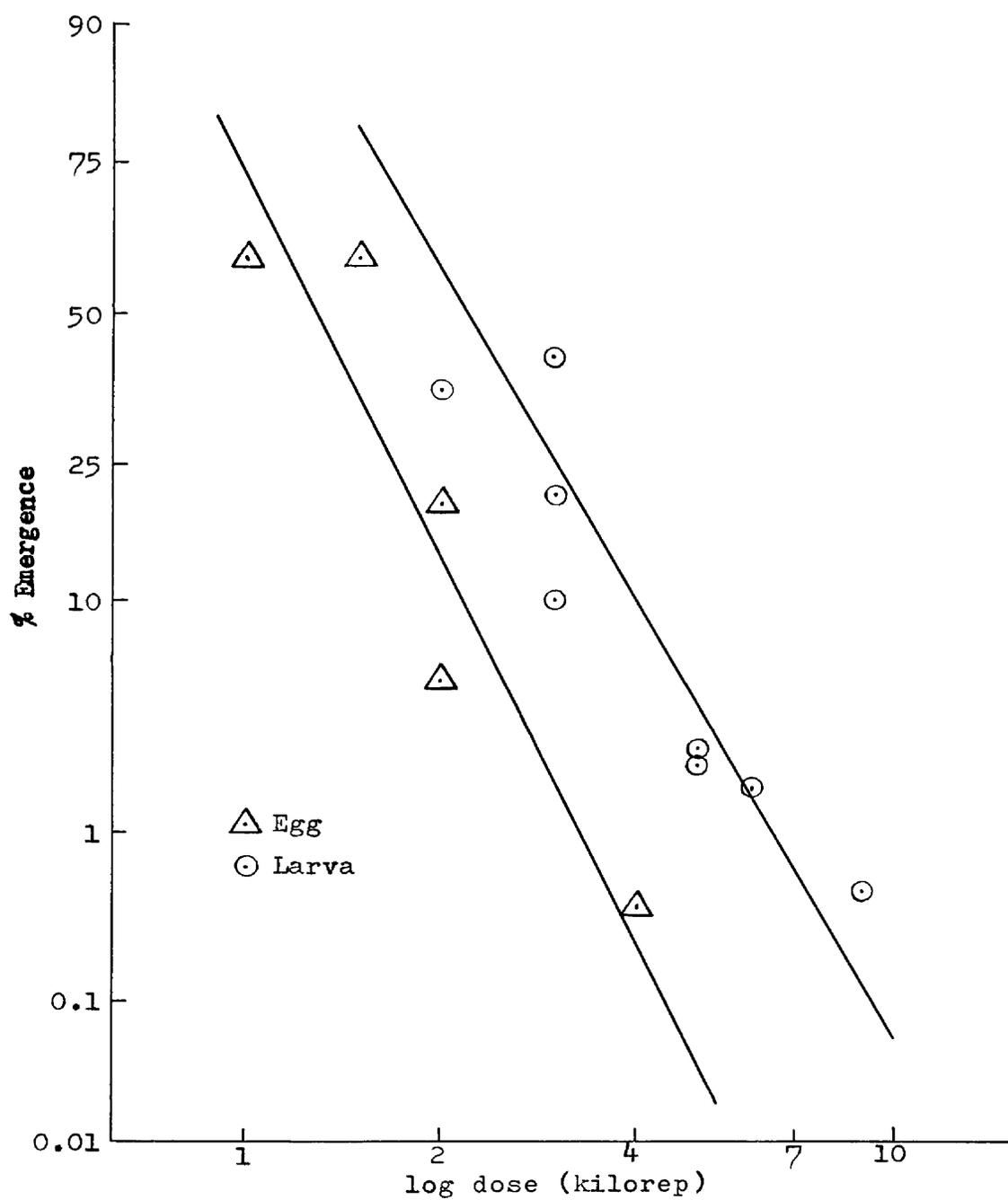


Fig. IX. Emergence of irradiated granary weevil eggs and larvae.

T-4: Five samples of about 50 adults each were prepared and treated in a manner similar to that of test T-3. Table 23 gives the results of the few counts made of the number of live adults.

TABLE 23
SURVIVAL OF IRRADIATED
GRANARY WEEVIL ADULTS
(NUMBER OF LIVE ADULTS)

Dose (rep)	Days after irradiation		
	0	1	10
0	49	44	18
4,000	48	46	0
6,000	48	46	1
8,000	50	46	2
10,000	47	46	0

The results are only suggestive because of the high mortality of the controls, the short observation period, and the high mortality of controls in previous tests.

T-12: Over 200 adults were placed in a small Petrie dish, covered with Saran Wrap, and irradiated at 10,000 rep by stationary method. Counts were made of the number of survivors every three days; at the same time the remaining live adults were transferred to new jars of uninfested wheat. After the 12th day, the three remaining adults were left in their jar and were not observed again until the 36th day by which time they were all dead. The jars in which these beetles had been kept were examined about three months after irradiation. No second generation adults were found in any of the jars. The mortality curve for these 227 adults is given in Figure X with the results of test T-73 described below. Unfortunately no control was run with this test.

T-73: Four samples of about 100 adults each were placed in small Petrie dishes, covered with Saran Wrap, and irradiated at four doses by conveyor method. The samples were counted the third day after irradiation and every two days thereafter and at each count the remaining live adults were placed in new jars of uninfested wheat until the 15th day. From the 15th day on, the insects were returned to the same jar. By the 17th day, 87 out of the original 95 control adults were still alive; the number of adults irradiated at 5,000 rep had been reduced from 100 to 7; all of the adults irradiated at 10,000 and 20,000 rep were dead. Figure X is a plot on probability paper of the percent survival against time, corrected for the control mortality.

If there is a marked difference in the death rate produced by the three doses 5,000, 10,000, and 20,000 rep, Fig. X does not reveal it beyond the fact that at 5,000 rep five adults survived through the 19th day. The one curve, from a previous test, T-12, does appear to be different, especially since the dose was the same as one of the T-73 doses. Several comments on the different circumstances of the two tests are pertinent. First, the insects in test T-72 are known to be, generally, younger adults than those of T-12. Second, the rearing room conditions prevailing during T-73 can be presumed to better for granary weevils than conditions prevailing during T-12 (cf. results of all previous granary weevil tests vs. T-72, T-73, and T-74). Third, there is no correction to the T-12 test for the natural mortality of the adults, and, if the experiences of some of the concurrent granary weevil tests are applicable, that natural mortality was high; moreover, the corrections

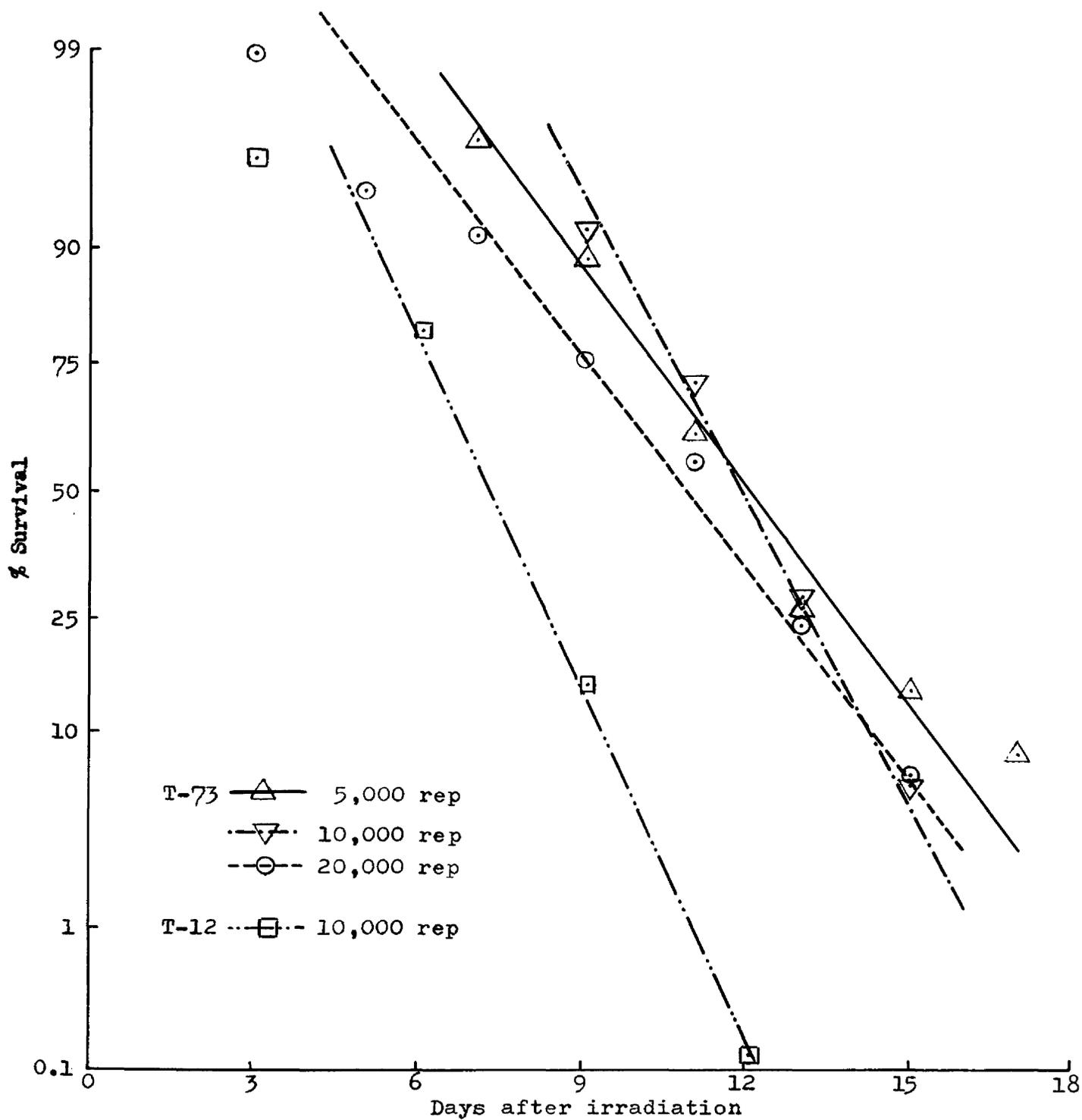


Fig. X. Order of death of irradiated granary weevil adults.

for natural mortality would move the points up and bring the curve more closely in line with the T-73 curves.

The jars in which the treated and control adults of this test were placed after each count were counted to determine the fertility of the irradiated adults. The counts were made periodically until the number of second generation adults remained constant. Table 24 gives the number of second generation adults that emerged from each sample. The emergence in the control samples shows a general increase, particularly after the first period, which was three rather than two days. The same loss of fertility is shown by the adults irradiated at 5,000 rep as that shown by the lesser grain borers.

TABLE 24

NUMBER (LIVE/DEAD) OF SECOND GENERATION
ADULTS FROM EGGS LAID BY IRRADIATED
GRANARY WEEVIL ADULTS

Dose (rep)	Egg laying period (days after irradiation)						
	0-3	3-5	5-7	7-9	9-11	11-13	13-15
0	259/7	224/4	246/4	287/1	318/7	316/7	329/3
5,000	12/0	10/2	2/0	0	0	0	0
10,000	0	0	0	0	0	0	0
20,000	0	0	0	0	0	0	0

T-38: A culture of wheat heavily infested with all the life forms of the granary weevil was spread on trays and irradiated by conveyor at 10,000 rep. The size (two kilograms) and extent of infestation of this culture encouraged only casual inspection. Activity proceeded normally for about one week, then the culture seemed to have lost all life, but by the end of 107 days after irradiation,

long enough for two or three generations, adults were again observed and within a week afterwards the colony was again thriving. The conditions of the test did not obviate the possibility of a dose of less than 10,000 rep, a dose known to have been lethal to all the subjects tested previously, delivered at some point in the sample. However, if the order of death is, in fact, log-normal, then the emergence curve of Fig. IX shows that one out of every 2,000 larvae would survive 10,000 rep. This culture probably contained many more larvae than 2,000.

Sitophilus oryza, the rice weevil

T-9: Twenty-six 17-gram samples of wheat in which adults had oviposited for three days were irradiated in small Petrie dishes at six doses by stationary method. These samples were counted every other day from the 40th to the 48th day after irradiation; only 12 insects emerged in the control samples, and these by the 40th day, and none in any of the irradiated samples (1,000 rep and higher). An analysis of variance of the data shows no significant difference between control and irradiated samples.

T-19: Five 130-gram samples of wheat in which adults had oviposited for three days were spread on trays and irradiated at five doses by conveyor method. These samples were examined 77 days after irradiation; no adults were found in any of the samples.

T-50: Twelve 10-gram samples of wheat in which adults had oviposited for three days were placed in small Petrie dishes and irradiated by conveyor method at four doses. The samples were examined 39, 59, and 73 days after irradiation. Table 25 gives the number of adults that had emerged by the 59th day after irradiation. Only one more adult had emerged between the 59th and 73rd days, and that one was a granary weevil. That sample had been dropped on the floor during handling and could have been contaminated when the grains were recovered; therefore, the granary weevil was not counted. The analysis of variance shows a significant difference between the control and irradiated samples, but none among irradiated samples. These samples were all infested with Indian-meal moth.

TABLE 25
EMERGENCE FROM REPLICATE SAMPLES
OF IRRADIATED RICE WEEVIL EGGS
(NUMBER OF ADULTS)

	Dose (rep)			
	0	2,000	4,000	8,000
	18	2	1	0
	17	0	0	0
	11	0	0	0
Total	46	2	1	0
%	100	4	4	0

T-15: Five 90-gram samples of wheat in which adults had oviposited for three days were spread on trays and irradiated, 24 days after egg laying ceased, at five doses by conveyor method. Counts of the number of live and dead adults that emerged were made 16 and 34 days after irradiation. Two of the adults were granary weevils; these two are not included in the data of Table 26, which gives the number of adults that emerged by the 34th day after irradiation (the 58th day after egg laying ceased). Percent survival means percent of the adults that emerged at that dose that survived.

TABLE 26
EMERGENCE AND SURVIVAL OF RICE WEEVIL ADULTS
FROM IRRADIATED LARVAE (NUMBER OF ADULTS)

Dose (rep)	Live	Dead	Percent emergence	Percent survival
0	92	5	100	95
2,000	13	9	23	59
3,000	6	5	11	55
5,000	0	2	2	0
7,000	0	1	1	0

T-53: Twelve 10-gram samples of wheat in which rice weevil adults had oviposited for three days were placed in small Petrie dishes and irradiated, seven days after egg laying ceased, at four doses by conveyor method. Counts were made of the number of adults emerged on the 39th, 59th and 73rd days after egg laying. Table 27 gives the number of adults emerged by the 59th day; only one adult emerged after the first count.

TABLE 27
NUMBER OF ADULTS EMERGED FROM
REPLICATE SAMPLES OF IRRADIATED
RICE WEEVIL LARVAE

	Dose (rep)			
	0	3,000	5,000	7,000
	75	4	0	0
	68	2	0	0
	56	1	0	0
Total	199	7	0	0
%	100	3.5	0	0

The analysis of variance shows a highly significant difference between the control and irradiated samples, but no significant difference among irradiated samples. The emergence percentages from this test and the previous larva test, T-15, are plotted on log-normal coordinates (Fig. XI). The single point that is far from the line is from T-53 in which the larvae were irradiated at an earlier stage in their development. All the samples in this test were heavily infested with Indian-meal moth.

T-13: Over 200 adults were placed in a small Petrie dish, covered with Saran Wrap, and irradiated at 10,000 rep by stationary

method. One hundred of these were transferred immediately to a jar containing uninfested wheat. Counts were made every third day after irradiation and the remaining live adults were transferred to new jars after each count. Figure XII is a plot on probability paper of the survivors. On the 12th day of counting, it was observed that two of the five dead weevils were granary weevils. A count was made at once of the irradiated beetles that were not used in the test. The count revealed 134 dead rice weevils, 15 dead granary weevils, and four live granary weevils; these four died within a few days. Probably the 100 insects used in the test contained no higher a fraction of granary weevil contamination than that of the unused portion (about 10%). The 100 supposed rice weevils used were counted as they climbed up the side of a glass jar, an ability that the granary weevil does not have to the same degree.

The jars in which the dying weevils had stayed for successive three-day periods were examined about four months after the weevils were irradiated. No evidence of life was found in any of the jars. Unfortunately, no control was run with this test.

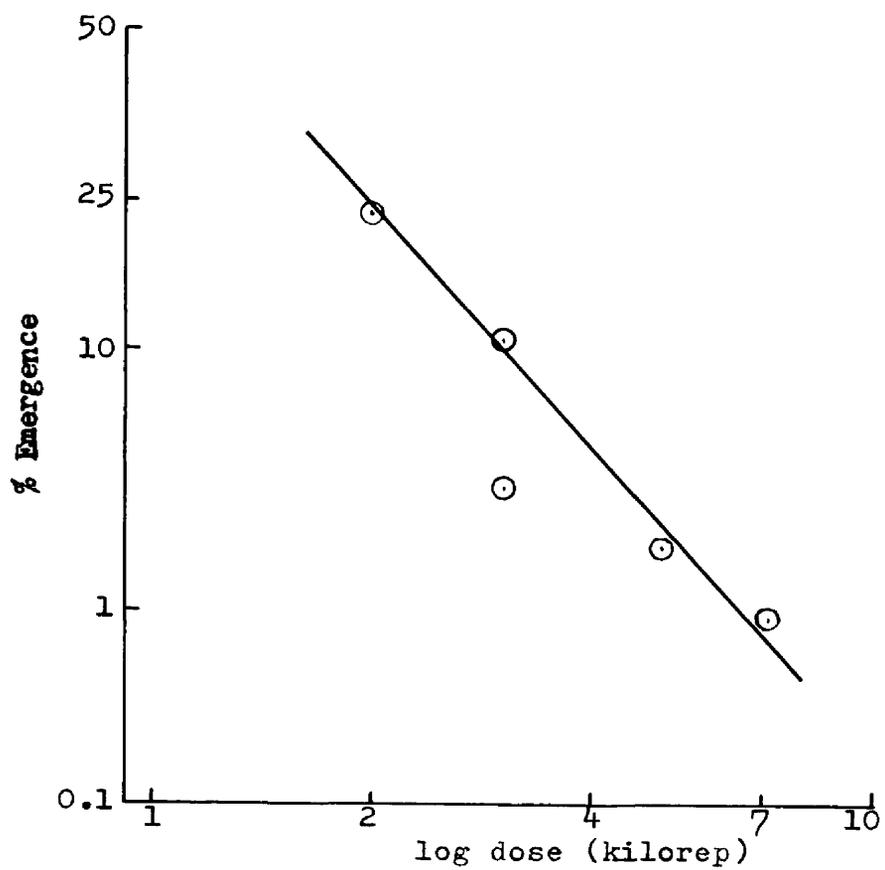


Fig. XI. Emergence of irradiated rice weevil larvae.

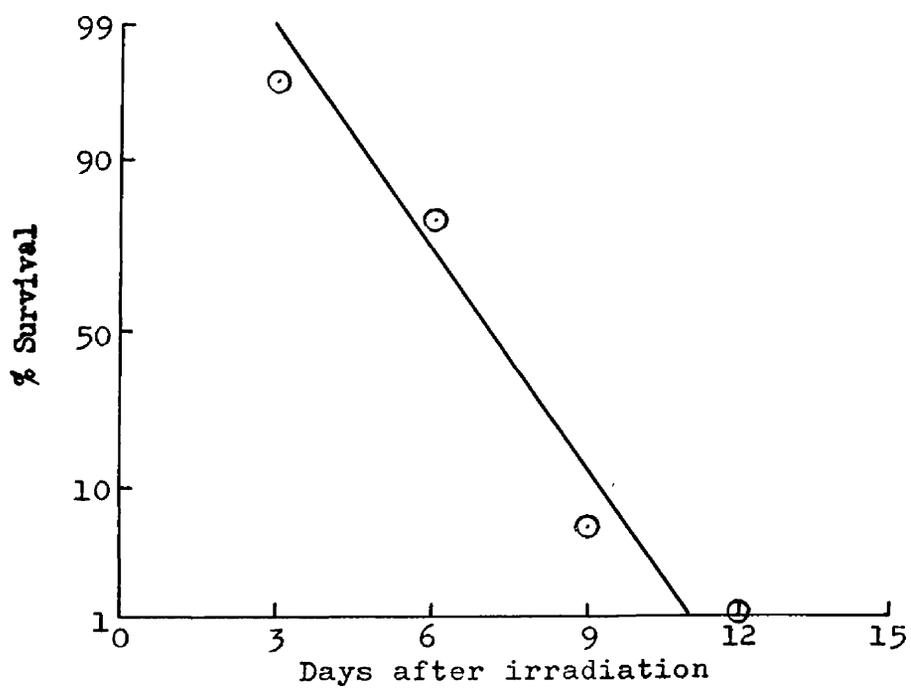


Fig. XII. Order of death of irradiated rice weevil adults.

Sitotroga cerealella, the Angoumois grain moth

T-27 Six 104-gram samples of corn in which adults had oviposited for four days were spread on trays to a depth of about one kernel and irradiated by conveyor method at three doses, two replicates at each dose. The counts of emerged adults at the end of 39 days are given in Table 28. An analysis of variance of the data shows no significant difference among doses. However, the data do show that 8,000 rep is insufficient to make any appreciable reduction in emergence.

TABLE 28

EMERGENCE OF ANGOUMOIS GRAIN
MOTHS FROM IRRADIATED EGGS
(NUMBER OF ADULTS)

	Dose (rep)		
	0	4,000	8,000
	109	112	44
	96	59	35
Total	205	171	79
%	100	83	39

T-34: Six 120-gram samples were prepared and irradiated as in test T-27, with the exceptions that only two days were allowed for egg laying and that six doses with no replications were used. The samples were not examined until over four months after irradiation. The results were as follows: both the control and 8,000-rep samples were vigorous colonies; the 5,000-rep sample contained one live and one dead moth; the 12,000- and 20,000-rep samples each contained two dead moths; the 30,000-rep sample

contained one dead moth. The skip at 5,000 rep (one of the two experienced in over 70 tests in this study) is difficult to explain. The result at 8,000 rep is consistent with the previous egg test, T-27. There are at least three possible sources for the adults in the 5,000-, 20,000-, and 30,000-rep samples. One, of course, is growth from the original infestation, in which case, 30,000 rep must be regarded as insufficient to prevent the eggs from eclosing; a second source is the failure to remove all the adults used to infest the culture; a third source is secondary infestation. The presence of both a live and a dead adult in the 5,000-rep sample suggests secondary infestation because these moths do not live for four months.

T-57: Three hundred grams of wheat in which adults were allowed to oviposit for three days was divided into eight samples on a Boerner sampler. The sub-samples were placed in large Petrie dishes and irradiated at five doses by stationary method. Table 29 gives the number of adults found in each sample 65 days after irradiation. This period is very close to being long enough for two generations; however, the samples were observed often enough to obviate the possibility that second generation were counted. These samples were counted after first killing the moths with ether and then opening the jars; therefore, only one count could be made. No analysis of variance of the data was made because the wide discrepancy in the counts of the two 5,000-rep samples may have been simply an inadvertant mixing of the sub-counts of the two samples. The results are in fair agreement with those of the two previous tests, T-27 and T-34. The failure of the high doses of 20,000 and 40,000 rep is confirmed by this test.

TABLE 29

EMERGENCE OF ANGOUMOIS GRAIN MOTHS FROM
IRRADIATED EGGS (NUMBER OF ADULTS)

	Dose (rep)				
	0	5,000	10,000	20,000	40,000
	181	267	7	2	3
	151	22	5	—	—
Total	332	289	12	2	3
%	100	87	3.6	1.2	1.8

T-64: Four half-pint samples of wheat (obtained by dividing on a Boerner sampler) in which adults had oviposited for three days were spread to a one-kernel depth on trays and irradiated at four doses by conveyor method. Adults were first observed in the control samples 27 days after irradiation and in the 15,000-rep sample 33 days after irradiation. The samples were counted on the 40th day. The results are given in Table 30.

TABLE 30

EMERGENCE OF ANGOUMOIS GRAIN
MOTHS FROM IRRADIATED EGGS

Dose (rep)	No. adults	Percent
0	314	100
15,000	26	8.3
30,000	3	0.96
60,000	0	0.0

Figure XIII is a log-normal plot of the emergence percents from the three egg tests T-27, T-57, and T-64. Some adjustment should be made to the doses for the one test in which the eggs were on corn, because 1-Mev electrons do not penetrate corn as

well as wheat. A precise adjustment of the dose would take account of the probable position of the eggs, the ionization-vs.-depth function, and the percent emergence-vs.-dose function. The net effect of an adjustment would be to shift the two points for corn further to the left in the figure. The LD_{50} for eggs lies at about 7,500 rep, higher than that for any of the Coleoptera tested.

T-28: Six 65-gram samples of corn in which adults had oviposited for four days were spread on trays to a one-kernel depth and irradiated at three doses by conveyor method, two replicates at each dose. The irradiation was done 12 days after egg laying ceased. The samples were examined 40 days after egg laying. The results are given in Table 31. An analysis of variance of the data shows no significant difference among doses. A comparison of this test with the first egg test, T-27, reveals comparable numbers of adults in the control samples, a smaller reduction in emergence in the irradiated samples, and the failure of 8,000 rep to make any appreciable reduction in the population.

TABLE 31

EMERGENCE OF IRRADIATED
ANGOUMOIS GRAIN MOTH LARVAE
(NUMBER OF ADULTS)

	Dose (rep)		
	0	4,000	8,000
	101	87	76
	71	76	33
Total	172	163	109
%	100	95	63

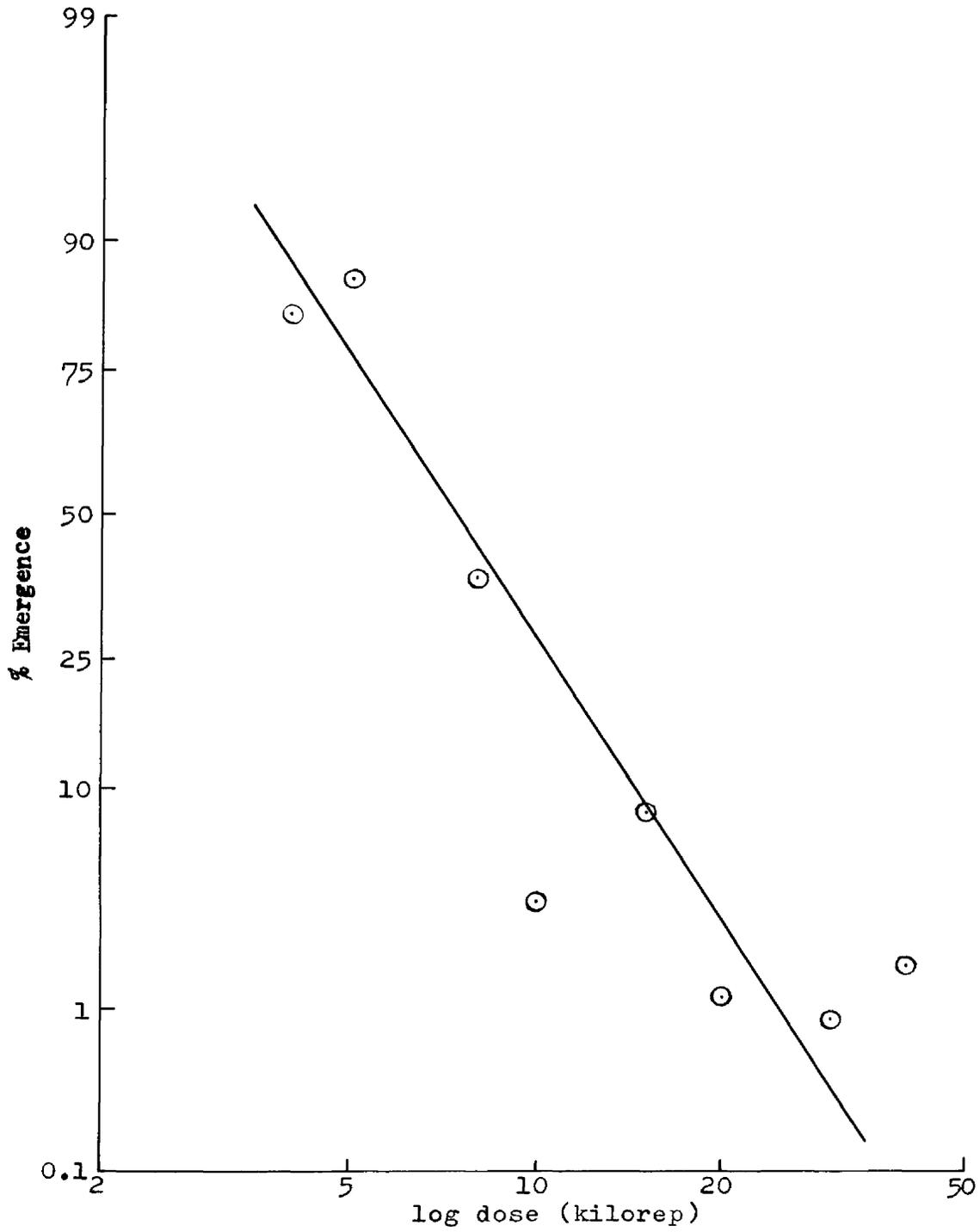


Fig. XIII. Emergence of irradiated Angoumois grain moth eggs.

T-39: Six 120-gram samples of corn in which adults had oviposited for two days were spread on trays to a one-kernel depth and irradiated at five doses 14 days after egg laying ceased. The samples were examined 133 days after egg laying. The results are given in Table 32. The criticisms of this test are similar to those of the egg test T-34.

TABLE 32
RESULTS OF THE IRRADIATION OF
ANGOUMOIS GRAIN MOTH LARVAE

Dose (rep)	Remarks
0 (two samples)	Both vigorous colonies
10,000	11 dead adults
18,000	7 dead adults, one meal moth pupa
30,000	2 dead adults
60,000	one live and one dead adult

T-58: Eight 38-gram samples of wheat in which adults had oviposited for three days were divided on a Boerner sampler, spread on trays to a depth of one kernel, and irradiated at five doses 10 days after egg laying ceased. The results of a count made 55 days after egg laying are given in Table 33.

TABLE 33
EMERGENCE OF IRRADIATED ANGOUMOIS
GRAIN MOTH LARVAE (NUMBER OF ADULTS)

	Dose (rep)				
	0	5,000	10,000	20,000	40,000
	119	73	29	9	2
	96	59	28		
Total	215	132	57	9	2
%	100	61	27	8.4	1.9

T-68: Four half-pint samples of whole wheat in which adults had oviposited for three days were spread to a one-kernel depth on trays and irradiated by conveyor method at four doses 14 days after egg laying ceased. These samples were obtained by dividing on a Boerner sampler. Adults were first observed in the control sample 27 days after egg laying and in the 15,000-rep sample 33 days after egg laying. The samples were counted on the 44th day. The results are given in Table 34.

TABLE 34

EMERGENCE OF IRRADIATED
ANGOUMOIS GRAIN MOTH LARVAE

Dose (rep)	Number of adults	Percent
0	386	100
15,000	24	6.2
30,000	5	1.3
60,000	3	0.78

Figure XIV is a log-normal plot of percent emergence against dose for the data of larva tests T-28, T-58, and T-68. The data suggest that the larvae are slightly more resistant to radiation than the eggs; however, there is considerable scatter in the points. The LD_{50} for larvae lies at about 8,000 rep and the LD_{99} at about 33,000 rep. These larvae were irradiated at various times in their development period, which may account in part for the scatter of the points. The data obtained from larvae irradiated in corn are subject to the criticisms mentioned in the egg test discussion; however, in the case of larvae, the adjustment may not be as great because the larvae are inside the kernels rather than outside.

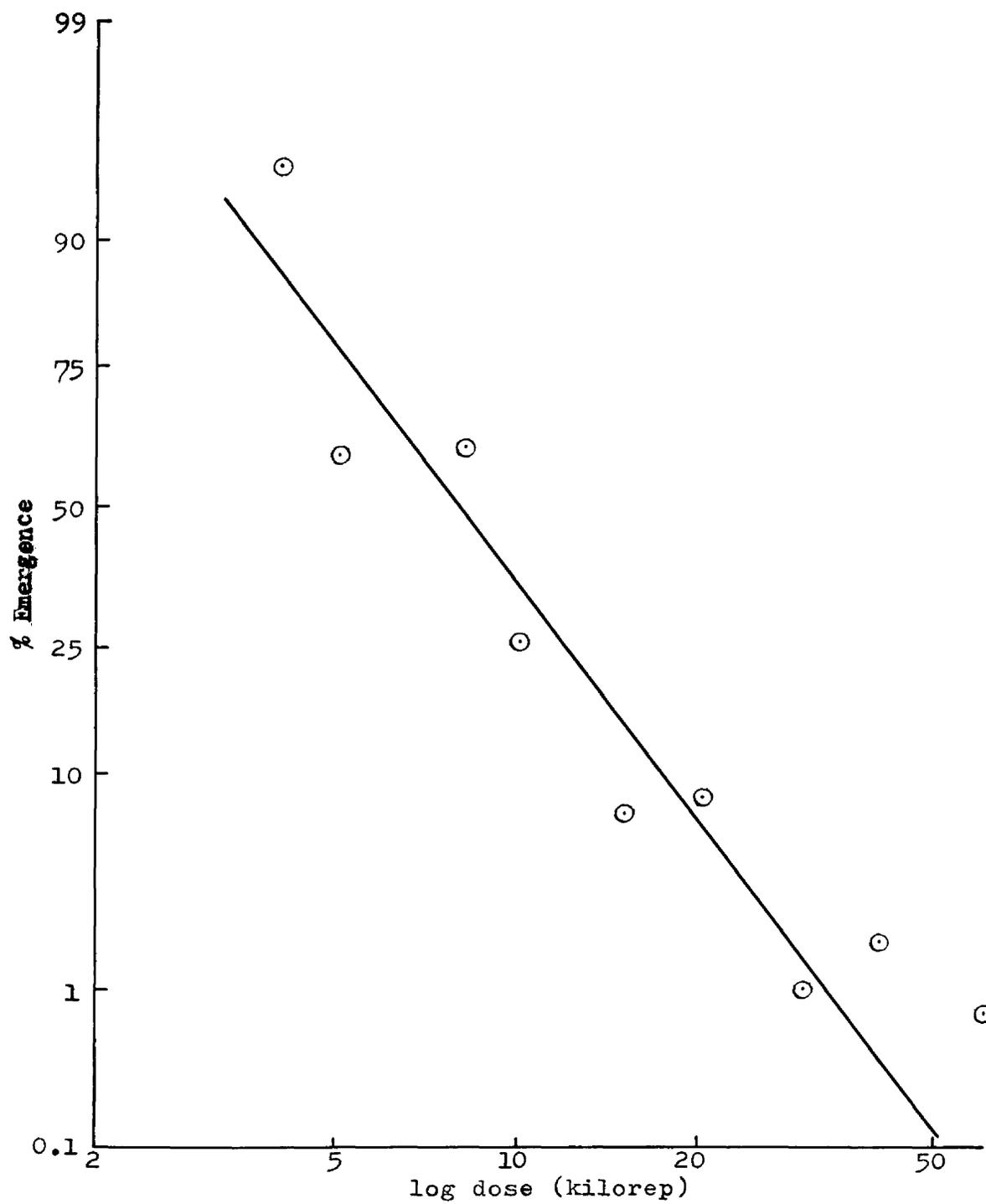


Fig. XIV. Emergence of irradiated Angoumois grain moth larvae.

T-61: Forty-five adult moths were placed, within about 12 hours of emergence, in small Petrie dishes containing about 10 grams of flour, covered with polyethylene, and irradiated at five doses by conveyor method. These moths were kept in individual jars and observed several times a day. Table 35 gives the number of days from the time of irradiation to death. The analysis of variance shows a highly significant difference between the mean life for adults treated at 160,000 rep and the other adults. There was some evidence that female moths lived much longer than male moths, but at the time of this test no one was available to sex the moths. If the guesses of the writer as to sex were correct, nearly two-thirds of the adults treated were females, and their average life span was about twice as long as that of the males. The data show that the life span of the adults is not appreciably affected by doses up to 40,000 rep.

TABLE 35

DAYS OF LIFE OF IRRADIATED
ANGOUMOIS GRAIN MOTH ADULTS

	Dose (rep)				
	0	2,000	8,000	40,000	160,000
	6.9	11.6	6.9	13.0	3.0
	11.6	10.0	5.9	11.7	2.5
	17.0	13.0	13.0	12.0	0.5
	5.0	11.1	7.3	11.5	5.5
	16.5	5.0	5.5	10.7	9.5
	11.3	14.0	14.0	12.0	0.1
	14.0	12.0	6.0	16.0	6.0
	13.4	5.0	10.8	11.5	0.0
	5.4	8.5	6.0	15.5	0.2
Mean	11.2	10.0	8.4	12.7	3.0

T-62: Twenty-eight mating pairs of adults which had emerged within 24 hours of treatment time were placed in small Petrie dishes

containing about 10 grams of wheat, covered with polyethylene, and irradiated at four doses by conveyor method. The pairs were observed several times a day to determine the length of life and the cultures were kept to determine the fertility. Table 36 gives the length of life of the treated adults. An analysis of variance shows a highly significant difference in mean life between adults irradiated at 160,000 rep and all other adults. The average life spans in this test are smaller than those of the previous test, T-61. Can it be that there is some longevity advantage enjoyed by unmated moths? These data cannot support such a contention. First, on the average, the adults that mated were half a day older at the time of irradiation. Second, the proportions of males and females, as determined by the moths, are equal; whereas, there is a suspicion that in T-61 the proportion of females was higher and, moreover, that the females lived longer. Third, after these two corrections the variance among samples can account for the remaining difference.

TABLE 36

DAYS OF LIFE AFTER IRRADIATION OF
MATING PAIRS OF ANGOUMOIS GRAIN MOTHS

	Dose (rep)			
	0	10,000	40,000	160,000
	5.0	6.7	7.5	0.1
	8.7	9.3	10.9	7.4
	4.5	5.4	1.2	0.7
	6.0	8.0	6.6	5.0
	5.5	6.6	5.0	0.7
	7.5	6.6	11.5	2.5
	5.0	7.0	7.0	0.7
	8.0	9.1	8.0	0.7
	4.5	8.6	6.5	0.7
	6.5	8.6	9.5	6.1
	6.0	6.0	7.5	0.2
	8.0	8.1	8.1	3.4
	7.0	6.3	8.1	2.0
	<u>7.0</u>	<u>8.1</u>	<u>12.0</u>	<u>2.0</u>
Mean	6.4	7.5	7.8	2.3

The results of the fertility part of this test are given in Table 37. The analysis of variance of the data shows a significant difference between the number of second generation adults in the control samples and the irradiated samples. The reduction in fertility measured in terms of second generation adults is 87% at a dose of 10,000 rep. Evidently the conditions for raising this moth were good since the average number of second generation adults is nearly twice the average number of eggs laid given in Appendix A. The counts were made about 62 days after irradiation of the adults; the first emergence was observed 33 days after irradiation.

TABLE 37

NUMBER OF SECOND GENERATION
ANGOUMOIS GRAIN MOTHS FROM
IRRADIATED MATING PAIRS

	Dose (rep)			
	0	10,000	40,000	160,000
	84	9	0	0
	135	7	0	0
	126	42	0	0
	134	3	0	0
	114	24	0	0
	148	10	0	0
	156	25	0	0
Mean	128	17	0	0

As a matter of interest, these moth pairs were bred in jars containing 35 grams, or about 350 kernels, of wheat. Each second generation adult means the loss of one kernel of wheat; the average number of second generation adults (unirradiated) was 128.

Tenebrio molitor, the yellow mealworm

T-33: Six 20-larvae samples of yellow mealworm were placed in 10 grams of mixed wheat and flour in small, polyethylene-covered Petrie dishes and irradiated by conveyor method at six doses. Periodic counts made of the number of live larvae are given in Table 38. The larvae-to-pupa period is given by different authors as 6-9 months and 31 days. The fact that nearly half of the larvae in the control sample had pupated by the 35th day after irradiation, and the fact that the larvae were nearly all the same size indicate that, if the 6-9 months figure is correct, the larvae were fairly old. It is not known whether the five larvae irradiated at 20,000 rep and still surviving 52 days after irradiation would have lived to become adults or not.

TABLE 38

MORTALITY OF IRRADIATED YELLOW
MEALWORM LARVAE (NUMBER OF LARVAE)

Dose (rep)	Days after irradiation									
	0	1	2	3	5	10	16	25	35	52
0	20	20	20	20	20	19 ¹	18 ²	18 ²	13 ⁷	-
20,000	20	20	20	20	20	18	14	12	10	5
40,000	20	19	19	19	19	12	7	3	1	0
60,000	20	19	19	19	18	2	2	0		
90,000	20	20	18	18	15	0				
150,000	20	20	20	20	7	0				

¹the superscripts give the number of larvae that pupated

It is particularly difficult to tell the exact time of death of these larvae. Some days after death, the bodies become blackened. Doubtful cases were decided by whether or not the larva twitched when the side of the glass container was struck. These data show the decrease in time of kill with increasing dose. Figure XV is a linear plot against dose of the time at which half the treated larvae were dead. The time was determined by a linear interpolation between counts bracketing the 50% point. A plot of the 0.5-survival times against the logarithm of the dose is nearly a straight line except for the first point. Since the number of subjects is small, no further analysis of the data was attempted. The LD_{50} lies below 20,000 rep, but how far below is not known.

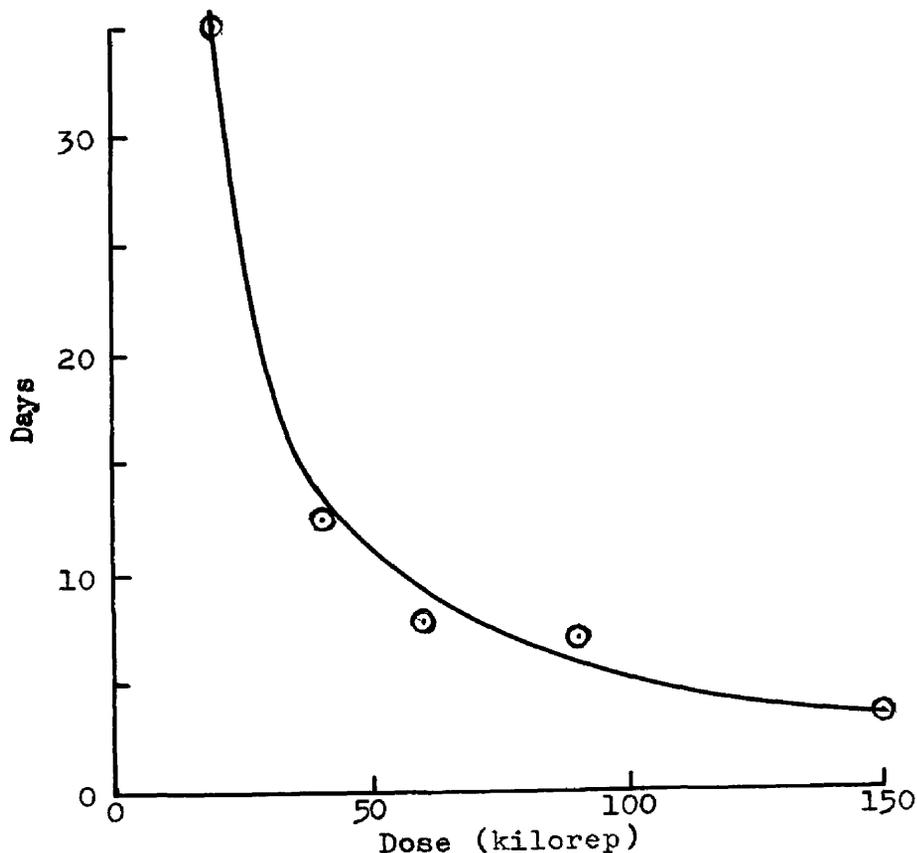


Fig. XV. Time of 50% survival of yellow mealworm larvae.

Tenebriodes mauritanicus, the cadelle

T-36: Six 20-egg samples were placed in otherwise empty Petrie dishes and irradiated at six doses by conveyor method. The eggs were examined periodically for a period of 40 days. The expected time for the eggs to eclose is about 10 days; no eclosion was observed in any sample.

T-16: Twelve samples of about 23 larvae each in about 100 grams of wheat were spread on trays, covered with polyethylene, and irradiated at four doses by conveyor method, three replications at each dose. Counts were made every other day for 18 days and a final count was made 24 days after irradiation. Table 39 gives the percent survival (replicates grouped together) as a function of time. The data suggest that the radiation has a salubrious effect on the larvae, but an analysis of variance of the survival percents on the 8th, 12th, and 18th days shows that, although the difference between survival of the control and irradiated samples is significant on the 8th day, the differences are not significant on the other two days. A plot (not given) of the percent survival against time for the control shows a more or less linear decrease up to about the 16th day; the decreases in the irradiated samples are roughly linear and grouped together; however, as stated, the scatter among samples was too wide to show statistical significance beyond the 8th day. Seven thousand rep makes no appreciable reduction in live larvae compared with the control.

TABLE 39
PERCENT SURVIVAL OF IRRADIATED CADELLE LARVAE

Dose (rep)	Days after irradiation										
	0	2	4	6	8	10	12	14	16	18	24
0	100	100	100	94	85	75	66	54	49	42	39
3,000	100	100	100	97	97	91	87	76	71	65	49
5,000	100	100	97	97	91	91	85	79	66	58	37
7,000	100	100	100	100	99	96	80	71	68	65	52

T-23: Six samples of about 20 larvae each in 150 grams of wheat were spread on trays, covered with polyethylene, and irradiated at five doses by conveyor method. Periodic counts were made until the 15th day after irradiation; two final counts were made much later. Table 40 gives the percent survivals as a function of time after irradiation; the control figures are the averages of two samples.

TABLE 40
PERCENT SURVIVAL OF IRRADIATED CADELLE LARVAE

Dose (rep)	Days after irradiation									
	0	3	5	7	9	12	15	50	67	
0	100	100	95	90	88	82	68	25	12	
10,000	100	100	100	100	90	90	70	5	0	
20,000	100	95	95	90	86	81	81	0		
40,000	100	94	94	94	89	61	28	0		
60,000	100	100	100	89	74	37	16	0		

Cannibalism is the rule among the cadelle larvae. The high mortality of the control larvae (and, unhappily, some of the irradiated larvae) was probably caused by the cannibal habit rather than other possible sources such as rough handling or unsuitable

habitat. Sometimes it was possible to catch the larvae in the act, but that evidence alone was not sufficient to establish cannibalism in any particular instance because the attacked larva may have been dead before it was attacked and eaten. If constant mobility and constant appetite are assumed for individual larvae, then the number of larvae dying of cannibalism will be proportional to the number of larvae present, provided a fixed volume of grain is available for movement. The volume of grain available for movement in T-23 was about 50% greater than that in T-16, and the number of survivors was uniformly higher. Also, if a loss of appetite is assumed for irradiated larvae, then the higher initial survival can be qualitatively accounted for. The order of death, if sigmoid, will be different for irradiated larvae -at least for those irradiated at lethal doses.

T-25: Eight 20-larvae samples in 15 grams of flour were placed in small Petrie dishes, covered with polyethylene, and irradiated at two doses by stationary method, four replicates at each dose. The results of periodic counts are given in Table 41. An analysis of variance of the number of survivors at each count shows significant differences at the 5% level or higher for each count except that on the 8th day, which is near the cross-over point on the survivor curves. Figure XVI is a linear plot against time of the percent survival. The orders of death are different.

These data, from the three larva tests, show the decrease in time before death with an increase in dose; however, the separation of radiation effects is not possible without some knowledge of the natural mortality of these larvae in the absense

of cannibalism. Ten thousand rep was ultimately lethal to all of 20 larvae, and all higher doses were also lethal to all the larvae treated. However, if 10,000 rep were to be used as a deinfestation dose, a practical question enters the picture as to whether, although the irradiated larvae are about to die, it is economical to permit them to live as long as 67 days before they die. The cadelle larvae are nibblers; instead of spending their lives in one kernel of grain, like many of the beetles, they wander about in the grain eating small portions of many kernels. On the other hand, it may be that the irradiated larvae eat little or nothing. One phenomenon frequently encountered in the examination of dead and dying irradiated cadelle larvae was the presence of an anal exudate, suggestive of internal breakdown of the system.

TABLE 41

NUMBER OF SURVIVORS OF REPLICATE SAMPLES OF
IRRADIATED AND NON-IRRADIATED CADELLE LARVAE

Days	Control				Mean (%)	100,000 rep				Mean (%)
0	20	20	20	20	100	20	20	20	20	100
2	18	18	16	16	85	20	20	20	19	99
5	13	13	13	9	60	17	18	14	14	79
8	12	11	11	6	50	6	6	6	11	36
15	7	5	4	5	26	1	0	1	1	4
22	6	5	3	3	21	0		0	0	0

As a matter of interest, Fig. XVII, a plot of the logarithm of percent survival of non-irradiated larvae against time, is offered in support of the contention that cannibalism proceeds according to a logarithmic function, and therefore, can be differentiated from radiation death.

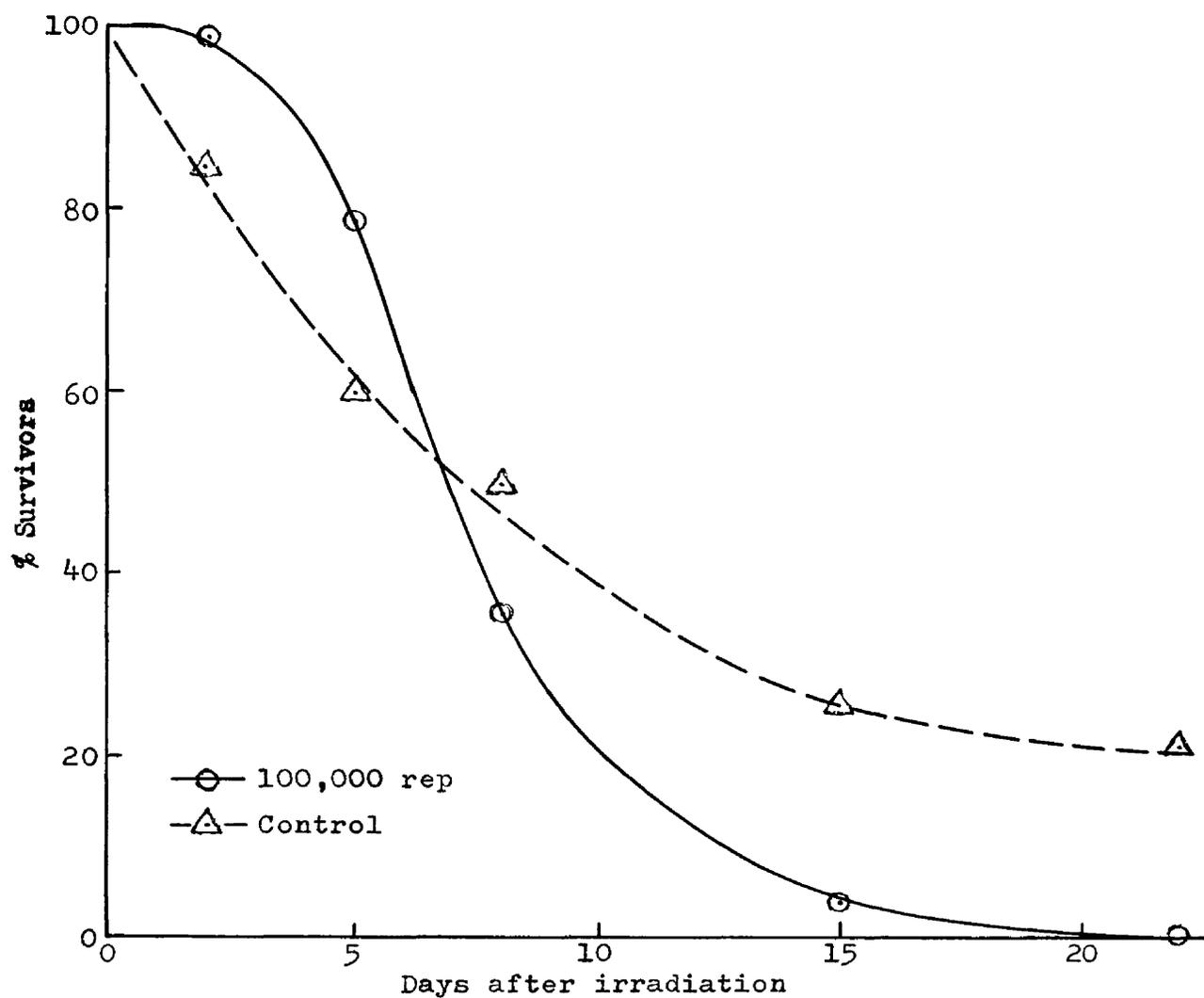


Fig. XVI. Survival of irradiated and non-irradiated cadelle larvae.

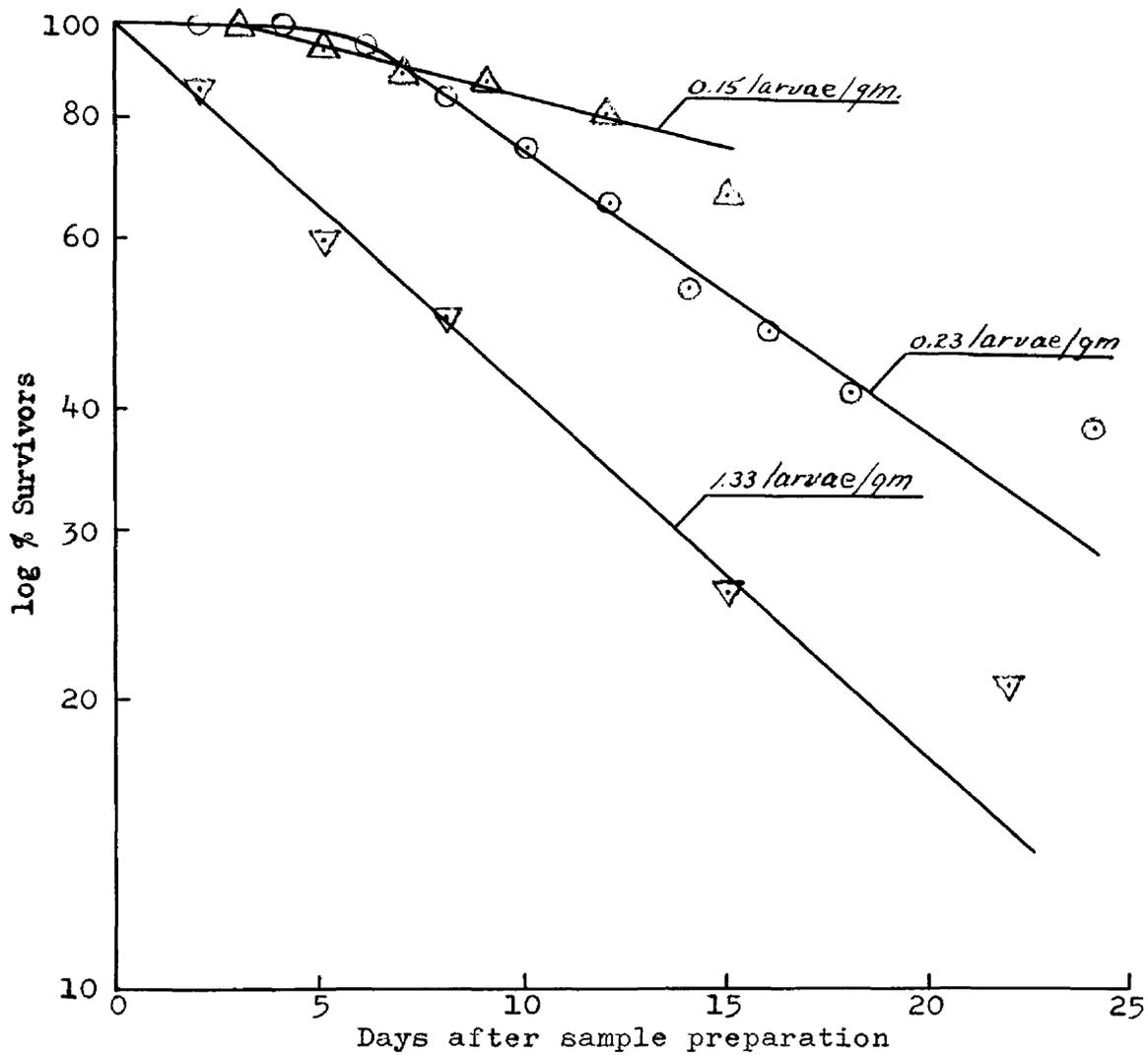


Fig. XVII. Survival of non-irradiated cadelle larvae in various amounts of food.

Tribolium confusum, the confused flour beetle

T-2: Forty-eight 13-gram samples of flour in which adults had oviposited for two days were irradiated in small, uncovered Petrie dishes at six doses by stationary method. To half of the samples, about 20 grams of flour was carefully added on top of the infested flour to determine the protective effect of the added flour. The samples were counted on either the 39th or 40th day after irradiation. Twelve of the samples contained some larvae (no more than two in any sample), and four samples contained some pupae (no more than three in any sample). These larvae and pupae were counted as members of the same generation as the adults. Many of the samples contained dead adults, but no more than four in any sample. The final counts given in Table 42 are the sums of live and dead adults, larvae, and pupae.

TABLE 42

NUMBER OF SUBJECTS FROM REPLICATE SAMPLES
OF IRRADIATED CONFUSED FLOUR BEETLE EGGS

Dose (rep)	13 grams flour								33 grams flour							
	Replicates				Sum	Mean	%	Replicates				Sum	Mean	%		
0	176	172	146	154	648	162	100 ¹	175	183	182	164	704	176	100 ¹		
500	161	173	160	162	656	164	97.2	155	165	188	134	642	160	95.1		
1,000	77	88	87	86	338	84	50.1	161	165	166	172	664	166	98.4		
1,500	41	42	32	43	158	40	23.4	136	139	169	145	589	147	87.3		
2,000	16	28	27	27	98	24	14.5	120	136	141	133	530	132	78.5		
4,000	0	3	5	3	11	3	1.6	120	118	120	109	469	117	69.2		

¹based on the average of all eight control samples

The analysis of variance shows a significant difference in emergence among all doses with 13 grams of flour except between control and 500 rep; the samples containing 33 grams of flour are not different through 1000 rep, but the 4,000-rep sample is different from the 1,500-rep sample, the 2,000-rep sample is different from the 1,000-rep sample, and the 1,500-rep sample is different from the 1,000-rep sample. The mean difference for significance at the 5% level is about 15 insects.

The differences in percent emergence between the two amounts of flour provide one means of checking the adequacy of the depth-dose curve (Appendix C). Thirteen grams of flour in an 8.9-cm diameter Petrie dish corresponds to about 0.26 cm equivalent to a density of 1 gm/cm^3 (based on an assumed density of flour of 1.2 gm/cm^3); 33 grams of flour corresponds to 0.65 cm-equivalents. Since the added 20 grams of flour was placed on top of the infested portion, the infested flour must lie between 0.39 and 0.65 cm-equivalents. According to the derived depth-dose curve, the maximum ionization will be nearly zero; that is, complete protection. But the data show a significant reduction in emergence even with the added flour. At 4,000 rep (33 grams of flour) the emergence corresponds to the emergence at about 750 rep with 13 grams of flour. Therefore, some adjustment to the depth-dose curve should be made.

T-5: Six samples of about 50 larvae each were placed in 10 grams of flour in small, uncovered Petrie dishes and irradiated at six doses by stationary method. Counts were made of the samples immediately after irradiation and any dead larvae were removed.

The one dead larva found in the 2,000-*rep* sample was assumed to have been dead at the time of irradiation. Table 43 gives the results of counts made immediately after irradiation, 0 days, and the counts made 18 days after irradiation.

TABLE 43
SURVIVAL OF IRRADIATED CONFUSED FLOUR
BEETLE LARVAE (LIVE/DEAD)

Dose (<i>rep</i>)	0 days	18 days			Total subjects
	Larvae	Larvae	Pupae	Adults	
0	48/0	0/2	0/0	38/0	38/2
1,000	49/0 ¹	1/0	3/0	36/1	40/1
1,500	41/0 ¹	0/0	1/0	46/0	47/0
2,000	48/1 ²	2/1	23/1	12/5	37/7
4,000	46/0 ²	0/22	3/16	2/2	5/40
7,000	45/0	3/36	0/2	0/1	3/39

¹7 pupae

²2 pupae

The expected larval period for this species is given as 31 days. The fact that all but one larva in the control and 1,000-*rep* samples had become adults by the 18th day after irradiation gives a lower limit on the age of the larvae. There are discrepancies between the total number of subjects counted initially and the number found at the end of 18 days. These discrepancies can be attributed to poor counting technique; moreover, they make a quantitative interpretation of the data difficult. A final count was made 27 days after irradiation. Table 44 gives the results of the final count. The percent of larvae that became adults includes all those adults found; however, at the higher doses many died before living a normal life span.

TABLE 44

SURVIVAL OF IRRADIATED CONFUSED
FLOUR BEETLE LARVAE (LIVE/DEAD)

Dose (rep)	Adults at 27 days	Percent Adults ¹
0	38/0	79
1,000	41/0	89
1,500	44/1	98
2,000	28/4	70
4,000	2/3 ²	15
7,000	0/0 ²	2

¹based on highest number of
adults found

²three dead larvae

T-11: Seven samples of about 50 larvae each were prepared and irradiated in the same manner as the previous larva test, T-5, except that different doses were applied. Table 45 gives the counts made 28 days after irradiation. The results of this test are comparable to those of T-5; however, no adults emerged in the later test at doses above 4,000 rep. Generally, as the dose increased, the subjects died at an earlier stage of development.

TABLE 45

SURVIVAL PATTERN OF IRRADIATED
CONFUSED FLOUR BEETLE LARVAE

Dose (rep)	Number (live/dead)			%Adults
	Larvae	Pupae	Adults	
0	0/1	0/0	47/2	98
2,000	0/3	0/2	10/28	89
3,000	0/39	0/11	0/5	9
4,000	0/11	0/40	0/0	0
5,000	0/8	0/42	0/0	0
7,000	0/50	0/1	0/0	0

T-10: Six samples of about 50 pupae each were placed in 10 grams of flour and irradiated in small, uncovered Petrie dishes at six doses by stationary method. The samples were examined 29 days after irradiation; the results are given in Table 46. The data show two aspects of death by irradiation. First, the increase in failure to reach the adult stage with increasing dose, and, second, the increasing mortality of those insects that did become adults. These data are simply another illustration of the delayed nature of radiation effects.

TABLE 46

EMERGENCE AND SURVIVAL OF IRRADIATED CONFUSED
FLOUR BEETLE PUPAE (NUMBER OF ADULTS)

Dose (rep)	Adults		Dead pupae	% Adults ¹	% Adults ²
	Live	Dead			
0	38	3	0	100	93
2,000	25	10	0	100	71
3,000	10	16	22	54	21
4,000	4	35	10	80	8
5,000	0	32	20	63	0
7,000	0	25	25	50	0

¹based on adults that emerged

²based on live adults 29 days after irradiation

T-17: Three 50-pupae samples of confused flour beetle were placed in about 10 grams of flour in small, uncovered Petrie dishes and irradiated at three doses by stationary method. Daily counts were made of the beetles and a record of their progress toward the adult stage was made in an effort to determine, in more detail, the order of irradiation death. The several landmarks in their development reported are: reaching the adult stage with subsequent death;

reaching a pre-adult stage characterized by freedom of the legs and antennae but otherwise still in the pupa case; and death in the pupa stage. The history of the control sample is given in the following scheme in which the number of adults that emerged on the stated day after irradiation are given.

	Control						
Days:	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
Emerged:	0	0	1	3	38	3	0

Five of the fifty control pupae failed to reach the adult stage. Three of these died in the pre-adult stage and the other two died in the pupa stage. The average time of the controls to emerge was five days after irradiation; the expected pupal period of the species is 8.2 days (one authority gives 3-5 days), so that, presumably, these pupae were in the earlier part of the pupa stage. Of the 45 that emerged, two died before the end of the test (12 days after irradiation); one lived two days, the other nine days.

The history of the 5,000-rep sample is given below.

	5,000 rep					
Days:	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
Emerged:	0	0	0	3	1	0

Of these four adults, two died six days after emergence, one five days, and one eight days. Six of the pupae died without any obvious change in development. The remaining 40 pupae reached the pre-adult stage at times after irradiation shown in the following scheme, and subsequently died by the end of 10 days after irradiation.

	5,000 rep									
Days:	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>
Pre-adults:	0	0	0	0	16	10	9	5	0	0

The data suggest that the time of what might have been emergence

has been prolonged by the treatment. No record was made of the time at which these pre-adult pupae died.

The history of the adults that emerged from pupae irradiated at 10,000 rep is given below.

	10,000 rep				
Days:	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
Emerged:	0	0	2	4	0

Of these six adults, four lived 7 days after emergence, one lived six days, the last, one day. The average length of life is just barely less than the average length of life of the four adults in the 5,000-rep sample; however, there is no significant difference between the two averages. Thirteen of the pupae died in the pupa stage; the remaining 31 died in the pre-adult stage, which they reached at times shown in the following scheme.

	10,000 rep								
Days:	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>
Pre-adult:	0	0	0	0	6	17	5	3	0

There is no significant difference between the times at which the 5,000-rep and 10,000-rep pupae reached the pre-adult stage, although the average time for the 10,000-rep sample is slightly longer. The only difference between the two treated samples is in the number of pupae that showed any development at all, and, in the absence of replicate samples, there is no way to test the difference for significance. The results are summarized in Table 47. The results of this test are in only general agreement with those of test T-10, the first pupa test. The number of adults surviving was the same at 5,000 rep in both tests, but the number that became adults was considerably smaller in the second test. Whether the few adults that did emerge were fertile was not investigated.

TABLE 47
EMERGENCE AND SURVIVAL OF IRRADIATED
CONFUSED FLOUR BEETLE PUPAE

Dose (rep)	Pre-adults	Number of Adults ¹	Adults ²
0	45	45	43
5,000	44	4	0
10,000	37	6	0

¹emerged

²alive at end of test

T-3: Six samples of about 150 adults each were placed in small, otherwise empty Petrie dishes and irradiated at six doses by stationary method. These adults were placed in uninfested flour for three days and then removed. The samples in which the irradiated adults remained for three days were counted 41 days after irradiation and, therefore, 38 days after egg laying ceased. Table 38 gives the number of second generation adults from eggs laid by adults irradiated at the stated dose, corrected, proportionally, for the number of adults.

TABLE 48
EMERGENCE FROM EGGS LAID
BY IRRADIATED CONFUSED
FLOUR BEETLES

Dose (rep)	Number adults	Percent
0	569	100
1,000	283	48.5
1,500	201	35.4
2,000	108	18.5
4,000	8	1.4
7,000	2	0.36

These same adults were again observed 70 days after irradiation and, therefore, 47 days after having been transferred to the jars now to be discussed, time enough for second generation adults to appear. The single live beetle irradiated at 7,000 rep was killed at the 25-day count so that no results were obtained at that dose. The number of adults irradiated at 4,000 rep was reduced to 132 live adults; no larvae, pupae, or skin casts were found in that colony's jar. The remaining four jars were not counted, but numerous second generation larvae and pupae were noted. The jars of flour in which these irradiated adults had lived for twenty days (the 3rd to 23rd days after irradiation) were examined 74 days after the adults were irradiated and, therefore, 51 days after the adults were removed. The 7,000-rep jar contained 2 adults, no pupae, no larvae, and no accumulation of castoff skins. The 4,000-rep jar contained no adults, larvae, pupae, or castoff skins. The remaining four colonies were vigorous. In the three days of egg laying immediately after irradiation, the adults irradiated at 4,000 rep produced enough eggs to yield eight second generation adults; in the same period, the 7,000-rep adults produced two second generation adults. During the next 20 days of egg laying, the 4,000-rep adults produced no second generation; the 7,000-rep adults produced two adults. However, since this latter jar contained no castoff skins, and since the jars were uncovered, and since the also-uncovered granary weevil jars of a concurrent test contained confused flour beetles, the possibility of secondary infestation must be entertained. That these adults could lose fertility after irradiation was not expected at the time of this

test, but in the light of the results of the tests with the lesser grain borer and the granary weevil, such a conclusion is indicated.

T-4: Five samples of about 50 adults each were placed in otherwise empty, small Petrie dishes, covered with Saran Wrap, and irradiated at five doses by stationary method. Periodic counts of the number of survivors are given in Table 50. These results are in agreement with those of the previous adult test, T-3, in which one of 144 adults irradiated at 7,000 rep survived, and 136 of 150 irradiated at 4,000 rep survived. The counts were not made often enough to give a detailed picture of the order of death.

TABLE 50

SURVIVAL OF IRRADIATED CONFUSED
FLOUR BEETLE ADULTS
(NUMBER OF ADULTS)

Dose (rep)	Days after irradiation				
	0	1	10	14	22
0	50	50	49	48	48
4,000	49	49	45	45	45
5,000	49	49	45	38	38
6,000	53	53	25	3	1
7,000	52	52	16	1	0

T-7: Two hundred eighty-four adults were placed in an otherwise empty, small Petrie dish, covered with Saran Wrap, and irradiated at 10,000 rep by stationary method. These beetles were counted every other day until all were dead. Figure XVIII is a plot on probability paper of the percent survivors against time. No beetles died until the sixth day after irradiation; all were dead by the 18th day after irradiation. After each count, the remaining live adults were transferred to fresh flour; these flour samples

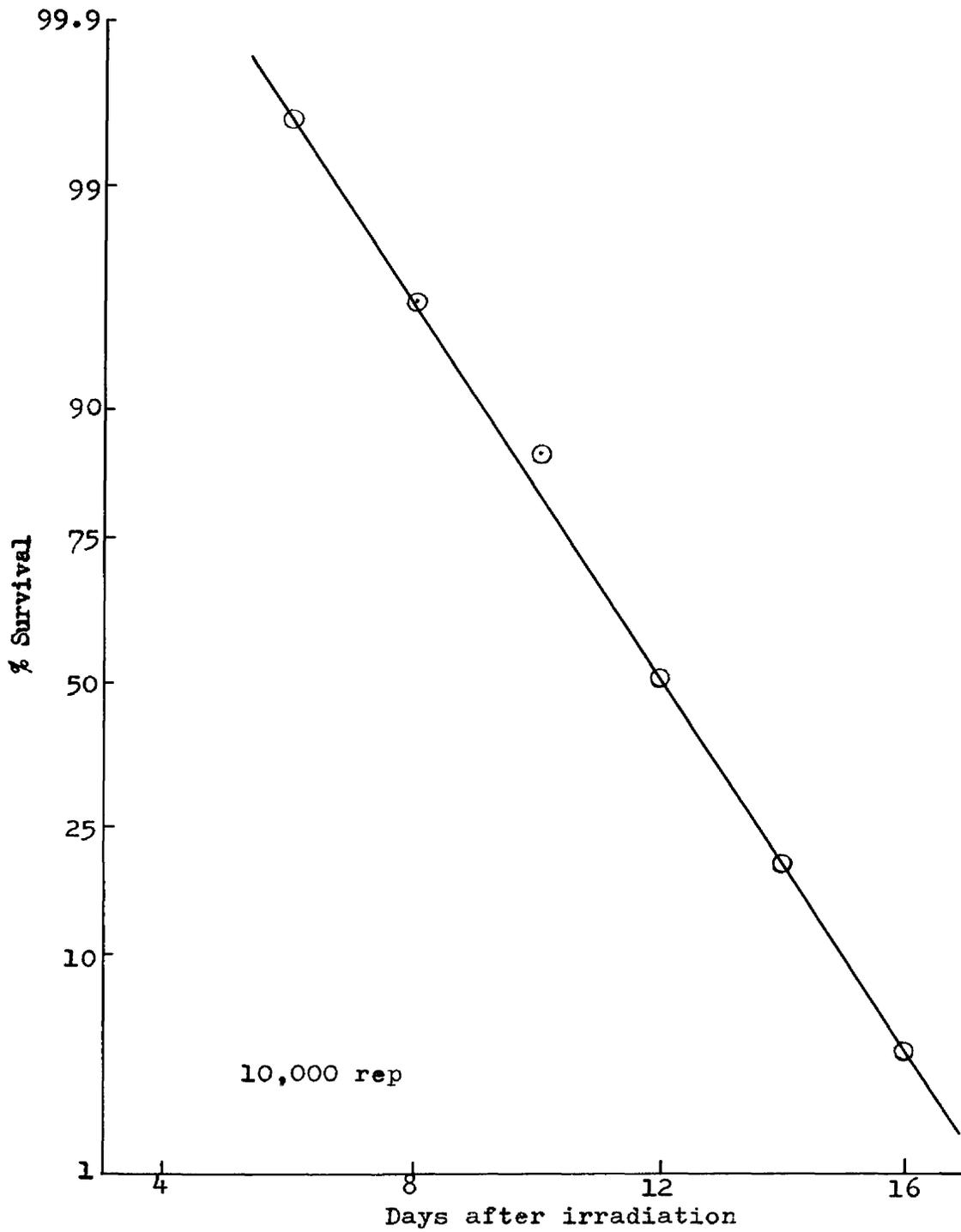


Fig. XVIII. Order of death of irradiated confused flour beetle adults.

were saved and examined 42 days after the adults were irradiated. No adults, pupae, larvae, or castoff skins were found in any of the samples. Unfortunately, no control was run concurrently with this test; however, a separate test, T-59, was run much later with 100 adults. Since the environment was not the same, this control is not strictly comparable with the 284 irradiated adults. The T-59 group was counted and transferred three times a week until the 17th day after the test began. Two beetles died in the course of the test; each jar into which the adults were transferred after each count was found to contain a vigorous colony of second and third generation beetles.

T-37: A 2-kilogram culture of whole wheat flour heavily infested with all life forms of confused flour beetle was irradiated at 10,000 rep. The culture was spread on trays to a depth of about 3 mm, the trays were covered with polyethylene and irradiated by conveyor method. This culture was observed casually every few days. At the end of about two weeks, activity was apparently at an end; however, by the 65th day after irradiation, adults were again observed, and by the 72nd day the colony was again thriving. Ten thousand rep was expected to destroy all the life forms of this species. The length of time for the colony obviously to recover is nearly long enough for two generations of beetles. The method by which the culture was irradiated does not preclude the possibility of a sub-lethal dose having been delivered to some of the subjects; moreover, if the order of death is truly log-normal, then probability considerations alone permit a few survivors in such a heavy infestation.

The results of the tests on the life forms of the confused flour beetle are given in Fig. XIX, a log-normal plot of the number of adults emerging from the given irradiated metamorphic form. These data are, in the case of the larvae, for instance, the percent of subjects that reached the adult stage, whether they lived a normal life span or not. As has been shown above, even though a particular irradiated life form reaches the adult stage, the probability of that adult living a normal life span is less than for non-irradiated subjects. The data show a general increase in resistance with development, based on the LD_{50} , which reads from egg to adult: 980, 2500, 4800, and 5000 rep. The LD_{50} for sterility, as measured by the number of second generation adults from eggs laid within the first three days after irradiation of the parents, is 1100 rep.

In cases in which the number of subjects was small, and in which the tests were performed on subjects of unknown, mixed ages, the scatter of points is large. The two cases (eggs and fertility) in which the number of subjects was larger (order of 500 controls), showed much less scatter. These data lend credence to the notion of a log-normal order of death. The failure of the 500-rep point for the egg test, T-2, to fall on the line perhaps deserves special comment. The most obvious cause for poor fit is that the log-normal law is not the law of death by irradiation; however, if the log-normal law is correct, then the poor fit may be caused by a systematic error in dose measurement, or a failure of the law at low doses, possibly because of a repair phenomenon. The exposures at 500 rep were of 2.5 seconds duration. A finite shutter opening

time could conceivably introduce a systematic time error. A nearly 50% reduction in the stated dose would be required to put the 500-rep point on the line.

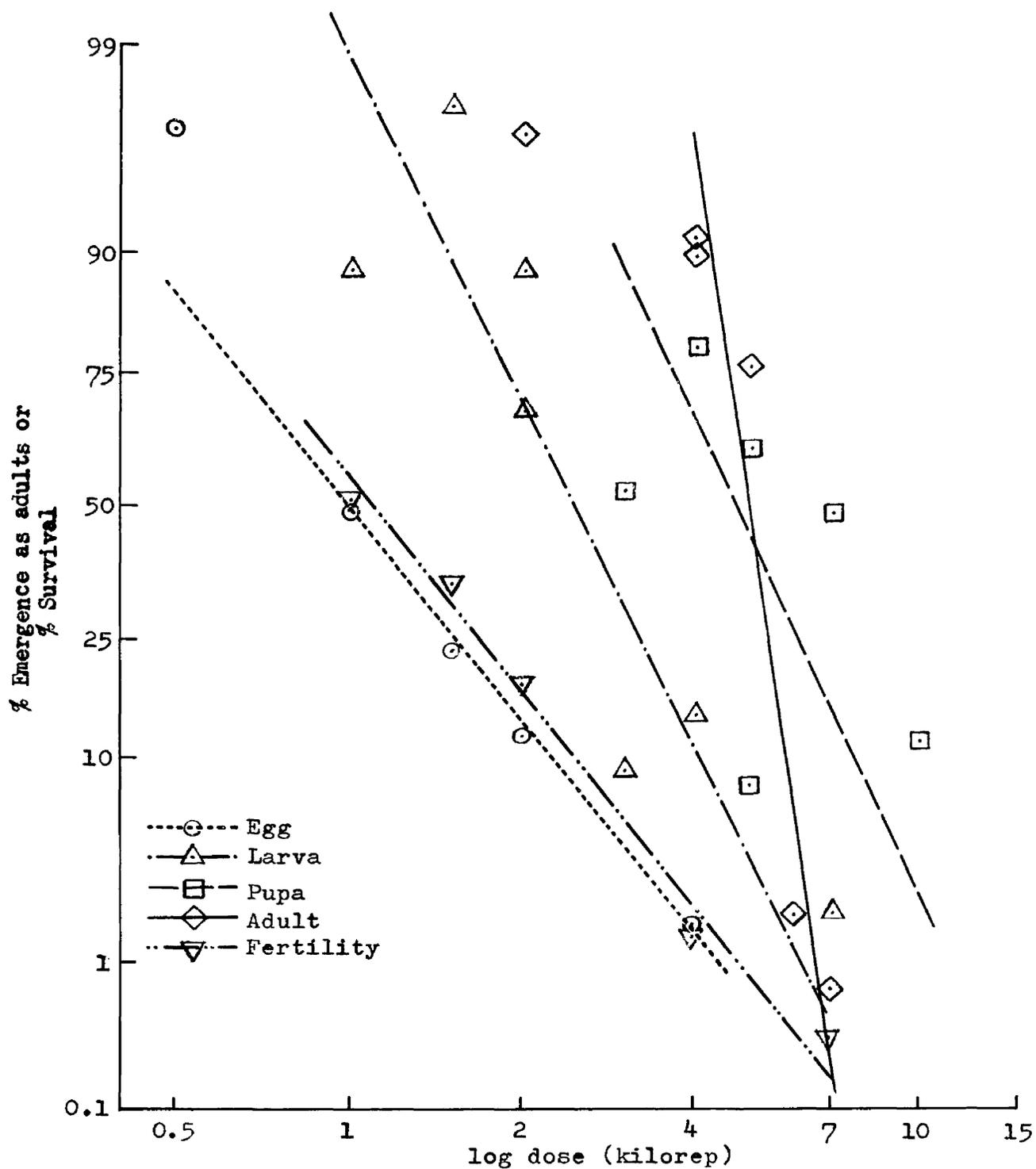


Fig. XIX. Emergence or survival of the metamorphic forms of irradiated confused flour beetles.

Mites

Four tests were made with mites. Because these animals can be, and are, a nuisance, the author did not trust himself to handle and observe these colonies, and he preferred to have an entomologist examine the samples, rather than run the risk of careless, amateur handling. For this reason, only portions of vigorous colonies, without regard to life form, were irradiated; moreover, the only method of evaluation was microscopic observation for live mites. The species of mite, other than that all four tests were performed with the same species, is not definitely known; however, the probable identification is Acarus siro (L.). All four tests were made in about the same way. Five grams of flour containing mites and five grams of uninfested flour placed in a small Petrie dish constituted a sample. The dishes were all covered as tightly as possible with Saran Wrap and, except when irradiated and observed, covered with regular Petrie dish covers. Periodic observations were made, and the result of each observation was either a yes (live mite observed) or a no (no live mite observed).

T-35: Six samples were prepared as described above and irradiated at six doses by conveyor method. The results are given in Table 51. No effort was made to estimate the strength of the samples.

Can one conclude that the dose required to kill mites lies between 60,000 and 90,000 rep? Since the colonies were kept for only 17 days there is no knowledge of 1) whether the colony treated at 60,000 rep would have succumbed after 17 days or, 2) whether the colony treated at 90,000 rep would have eventually shown life.

TABLE 51
SURVIVAL OF IRRADIATED
MITE COLONIES

Dose (rep)	Days after irradiation			
	4	7	12	17
0	+ ¹	+	+	+
30,000	+	+	+	+
60,000	+ ²	+	+	+
90,000	-	-	-	-
150,000	-	-	-	-
250,000	-	-	-	-

¹live mite

²no live mite noted

T-40: Twelve mite samples were prepared and treated in the same manner as the previous test, T-35, at four doses, three replicates at each dose. The samples were observed periodically up to 29 days after irradiation. The highest dose given, 60,000 rep, as well as all lower doses, failed to kill all the mites in any sample.

T-45: Four mite samples were prepared and treated as in the previous two tests at four doses. The results of the periodic observations are given in Table 52. Although these dishes were covered with Saran Wrap and, except as noted above, with glass Petrie dish covers, the results at 90,000 rep of this test and T-35 suggest reinfestation in the 90,000-rep sample of T-45.

T-49: Twelve mite samples were prepared and treated at four doses, three replicates at each dose. The results of weekly counts are given in Table 53.

TABLE 52
SURVIVAL OF IRRADIATED
MITE COLONIES

Dose (rep)	Days after irradiation			
	7	18	22	42
0	+ ¹	+	+	+
60,000	+	+	+	+
75,000	- ²	-	-	-
90,000	-	+?	+?	+

¹live mite

²no live mite moted

TABLE 53
SURVIVAL OF REPLICATE SAMPLES
OF IRRADIATED MITE COLONIES

Dose (rep)	Weeks after irradiation			
	1	2	3	4
0	+ ¹	+	+	+
	+	+	+	+
	+	+	+	+
80,000	- ²	+	+	+
	+	+	+	+
	+	+	+	+
100,000	-	-	+	+
	-	+	+	+
	-	-	+	+
200,000	-	-	-	+
	-	-	+	+
	-	-	+	+

¹live mite

²no live mite noted

Subsequent to this series of mite tests, nearly every insect sample in the rearing room became infested with mites. The conclusion that some of these data represent reinfestation seems valid. The method employed to confine the mites to their dishes was inadequate. A conclusion consistent with the results of these four tests is that the dose lethal to mite colonies of such strength is in the neighborhood of 80,000 rep, and that every mite in samples irradiated at doses higher than 80,000 rep is evidence of reinfestation. One can not conclude from the data that mites are more difficult to kill than any of the insects reviewed. These mite samples, although of comparable initial population, may very possibly have been reduced to something of the order of 0.01% of their initial number and still have appeared to be heavily infested. Because the initial and final numbers are not known, no inference can be made of the LD₅₀

Psocoptera

Two tests were made with this primitive, wingless insect in which an entire colony, without regard to life form, was irradiated. As in the mite tests, the only data collected were the answers, yes, a live psocid was seen or, no, no live psocid was seen. These samples consisted of about 10 grams of heavily infested flour.

T-44: Six colonies of book lice were irradiated in small, polyethylene-covered Petrie dishes at six doses by conveyor method. Four observations were made about a week apart. Living lice were found at every observation in the samples treated at 0, 8,000, 16,000, and 30,000 rep; no lice were found at any time in the samples treated at 60,000 and 120,000 rep. By the 23rd day after irradiation all the samples had become infested with Indian-meal moth. The samples were discarded on the 28th day.

T-46: Six samples were prepared and treated in the same way as those of the previous test, T-44. Nine observations of the samples were made extending to the 72nd day after irradiation. On no occasion were live lice found in the samples treated at 50,000, 75,000, 100,000, and 200,000 rep. Live lice were observed on every occasion in the sample treated at 25,000 rep. The control sample showed a peculiar pattern. For the four observations made between the 24th and 44th days after irradiation, not only were no live lice found in the sample, but no dead lice were found either. On the 51st day, live lice appeared again in the sample. The only reasonable explanation would seem to be that the dish became interchanged, for a time, with a similar dish containing no lice.

The results of the two tests are consistent with the conclusion that doses of 50,000 rep and higher will kill appreciable numbers of this species (identified only as to order), and that some of the species can survive doses of 30,000 rep. Fifty thousand rep would represent one upper limit on the LD₅₀, otherwise the data are subject to the same criticism as the mite data.

DISCUSSION AND CONCLUSIONS

Certain comparisons can be made with results already published. For the most part, work on insects has been done at doses many times those used in this study. In all those reports, all the subjects irradiated died. To that extent, the results of other workers is confirmed. In several instances, however, doses comparable with those herein described were used. In particular, Hassett's and Jenkins' (27) experiments included doses near those used herein; there are several instances in Baker's work (28) in which low doses were used; and the work of Davey (19) included sub-100%-lethal doses.

An attempt was made to determine the conversion factor from Davey's milliamperes-minutes to rep by comparing the corrected mortality of adults. To be sure, this comparison is very uncertain; the result of the analysis suggests that if the milliamperes-minutes are multiplied by 20, then the dose will be in rep. Once a comparison has been made on this basis, then no further comparisons with Davey's results are possible without circularity.

Baker's finding of adults from larvae irradiated at 1,000 rep is confirmed; his finding of adults from larvae irradiated at doses of 10,000, 25,000, and 50,000 rep is not disproved in this work, but the possibility of finding such is shown to be remote. The irradiation of confused flour beetles and granary weevils at 10,000 rep allowed the beetles to live for about a week. This result is confirmed. Baker found a 70% reduction in eclosion of eggs from adults irradiated at 1,000 rep and an 18% reduction in

second generation adults from these same eggs compared with the controls. The figure found in this study for the reduction in second generation adults from irradiated adults is about 50% at 1,000 rep.

Hassett and Jenkins obtained 100% mortality of rice weevils, confused flour beetles, and lesser grain borers (about 100 adults from each species) at 16,100 rep. These results are confirmed for the confused flour beetle and rice weevil based on 243 and 100 adults, respectively, at a lower dose (10,000 rep). The lesser grain borer results showed only an 85% and 98% mortality at 16,000 and 32,000 rep respectively. Proctor et al. (29) also found 100% mortality of lesser grain borer at a dose of 25,000 rep. This discrepancy is somewhat difficult to explain. If the results in the present study are correct, then high natural mortalities in the other two studies can explain the differences, but the mortality of the controls in the Hassett and Jenkins experiment was only 11% by the end of 48 days after irradiation. A systematic error in the dose could explain the differences, but the results in these two reports are in agreement and, moreover, the dosimetry of Proctor's work was based on three independent calculations. If one assumes that the error is in the present study, then a systematic error in dose measurement is more believable, but the results of work with other species would compare less well. The numbers of insects used in this test were considerably smaller, in fact, rather smaller than could be desired, but small numbers would probably accentuate the ease of kill, since the hardy beetles are rare. Possibly there is a difference in strains (sibling species)

of lesser grain borers. The criticism that the insects were placed in too much grain and, therefore, received a protective effect is inapplicable in that these adults were irradiated in the absence of food. Another point in which the lesser grain borer data do not agree are the times required for half the insects to die, 23 days at 16,100 rep (Hassett and Jenkins) compared with 19 days at 16,000 rep (this study), and 21 days at 32,200 rep compared with 14 days at a dose of 32,000 rep.

No effort was made in this study to examine the effect of dose rate, although there are differences to be expected. Davey (32) found that daily doses increased the life span of confused flour beetle adults; Hassett and Jenkins (op. cit.) found vastly reduced mortalities when a dose subsequently found to be 100% lethal was delivered over a longer period of time. These results hint at a repair phenomenon which is probably effective only within some definite limit of dose rate. In this work dose rates were not the same for each dose, nor were they the same for the same dose delivered by conveyor and stationary methods; however, these doses were all delivered in less than one minute. Further work on the influence of dose rate might reveal more information on the order of death by irradiation of insects.

The order of death has been frequently referred to. Actually, two separate phenomena have been indiscriminately grouped under this term: the scheme of death against time of a single group of insects irradiated at a fixed dose; and, the variation with dose of the number of survivors at a very long time after irradiation. Most of the studies reviewed show, for the first kind of order of

death, a sigmoid shape of the curve for each dose. All the tests in this work show the sigmoid shape. Another feature of this order of death is the variation with dose in the time taken by a fixed fraction of the irradiated insects to die. This feature was treated in detail by Davey (19); several of the other studies reviewed could be similarly analyzed. One test herein on the granary weevil showed results that are, perhaps, at variance with the other studies. In test T-73, three doses, increasing by a factor of two, showed the same slope and position of the order of death curves. Moreover, one of these curves, that for 10,000 rep, was a repeat of a previous experiment. The two 10,000-rep curves have the same slope, but the time for 50% of the insects to die varied by about four days, an appreciable fraction of the time required to die (see Fig. X). Davey found this same phenomenon, but did not comment further on it. A more thorough analysis of this aspect of the order of death might lead to some clues to protection against radiation.

A few of the general characteristics of death by irradiation and irradiation damage as confirmed by this work can be summarized: a prolongation of development periods; higher mortality among adults from irradiated immature forms; differential resistance among species and within a species depending on metamorphic form; and a suspected sex differential in the case of Angoumois grain moths. One point not studied, but one that should be before irradiation is put into practice, is the possibility of producing, through mutations, a strain of insects that are more resistant than those not previously exposed, particularly since irradiated insects will be in a rich mutation-producing environment.

Some comparisons among species and within species will be discussed before taking up the problem of deinfestation. The basis chosen upon which to make comparisons is that quantity called the LD_{50} . More properly, this quantity as used herein is the dose at which the emergence of adults from the irradiated metamorphic forms was 50% that of the controls. Therefore, the LD_{50} value for eggs, for example, obscures the fact that some eggs surely eclosed, but that the subject died in the larval or pupal stage. A true LD_{50} , a dose at which only 50% of the eggs eclose, would probably be higher. Also, the LD_{50} values reported take no account of the fact that the mortality of adults that do emerge from these irradiated immature forms is higher than that of non-irradiated insects; moreover, from the evidence of tests conducted in such a way as to reveal the fact, these adults have lower fertility. The reporting of fertility in a fashion analagous to the LD_{50} values involves two conflicting notions. First, if attention is directed to the number of adults that are capable of laying fertile eggs, then one LD_{50} value will be obtained. But, second, if the number of second generation adults produced for each first generation adult is used as the criterion, then a second, and lower, LD_{50} value will be obtained. Finally, any LD_{50} value will be to some extent a function of the analytical method used to get it. If a linear interpolation is made between values bracketing the LD_{50} , then that value will, in general, be different from an LD_{50} obtained from a log-normal plot of the data, as were all the LD_{50} 's in this study. However, the LD_{50} 's will not be appreciably different from those obtained by any other method of analysis. Had the initial

number of subjects in each test been known, then a probit analysis of the data would give the best straight lines that could be drawn from the data and, in addition, confidence intervals of the LD_{50} 's could have been determined. Nevertheless, a good notion of the probable confidence limits can be had by remarking the scatter of the points, which, after all, is partly the basis for analytical confidence limits. One advantage of the probit analysis is the ability to take into account those points for which 100% and 0% survival obtained. These points, of course, cannot be plotted on a probability scale.

Table 54 shows, in addition to the LD_{50} values, the strengths and weaknesses of this study. Nine of the 12 species only are included in the table; mites and book lice are omitted since only an entire colony was irradiated, and the cadelle is not included for reasons already discussed under that section of the data. Where a range is given in the table, rather than a single value, the significance is usually that at one (or both) of the two points either a 100% or 0% emergence was obtained, that no intervening doses were applied, and that, therefore, only one point, at best, could be plotted on a log-normal curve.

A final point to be made concerning comparisons restricted to discussion of the LD_{50} is the fact that differences in resistance revealed at the LD_{50} point may not hold at other points, and, in fact, may be reversed as to order.

A number of comparisons within a species can be made. Where the data are sufficient, the general trend shown throughout the four stages of the insect's life cycle is that higher and higher doses are required to give the same result in the adult stage.

TABLE 54

MEAN EMERGENCE AS ADULTS OF IRRADIATED
METAMORPHIC FORMS, AND STERILITY
OF IRRADIATED ADULTS (LD₅₀ IN REP)

Species	Egg	Larva	Pupa	Adult	Sterility
<u>Ephestia</u> <u>kühniella</u>	-	5,000	6-10,000	-	10-20,000 ¹
<u>Oryzaephilus</u> <u>surinamensis</u>	<2,000	2-4,000	5,000	8,000	6-8,000 ²
<u>Plodia</u> <u>interpunctella</u>	-	5-8,000	<25,000	-	-
<u>Rhyzopertha</u> <u>dominica</u>	<2,000	<4,000	-	7,400	4-8,000 ² <2,000 ³
<u>Sitophilus</u> <u>granarius</u>	1,300	2,200	-	<5,000	5-10,000 ² <5,000 ³
<u>Sitophilus</u> <u>oryza</u>	<2,000	1,200	-	<10,000	<10,000 ³
<u>Sitotroga</u> <u>cerealella</u>	7,500	8,000	-	-	10-40,000 ¹ <10,000 ³
<u>Tenebrio</u> <u>molitor</u>	-	<20,000	-	-	-
<u>Tribolium</u> <u>confusum</u>	970	2,500	4,800	5,000	4-7,000 ² 1,100 ³

¹50% pairs sterile

²fertility-sterility range

³50% reduction in second generation

The data for the confused flour show the phenomenon particularly well; from egg to larva the dose is more than doubled, and from larva to pupa the dose is nearly doubled; the adult LD_{50} dose is very close to that for pupae and the scatter of the points would not justify the conclusion that these two forms have different LD_{50} 's (cf. Fig. XIX, p. 117). The slope of the log-normal curve for adults is steeper than for any of the other forms, a fact that may be indicative of adulthood. The insect, once it reaches the adult stage, grows no more, in contrast to the other forms, in which great changes take place. The LD_{50} for sterility (confused flour beetle) as measured by the number of second generation adults, which lies close to the LD_{50} for eggs, can be contrasted with the LD_{50} for sterility as measured by the fertility-sterility range of 4,000-7,000 rep, which lies close to the LD_{50} for adults.

Similar conclusions can be drawn from the data for the sawtoothed grain beetle. As in the case of the confused flour beetle, the fertility-sterility range is in the neighborhood of the LD_{50} for adults. Other comparisons within species can be made though not with the same detail as those already discussed. The data do not show any exception to the general rule of increasing resistance with age of the insect.

The first striking result of a comparison among species is the Lepidoptera-Coleoptera comparison. Larvae, the only metamorphic form for which complete comparison can be made, show, for the three Lepidoptera, an LD_{50} range of 5,000 rep to 8,000 rep; for the five Coleoptera (the yellow mealworm must be omitted because an upper limit only is known) the range is from 1,200 to 4,000 rep.

Comparisons for the other stages cannot be made in the same way, but the data do not, in any instance, violate the conclusion that, of the species irradiated, the Lepidoptera are more resistant than the Coleoptera.

Three families are represented twice each among the species tested. For two of these, Pyralididae and Tenebrionidae, the data are too meagre to permit any comparison beyond the difference already noted between moths and beetles. The third family, Curculionidae, is represented by the rice and granary weevils, which belong to the same genus as well. Since there is no a priori reason to believe that related species must show the same resistance, it may be comforting to exhibit at least one clear difference. The LD₅₀ values for the larvae of the granary and rice weevils are 2,200 and 1,200 rep, respectively. Even though the value for the rice weevil is an extrapolated value (see Fig. XI), and even though the points for the granary weevil are widely scattered, (see Fig. XI), the data cannot be stretched to give an overlapping value for the larvae. Therefore, one difference can be shown for closely related species. Since the difference obtains, it follows that any general statements about the resistance of families, genera, and orders are suspect.

That physical size is not the only factor in determining radiation resistance can be shown by these data. The larva sizes can be divided into three groups: large, medium, and small (nearly mature larvae). To the first group belongs the yellow mealworm, to the second, the Mediterranean flour moth, Indian-meal moth, and cadelle, and to the third, all the other larvae in this

test. The largest larva,¹ the yellow mealworm, exhibited a 25% survival at the lowest dose applied (20,000 rep); no other larva had such a high resistance. The cadelle larvae, because of cannibalism, could not be analyzed. The other two members of the medium sized category have a higher resistance (as measured by the LD₅₀) than the Coleoptera larvae in the small category, but their resistance is less than or equal to that of the Angoumois grain moth, which is in the small category. And, the Angoumois grain moth larvae have a much higher resistance than any of the small Coleoptera larvae.

Since the ultimate objective of this study was the assigning of a deinfestation dose, it will be meet to examine in detail the implications of deinfestation in the light of the data presented above. Deinfestation was defined as the prevention of a second generation from the irradiated first generation metamorphic forms. There are, to be sure, other, and perhaps better, definitions of deinfestation. However, the problem of infested grain has certain differences from the use of insecticides on, for instance, a product that is in danger but once from insect attack, one in which a 99% kill would be most acceptable, and one that once grown and harvested is safe. Deinfestation of stored grain must, to be of real value, rid the grain of more than 99% of the insects present else, within a matter of a few months, the population will return to its original value, at which time re-irradiation may prove uneconomical (for example, already bagged flour). In addition, there is the aspect

¹As a matter of interest, these larvae are so large their body diameter exceeds that of the 1-Mev electron penetration.

of irradiation damage to the product itself that will be, to a great extent, cumulative and undesirable.

A complete analysis of the result to be expected of the irradiation of a grain product at a particular dose must take into account the dose, the species present, the extent of the initial infestation and the distribution of metamorphic forms of each species present, the nature of the order of death, and the storage conditions after irradiation. In most cases the initial population of a particular infestation will not be known. Without an exact knowledge of the initial infestation, one must make recommendations on the basis of expected infestation, some rough estimate of the cost of a given treatment balanced against the expected benefit, and a knowledge of the intended disposition of the irradiated product. Differences in treatment are indicated between grain that is to be consumed within two to three months and grain that may be stored for more than a year, especially where a few survivors can recontaminate an entire bin. In the case of individual packages, where inter-package reinfestation is not expected, the problem is different.

Suppose that the log-normal order of death holds and suppose that a quantity of flour is given a treatment of 7,000 rep, approximately the 99.9%-lethal dose, with a view to deinfestation of confused flour beetle. One of every thousand beetles can be expected to survive the treatment and, under favorable growth conditions, and an increase of 100-to-1, by the end of two generations, or about three months, the population will have grown to 10,000 beetles for every survivor from the irradiated adults, or an

increase of 10-to-1 for every adult in the initial population. A non-irradiated population would, under the same circumstances, have grown to 10,000-to-1. The fact that irradiated forms have decreased fertility and longevity adds a quantitatively unknown safety factor that makes the net reduction more favorable than 99.9%. The circumstance that the 99.9% levels lie, at least for the Coleoptera studied, at rather modest doses (order of 10,000 rep) would suggest 10,000 rep for most applications, but it should be born in mind that there is no assurance of killing all the insects. If a moth infestation (Angoumois) is expected, then treatments near the 99.9% point for that species (about 50,000 rep) are recommended.

The point should be raised: these low doses do not kill the insects immediately, why permit them to feed during the time it takes them to die? Several considerations enter into an answer to the question. First, although not proved in this study, nor in other studies, to the knowledge of the writer, anorexia seems to be a concomitant of death by irradiation, and, if it is, then feeding before death is a minor consideration. Also, since the treatment does not remove the insects from the product, but does immediately sterilize them, a matter of a week before death will not represent an increase in population. But, most important, other studies have shown that to produce instant death requires doses many times those recommended herein.

APPENDICES

- A. Pest names and some vital statistics
- B. Units of radiation dose
- C. Calibration of the electron-beam generator and the technique of treatment

Appendix A: Pest names and some vital statistics

<u>Scientific name</u>	<u>Family</u>	<u>Common name</u>
<u>Ephestia kühniella</u> Zell	Pyralididae	Mediterranean flour moth
<u>Oryzaephilus surinamensis</u> (L.)	Cucujidae	sawtoothed grain beetle
<u>Plodia interpunctella</u> (Hbn.)	Pyralididae	Indian-meal moth
<u>Rhyzopertha dominica</u> (F.)	Bostrichidae	lesser grain borer
<u>Sitophilus granarius</u> (L.)	Curculionidae	granary weevil
<u>Sitophilus oryza</u> (L.)	Curculionidae	rice weevil
<u>Sitotroga cerealella</u> (Oliv.)	Gelechiidae	Angoumois grain moth
<u>Tenebrio molitor</u> L.	Tenebrionidae	yellow mealworm
<u>Tenebriodes mauritanicus</u> (L.)	Ostomidae	cadelle beetle
<u>Tribolium confusum</u> Duv.	Tenebrionidae	confused flour beetle

These data were obtained from Fenton (40), Metcalf and Flint (41), Cotton (6), and the evidence presented in this work. The nomenclature is that of Fenton. The obvious inconsistencies in the development periods can probably be attributed to observations made at different temperature and relative humidity conditions. In some cases, average mill conditions were taken as a base, in others, optimum (but otherwise unspecified) conditions were the base, in the majority of cases no precise condition was specified.

Development periods, days,
unless otherwise specified;
range, average.

<u>Egg</u>	<u>Larva</u>	<u>Pupa</u>	<u>Adult lifespan</u>	<u>Number of eggs and manner of laying</u>
3-6	40	10	10 days	-
3-5 4	14	6-12 7	6-10 months to 3 years	45-285, loose, singly or clusters
4-6 3.7	36.2	6.9	12-19 days	350 or more in groups of 12-15; 200 ave.
11	30.5	6	-	300 to 500 singly or in clusters
4	19	5	7-8 months	300-400 one at a time in kernel
4	19	5	4-5 months	300-400 one at a time in kernel
7-10 4 ¹	20-24 24 ¹	5 ¹	8 days	40 ave. to 389; 60-90 singly or clusters of 20
2-3 ² 6.8	6-9 ³ 31	8.2	2-3 months	400-500 singly or in clusters
7-10 9.6	70-90; 94.5 7-14 ³	16.1	1 year average to 15-22 months	groups of 10-60, 1000 total
6.8 5	31	3-5 8.2	1 year or more	400-500

¹ minimum

² weeks

³ months

Appendix B: Units of radiation dose

The material of this appendix is taken largely from a summary article by Evans (42) which appeared in the first issue of Nucleonics in 1947. By now, over ten years later, the definitions and concepts of rep and roentgen have become common enough that the units are no longer defined in many journal articles. In fact, in some cases (cf. (29)) no distinction is made between the rep and the roentgen. The roentgen (r) is the basis of the definition of the rep.

<u>Unit</u>	<u>Symbol</u>	<u>Definition</u>
roentgen	r	"that quantity of X- or gamma-radiation such that the associated corpuscular emission per 0.001293 gm of air (1 cc of dry air at 0°C and 760 mm Hg) produces, in air, ions carrying 1 electrostatic unit of quantity of electricity of either sign."
roentgen equivalent, physical	rep	Energy lost by ionizations, produced by a primary source other than photons, in tissue, equal to the same energy loss for one roentgen in air.

That the roentgen is expressible as a dissipation energy derives from the combination of the average energy per ionization in air (32.5 electron volts) and the number of ion pairs in an electrostatic unit (2.083×10^9). The equivalent energy loss by ionization in air is, then, 83.8 ergs per gram of air. The definition takes account of dissipated energy only and nothing can be inferred as to the energy in the incident beam. The energy lost by ionization will be wavelength dependent; the energy transfer mechanism is a function of wavelength, and for hard X-rays, in the region of 1 Mev, Compton effect is the predominant mechanism. Energy loss by Compton effect in various materials is a function of electron density, and the comparative electron densities for air:water:tissue are

1.00:1.11:1.10 (17); consequently, in the case in which Compton effect predominates, the energy lost in water (and tissue, which is nearly the same) will be $83.8 \text{ ergs/gm} \times 1.11 = 93 \text{ ergs/gm}$ of water (or tissue).

As a result of the above considerations, a second definition of the rep is extant: that amount of particle radiation for which 93 ergs/gm of tissue is lost by ionization; that is, the energy loss is that equal to 1 r in tissue. The two rep units are often designated by subscripts, rep_{83} and rep_{93} .

Another quantity pertinent to radiation dosimetry is the relative biological effectiveness, RBE, defined as the ratio of r to rep doses required to produce the same effect (for example, LD_{50} doses for an insect species as determined by gamma-rays and electrons). The RBE for electrons is generally believed to be 1. The differences observed, particularly with the heavier ionizing particles such as α -particles and neutrons are connected with differences in ionizations per unit of path length.

The striking fact of radiation-induced biological effects is the small number of ionizations produced. In water treated with 1 r, for example, only about one ion pair for every 2×10^{10} molecules is produced.

References (16), (17), and (21) contain excellent, detailed discussions of RBE, effects on various tissues, and related matters.

Appendix C: Calibration of the electron-beam generator and the technique of treatment

In order to specify the dose of radiation received by material irradiated with the electron accelerator used in this work, two distinct aspects of the dosage problem must be considered. First is the variation of ionization with depth; this aspect is common to all material particles and is a function of the electron energies and the density of the irradiated material. Second is the form of the radiation field produced by the particular machine; this aspect is a function of machine construction and the geometry of applying the radiation to the sample.

Depth-dose Function

The depth-dose function for electron-induced ionization increases from the entrance surface to a maximum somewhere inside the medium and then decreases. Trump, Wright, and Clarke (43) have described a technique for obtaining the depth-dose function by recording the changes in ionization produced by successive thicknesses of aluminum placed between the electron beam and the ionization chamber. Lawton and Balwit (44) outline a similar scheme in the manual describing the characteristics of the General Electric 1-Mev electron accelerator. Their procedure was used to obtain the depth-dose function shown in Fig. XX. The two penetration scales are given to show the original data obtained with various combinations of thin aluminum sheets placed over the ionization chamber window, and to show the corresponding ionization at various depths to be expected with a material of 1 gm/cm^3 density. The second scale is a derived scale based on the assumption of an

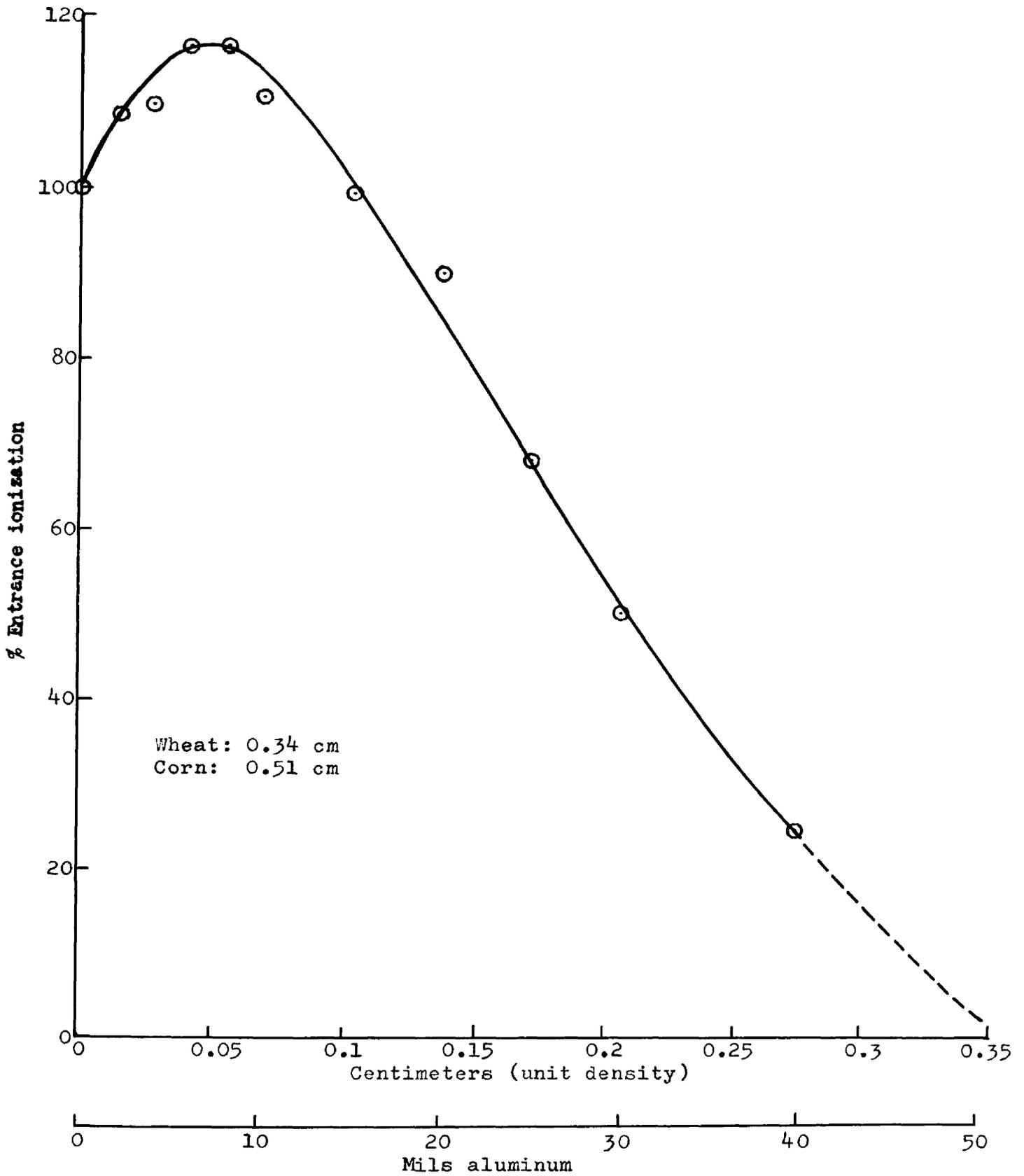


Fig. XX. Depth-dose function, MSU General Electric 1-Mev electron accelerator at 1 Mev.

inverse relation between penetration and density. Since over a small range of atomic numbers, the electron density is nearly proportional to density, the derived scale should be a fair representation. Between $Z=6$ (C) and $Z=13$ (Al), the error is about 9% according to Evans (42); when the comparisons are made on the basis of air ($Z=7.22$) the error in this range of atomic numbers is about 5%. These errors are in the relative stopping power of a medium, which decreases with Z , and, therefore, the relative stopping power of a medium of smaller atomic number than the reference medium will be relatively less and the penetration relatively more. In Fig. XX no correction has been made for this error. The atomic number of various kinds of tissue has been calculated by Lea (16) to be of the order of $Z=3.5$; it is, therefore, outside the range of 5% error reported by Evans (op. cit.). The penetrations corresponding to typical kernels of both wheat and corn are also shown in Fig. XX; these calculations are based on dimensions and specific gravities found by Zoerb (45) and were calculated on the assumptions that the smallest dimension of the kernel is parallel to the direction of the electron beam and that the moisture content of the grain is 10%. These two points must be regarded as only representative of these seeds because there will be variation with moisture content, variety, and size. The ionization chamber used in the determination of Fig. XX was furnished by the General Electric Company as part of the calibration equipment and has been described by Lawton and Balwit (op. cit.). The essential part of this chamber is its calibration in radiation units. The calibration is based solely on the volume of the chamber, the average energy for each ionization in air, and

the definition of the roentgen. Therefore, no corrections have been made for chamber geometry, scattering by the chamber window, or chamber wall material. The calculation includes the equivalence of 1 r to 83.8 ergs/gm of material. Since the definition of the rep is that amount of ionization energy equivalent to a roentgen, the large chamber constant in r/sec-microampere I_C (where I_C is the large chamber current) is numerically equal to the rep/sec-microampere I_C , but it must be remembered that the rep is here a rep_{83} . The effect of scattering is reduced by using a chamber with beryllium windows, because the scattering is least for low atomic number. Although curves analagous to Fig. XX are usually presented with the maximum ionization as 100%, the values in Fig. XX are based on entrance dose equal to 100% because the reported doses in this work are based on entrance dose calculations.

Radiation field of the MSU 1-Mev electron accelerator

The capabilities of this accelerator are: any constant, peak, electron energy, E , between 500 kv. and 1000 kv. and any beam-out current, I_{bo} , from 0 to 1000 μa . The voltage applied to the primary windings of the transformer is sinusoidal with a frequency of 180 cycles per second and, therefore, in the absence of any biasing voltage, the electron energies in the emergent beam range from zero up to the maximum (1 Mev in all these tests) with an r.m.s. voltage of $0.71E_{\text{Max}}$. The voltage is a measure of penetration only. The beam-out current is a measure of the number of electrons per second in the entire beam; the number of electrons is proportional to beam-out current. Once the generator is set at particular values of E and I_{bo} , the number of electrons per cycle (1/180 seconds)

at any one point in the field remains constant. This number of electrons will produce a fixed ionization current in the ionization chamber which can be converted to $\text{rep/sec} - \mu\alpha I_{bo}$. Therefore, the dose can be calculated by knowing the peak electron energy, E , the beam-out current, I_{bo} , the position of the sample in the field, and the time, t , spent in the field. This assigned dose value will apply only to the entrance dose, and the dose at any other point in the sample must be inferred from Fig. XX.

The generator is mounted in the radiation room in such a way that the electron beam is directed toward the floor and is perpendicular to it. Figures XXIA and XXIB show some of the electron field characteristics as determined from calibration measurements. A cylindrical coordinate system was chosen to describe the field intensity, d , the $\text{rep/sec} - \mu\alpha I_{bo}$, with origin at the center of the tube window and the positive z -axis along the beam axis. The most interesting feature of the field intensity, d , taken in a plane perpendicular to the z -axis is its conformity to a circularly symmetric normal error curve of the form

$$d(r, z_o) = A_{z_o} \exp(-r^2/2a_{z_o}^2)$$

where a_z corresponds to the standard deviation of the normal error curve, r is the radial distance from the z -axis in the z_o -plane, and A_{z_o} is the $\text{rep/sec} - \mu\alpha I_{bo}$ at z_o and $r=0$. In Fig. XXIB A_z is given in arbitrary units. Figure XXII shows a plot on arithmetic probability paper of the cumulative percent radiation received by an object passing entirely through the beam along a radius at constant velocity in the $z=47\text{cm}$ plane. From this plot a_{47} can be read directly (at the 84% and 16% points). Figure XXII is one

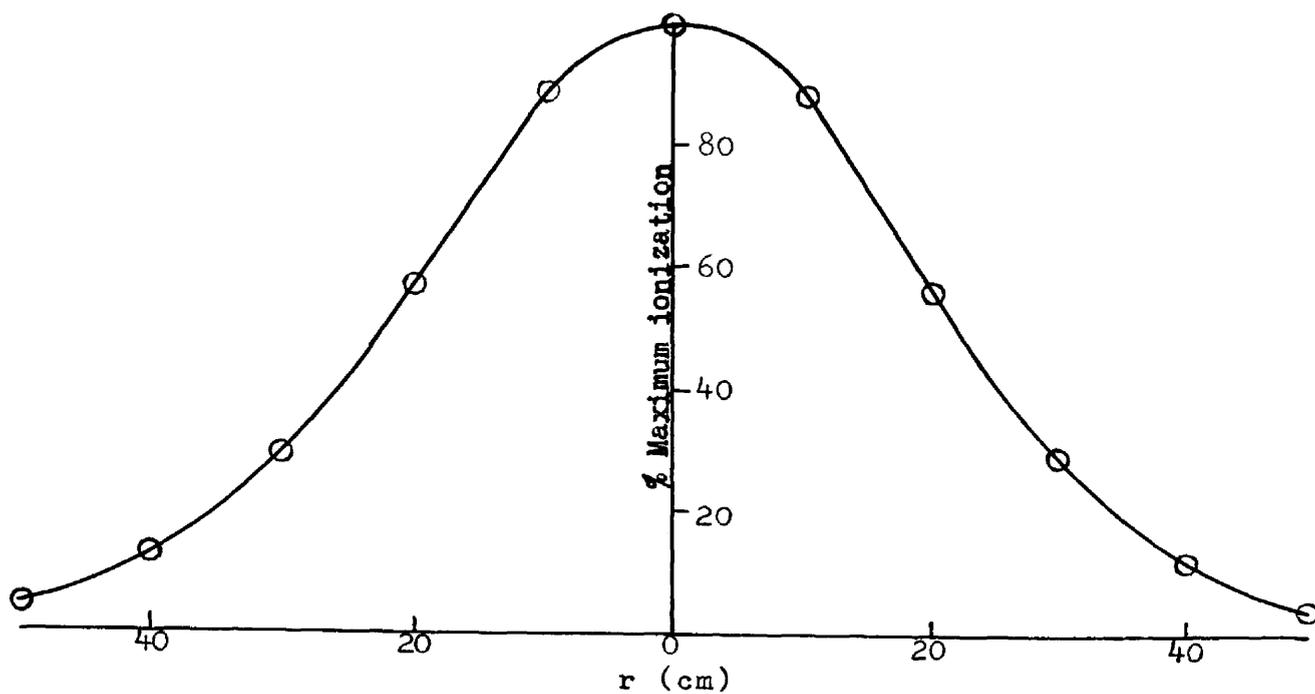


Fig. XXIa. Ionization intensity along a radius at $z=47$ cm.

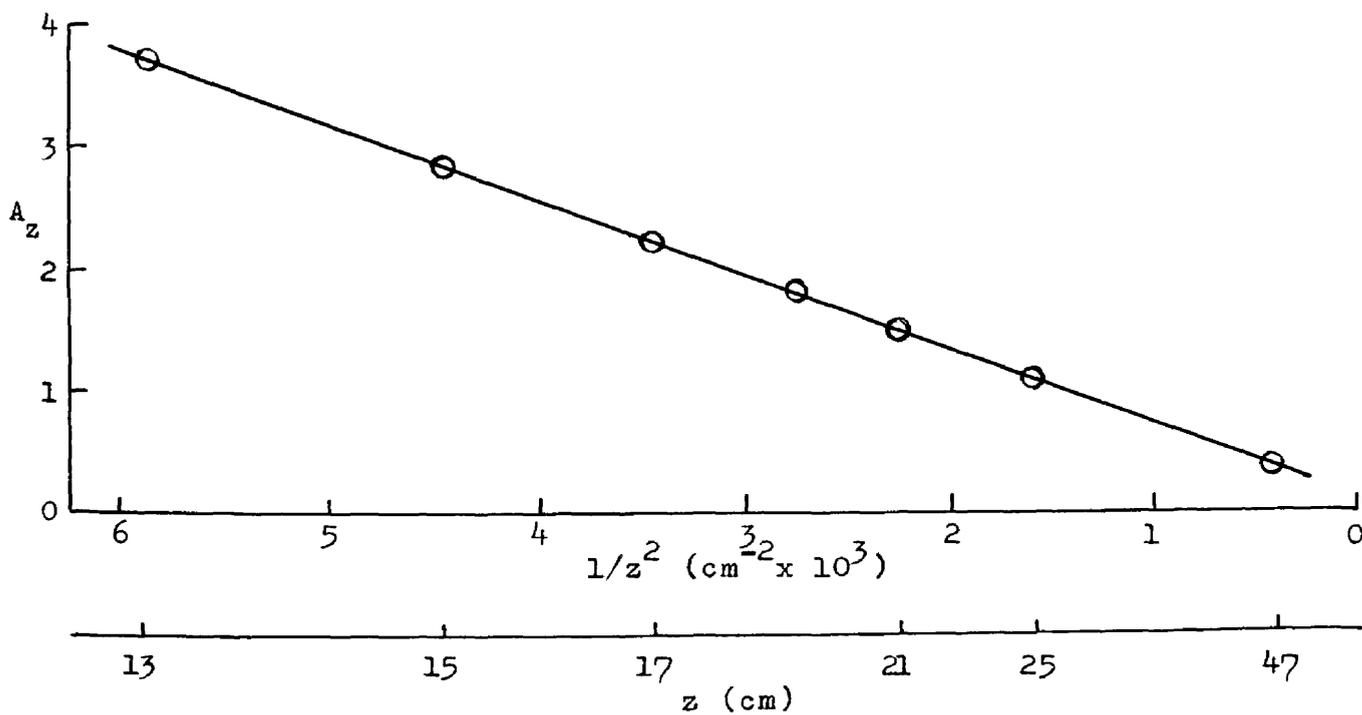


Fig. XXIb. Intensity as a function of distance from the window ($r=0$).

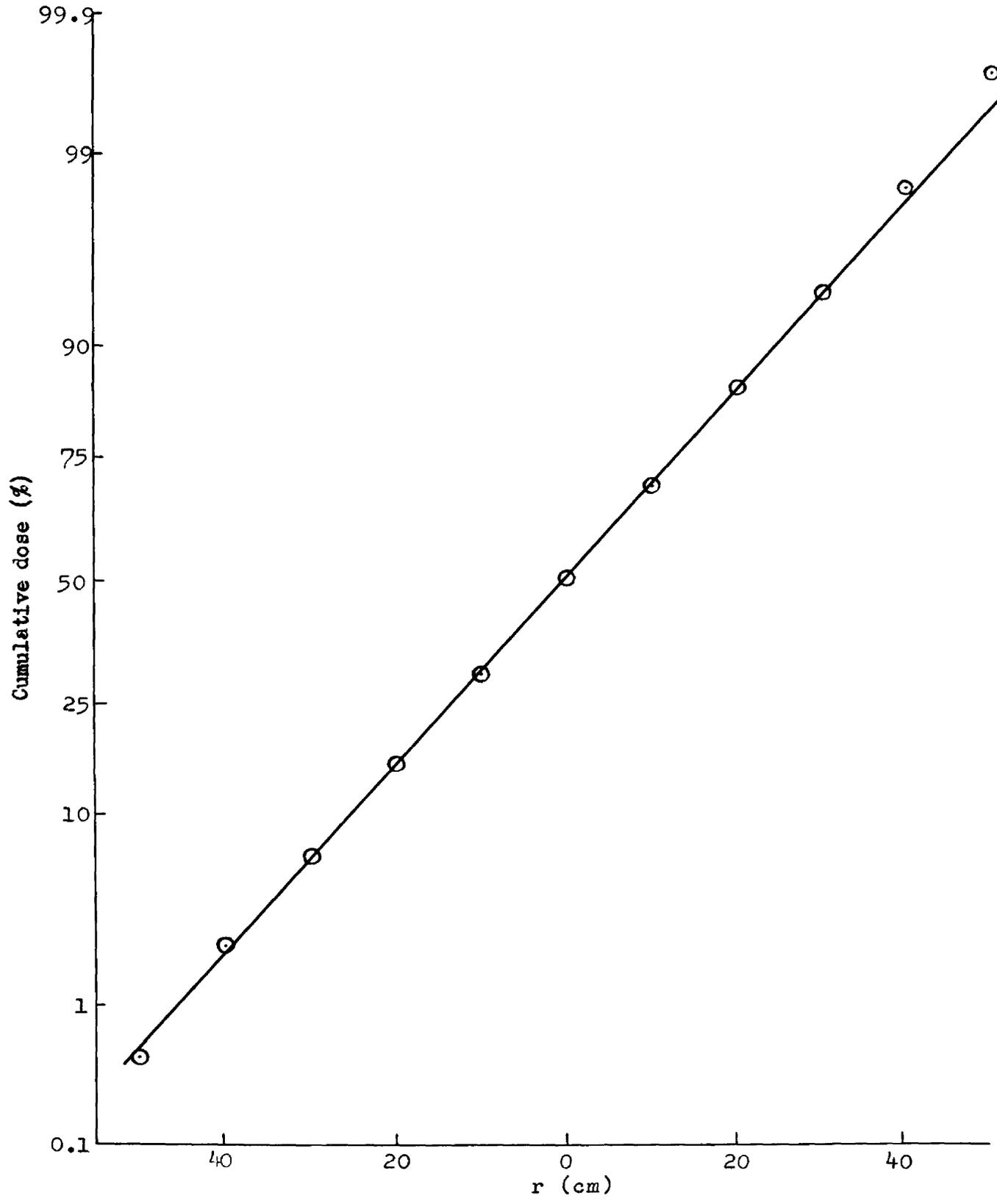


Fig. XXII. Cumulative dose along a radius at $z=47$ cm.

of six curves, all equally straight on probability paper, taken in six different directions through the beam axis in the $z=47$ cm plane, $\theta = 0, 15, 30, 45, 60,$ and 120° . These curves are the justification for the use of the normal error curve as descriptive of d and of the assumption of circular symmetry of the field.

As previously mentioned, treatments were delivered by either a stationary or conveyor method. In the stationary method the samples were placed at some fixed z with the center of the sample at $r=0$. The total dose, D , received by that part of the sample r cm from the center in time, t , is

$$D = A_z I_{bo} t \exp(-r^2/2a_z^2) \dots \dots \dots (i)$$

In the conveyor method the sample was placed on a conveyor and moved along a radius at constant velocity from one side of the measurable field to the other. The total dose received by a sample so treated traveling at velocity, v , is

$$D = (I_{bo}/v) \int_{-\infty}^{\infty} A_z e^{-r^2/2a_z^2} \delta r = A_z \sqrt{2\pi} a_z I_{bo}/v \dots (ii)$$

For stationary exposures equation (i) gives the dose received at any radial distance, r , from the sample center; the factor $\exp(-r^2/2a_z^2)$ is a measure of the decrease in dose from the center. If a circular Petrie dish of radius, R , is used, then the minimum entrance dose is

$$D_{min} = \exp(-R^2/2a_z^2) D_{Max},$$

and the average over the sample is

$$\bar{D} = I_{bo} t \int_0^R A_z 2\pi e^{-r^2/2a_z^2} r \delta r \Bigg/ \int_0^R 2\pi r \delta r$$

$$\bar{D} = 2(a_z/R)^2(1 - e^{-R^2/a_z^2})D_{Max}.$$

The above expression gives for an 8.9 cm-diameter Petrie dish (small) and a 13.9 cm-diameter Petrie dish (large) $0.998D_{Max}$ and $0.965D_{Max}$, respectively.

For 15 cm-wide trays of material passing along a radius at constant velocity, the dose along the center of the tray will be

$$D = (I_{bo} A_z/v) \int_{-\infty}^{\infty} e^{-r^2/2a_z^2} \delta r = I_{bo} A_z \sqrt{2\pi} a_z/v.$$

A dose at a distance, y , from the center of the tray measured perpendicular to the line of travel will get

$$D = (I_{bo} A_z/v) e^{-y^2/2a_z^2} \int_{-\infty}^{\infty} e^{-r^2/2a_z^2} \delta r = e^{-y^2/2a_z^2} D_{Max},$$

from equation (ii). For the trays used in these tests, $y=7.5$ cm and $D=0.92D_{Max}$.

These minima and averages are within the uncertainty of ability to set a constant beam-out current on the generator and well within the depth variation of dose. The point to be made is that this degree of uniformity of dose across the sample has been achieved at the expense of efficiency of beam utilization.

A_z and a_z were experimentally determined, A_z with the large chamber previously described and a_z with a small chamber the window of which is small enough (1/4 in.) to make more careful measurements at distances from the beam axis where the intensity varies most rapidly. The ionization currents for both chambers are linear with beam-out current, at least within the range used in the calibration. The conveyor velocity was determined by measuring the

time of travel, exclusive of starting and stopping, between two points on the conveyor rails. This conveyor is driven by a variable speed motor the dial setting of which is proportional to velocity.

The details of a calibration curve determination follow.

large chamber constant, $k = 0.359 \times 10^4 \text{ rep/sec-}\mu\text{AI}_C$

$I_C = 1.45 \times 10^{-2} I_{bo}$; $I_C = 1.32 \times 10^{-3} I_{bo}$ (at 47cm)

therefore, $I_C = 11.04 I_c$

$a_{47} = 18.7 \text{ cm}$

$v = 0.566S$ (S is the conveyor dial setting)

$$A_{47} = \frac{1.32 \times 10^{-3} \mu\text{AI}_c}{\mu\text{AI}_{bo}} \cdot \frac{11.04 \mu\text{AI}_c}{\mu\text{AI}_c} \cdot \frac{0.359 \times 10^4 \text{ rep}}{\text{sec-}\mu\text{AI}_c} = 52.2 \text{ rep/sec-}\mu\text{AI}_{bo}$$

$$D_{\text{Max}}(\text{stationary}) = (52 \text{ rep/sec-}\mu\text{AI}_{bo}) I_{bo} t$$

$$\begin{aligned} D_{\text{Max}}(\text{conveyor}) &= (52.2 \text{ rep/sec-}\mu\text{AI}_{bo}) I_{bo} (2\pi 18.7\text{cm})/0.566\text{Scm/sec} \\ &= (4.3 \times 10^2 \text{ rep}/\mu\text{AI}_{bo}) I_{bo}/S \end{aligned}$$

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