

Reproduced by

Armed Services Technical Information Agency
DOCUMENT SERVICE CENTER

KNOTT BUILDING, DAYTON, 2, OHIO

AD -

7	4	7	1
----------	----------	----------	----------

UNCLASSIFIED

AD No. ~~7471~~

ASTIA FILE COPY

423774

423779

LIBRARY OF CONGRESS
REFERENCE DEPARTMENT
TECHNICAL INFORMATION DIVISION
(NAVY RESEARCH DIVISION)

SEP 2-1952

FINAL REPORT

JUNE 1, 1952

PROJECT No. NR-117-58

CONTRACT N8onr-534 - TASK ORDER 1.

SUBMITTED BY

LEONARD B. CLARK

DEPARTMENT OF BIOLOGY

UNION COLLEGE

SCHENECTADY, N. Y.

OUTLINE.

	Page
Personnel-----	1.
I. Comparison of the biological effects of low (124 Kv.) and high (50 Mev.) voltage X-rays--	3.
II. Radiation Genetics of the Parasitic Wasp, Habrobracon----	7.
III. The Biochemical Activities of X-ray Induced Yeast Mutations-----	12.
Publications-----	16.

PERSONNEL

Nancy-Del Coons
B.A. Smith College - 1951
Major: Bacteriology
Born - Saugerties, N. Y.
U.S. Citizen
July 15, 1951 -

Gertrude Heidenthal
B.A., M.A., Mount Holyoke College
Ph.D., University of Chicago
Associate Professor; Biology Department
Russell Sage College
Major: Zoology - Genetics
Born - Carbondale, Pennsylvania
U. S. Citizen
September 1, 1951 -

Vincent P. Donnelly
B.S. Union College 1951
Major: Science
Born - Schenectady, N.Y.
U.S. Citizen
August 15, 1951 -

In charge:

Leonard B. Clark
B.A., B. Sc., M.A. University of Manitoba
M.A., Ph.D., The Johns Hopkins University
Major: Zoology - General Physiology
Born - Oakland, California.
U.S. Citizen.

Co-operating:

Co-operating in a comparative study of the depth-dose effects of low and high voltage x-radiation.

Kenneth E. Olson
B.S., University of Washington, 1929
M.D., Harvard Medical School, 1933.
Resident: Lally Institute of Pathology, 1933-34.
Intern and Resident: Surgery, Presbyterian
Hospital, New York City 1934-39.
Research Fellow: Columbia University 1939-40.
Pathologist: Olive View Sanatorium,
Seattle, Washington 1943-45.
Fellow in Oncology, Albany Medical College.
Born - Seattle, Washington.
U.S. Citizen.

Donald G. Remp
A.B., College of Wooster
Ph.D., Western Reserve University
Major: Biochemistry
Associate Professor of Biochemistry
Albany Medical College
Born - Huron, South Dakota
U.S. Citizen.

Co-operating in a study of the Biochemical
activities of X-ray induced yeast mutations.

David Paretsky
B.S. City College - New York - 1939
Ph-D. Iowa State College - 1948
Research Associate - Bacterial Section
Iowa Agricultural Experiment Station 1942-43,
1946-48.
Major: Bacterial Chemistry
Assistant Professor Biology -
Fennselaer Polytechnic Institute - Troy, N.Y. 1948
June 15, 1951 - September 15, 1951.

The project which closed 31 May 1952 under Contract N8onr-534 - Task order I. Project No. NP-117-58 is being continued with modification under A.F.C. contract (AT (30-1)-1387. Therefore this final report does NOT imply completion of all phases of the work. (was NR 171-58)

I. Comparison of the biological effects of low (124 Kv.) and high (50 Mev.) voltage X-rays.

It was early realized that the problem must be broken down into a number of subsidiary projects. This was done as follows:

(1) A comparison of the biological effects of high and low voltage x-rays when the distribution of ionization through the test organisms is essentially the same. To do this effectively it was necessary to determine the contribution to the biological effects of high voltage radiation of neutron production and induced radioactivity.

(2) A comparison of biological effects of low and high voltage when similar volume doses are given but the geometry of the system is such as to stress the difference in distribution of ionization in the test organism.

A. Facts Pertinent to the Problem:

1. Ionization in tissues due to x-rays is mainly brought about by the photoelectric effect up to 100 kv; by the compton effects from 100 kv. to 1-4 Mev. and by pair formation at energies greater than about 4 Mev. It is possible, although not probable, that differences in biological effects with low voltage (124 and 200 Kv.) and high voltage (2 and 50 Mev.) X-rays might be expected due to differing methods of ionization.
2. With x-rays up to about 1 Mev., depth-dose decreases exponentially with depth. With x-rays of greater energies, ionization increases with depth until a maximum is reached, after which it gradually decreases. The amount of maximum increase over the surface dose and the depth at which the maximum occurs depends on the energy of the x-rays. (Charlton and Breed, Amer. Jour. Reent. & Rad. Therapy, 40 1948).

3. Beginning with energies of about 22 Mev, X-rays induce radioactivity in certain atoms. (Mayneord, Martin and Layne, Nature, 104, p. 728, 1949).
4. Neutrons are produced by high voltage x-rays. (Baldwin and Elder, Report #RL - 302, Dec. 1949). For some biological systems, neutrons seem to be more efficient in causing damage than x-rays. The part neutron production would play in any differential effects of low and high voltage x-rays needed clarification. (Evans, Radiology, 50, p. 811, 1948).

B. Results.

- (1) Comparison of effects of low and high voltage radiation when distribution through the test organism is essentially similar for the two types of radiation.
 - (a) In killing of yeast - no difference demonstrated (Figure 1).
 - (b) In retardation of growth of wheat seedlings - no difference demonstrated (Figures 2, 3).
 - (c) In killing of first meiotic metaphase eggs of Habrobracon - no difference demonstrated (Figure 4).
 - (d) In comparison of survival times of mice (Figures 5, 6 & 7), two criteria were used: the time after radiation for 50% deaths and the percentage survival at end of 30 days (Figures 8 & 9), - no difference demonstrated.
 - (e) In comparison of weight loss of mice after radiation (Figures 10, 11 & 12).. Three criteria were used: maximum weight loss after radiation (Figure 13), time after radiation when weight loss reaches its maximum (Figure 14), and days after radiation when pre-radiation weight is recovered (Figure 15), no difference demonstrated.
 - (f) A comparison of residual survival of irradiated mice. After about 30 days the survival curve approaches an asymptote. It seemed possible that differences in effects of high and low voltage X-rays might appear very late and be reflected in decreased longevity. Populations consisting of mice which had

B. Results (cont.)

survived 50 days post radiation and controls were followed. Although irradiated "recovered" mice showed an increased death rate over the controls, there was no demonstrable difference between groups receiving the same dose of high and low voltage radiation (Figures 16 & 17).

- (2) A comparison of the effects of low and high voltage X-rays on incidence of cataract.

Evans (Radiology, 50 p. 811, 1948) and others indicated that neutrons are approximately seven fold as efficient as X-rays in producing cataract. Baldwin and Elder (Loc. Cit.) demonstrated the production of neutrons with 50 Mev. X-rays. If neutrons are produced in biologically significant amounts, it was believed that incidence of cataract indication would yield information. Controls and mice irradiated with 200 and 400r whole body low or high voltage X-rays were followed, - differences indicating greater efficiency of low voltage X-rays, opposite to that anticipated if neutrons were present in biologically significant numbers, were found. It is not believed that differences are significant (Figures 18 & 19), but further work should be done.

- (3) Induced Radioactivity in Biological Systems.

Mayneord, Martin and Layne (Loc. Cit.) demonstrated induced radioactivity in rats exposed to 22 Mev. X-rays. Inasmuch as this is near the threshold energies, it seemed desirable to extend studies into 100 Mev. X-rays. Blocks of yeast were radiated and counts, beginning .2 seconds after cessation of exposure made (Figures 20, 21 & 22). Induced radioactive oxygen, nitrogen and carbon was demonstrated but after exposures to 3050r, 68% of the total induced radio activity amounted to less than 0.005r. Unless induced radioactivity produces unique biological effects, it is believed that induced radioactivity plays an insignificant role.

- (4) A comparison of the effects of differing dose distributions upon weight, survival time and testes and spleens in rats.

To take advantage of different depth dose distribution in low and high energy X-rays, rats snugly fitted into a plastic cylinder, 6.5 cm. in

(4) Cont.

diameter enclosed in a prestwood phantom, 14.5 x 15 x 28 cm. long, were irradiated lengthwise with the posterior end of the animal facing the target. Equal total body doses (equal volume doses) were given with 124 kv., 200 kv. or 50 Mev. X-rays. The distribution of ionization for 900r is given in Figure 23.

A comparison of percent survival is given in Figure 24, on spleen in Figures 25 & 26, on testes, Figure 27 and the blood picture, Figure 28.

C. Summary.

1. No wave length effect with 124 kv, 2 Mev. and 50 Mev. X-rays were demonstrated, either quantitatively or qualitatively.
2. No effect of neutron production by 50 Mev. X-rays could be demonstrated on cataract formation. Neutron formation plays an insignificant role in cataract formation as compared to X-rays.
3. Induced radioactive oxygen, nitrogen and carbon, unless exhibiting unique biological effects, is such an insignificant part of the total radiation, 0.005r in 3050r, that it is thought to be without significance.
4. A striking biological difference in survival times was demonstrated when comparing 124 kv. or 200 kv. and 50 Mev. X-rays when the geometry of radiation was such as to take advantage of the features of the distribution of ionization unique to each. Studies so far pursued on liver catalase, blood picture, spleen and testes does not correlate with the striking differences in the specific doses given the organs and with the striking differences in survival times.

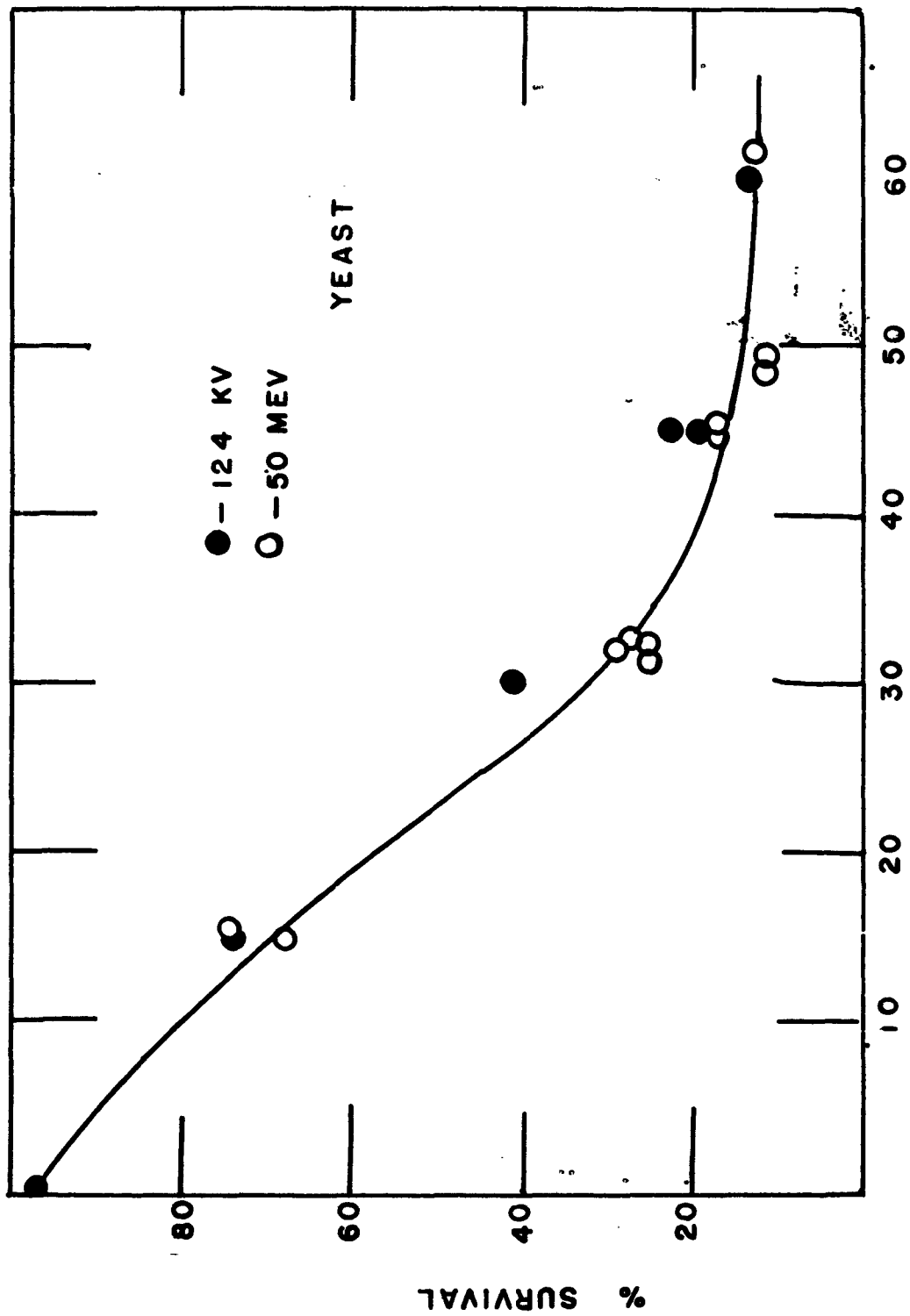


Fig. 1

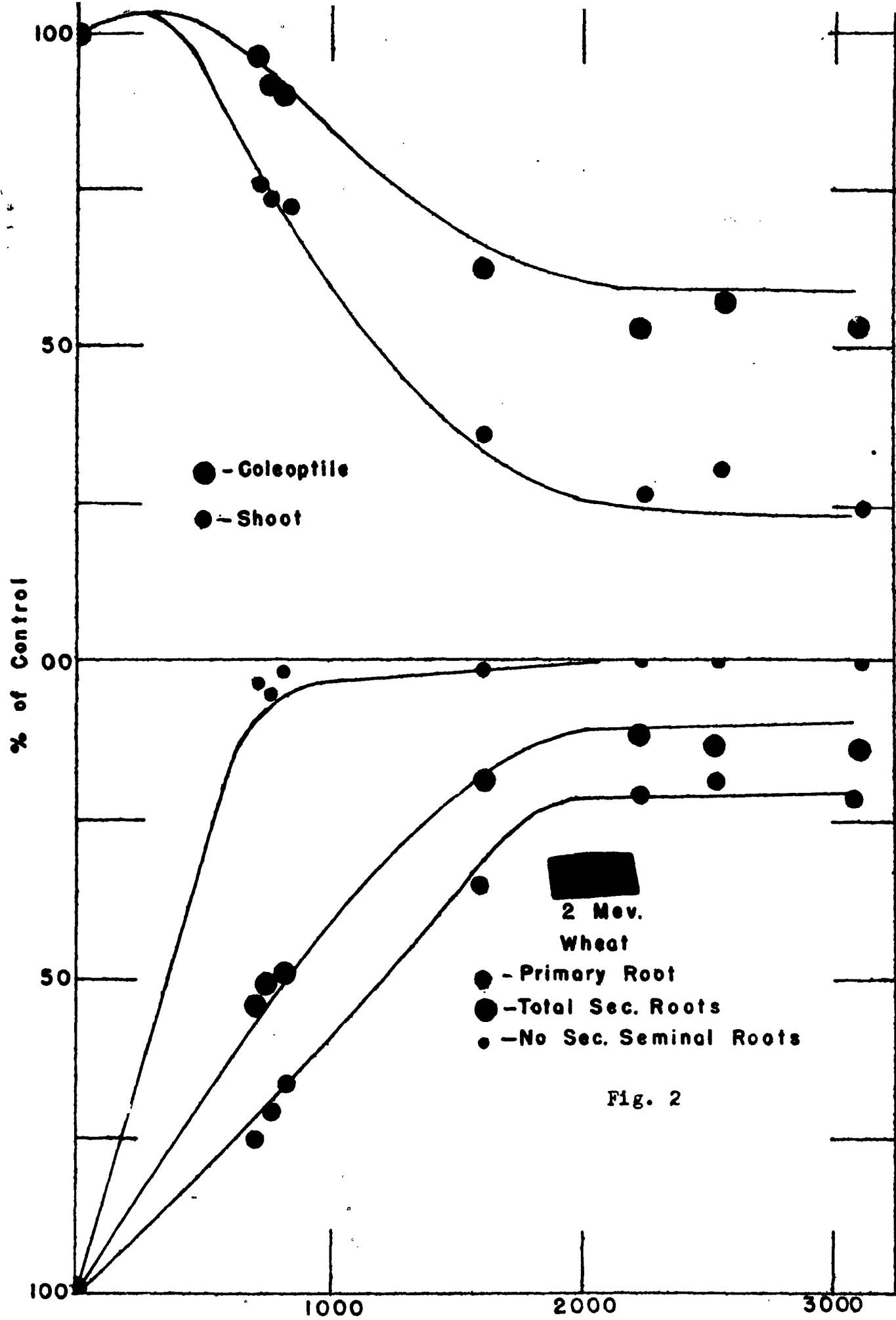


Fig. 2

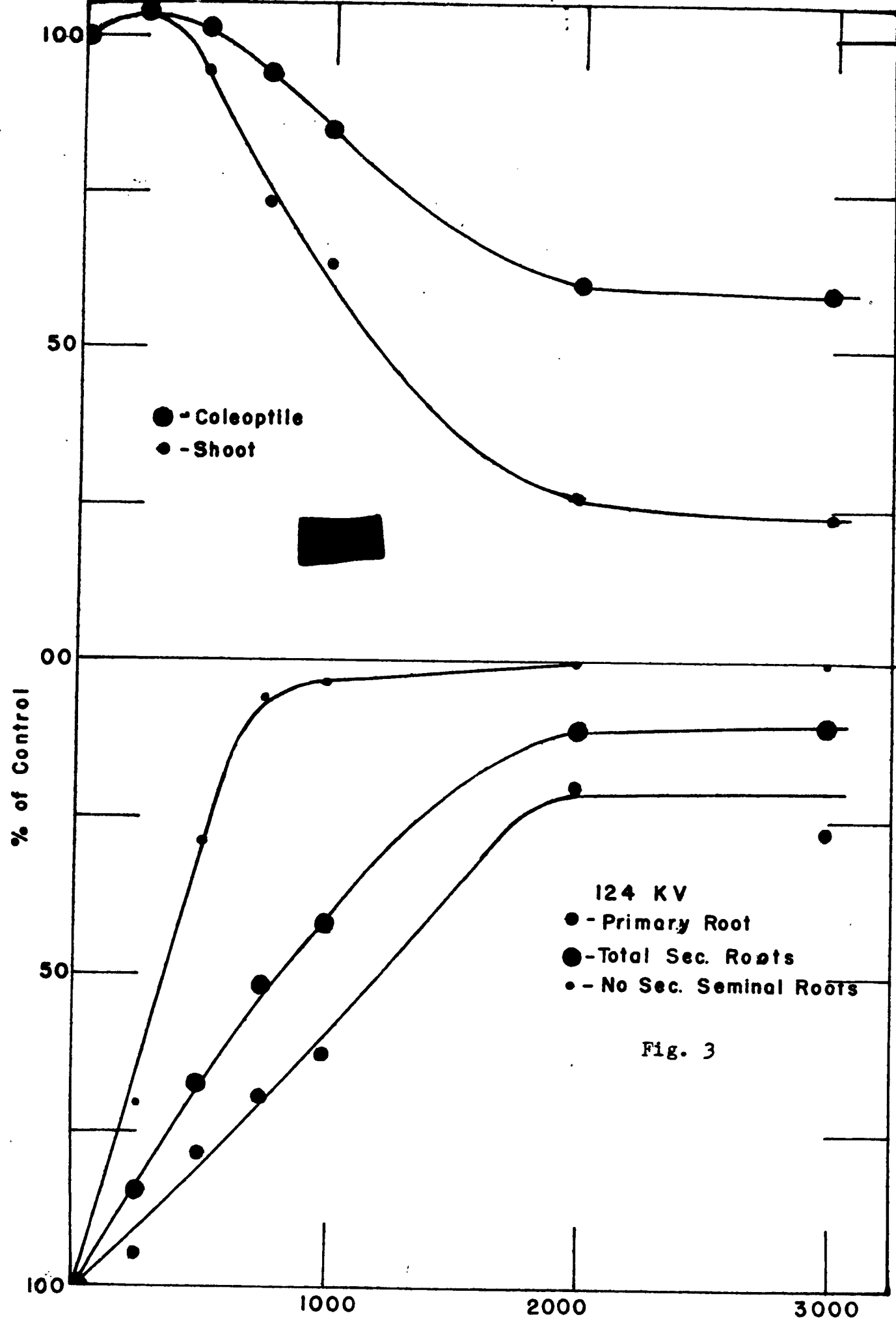
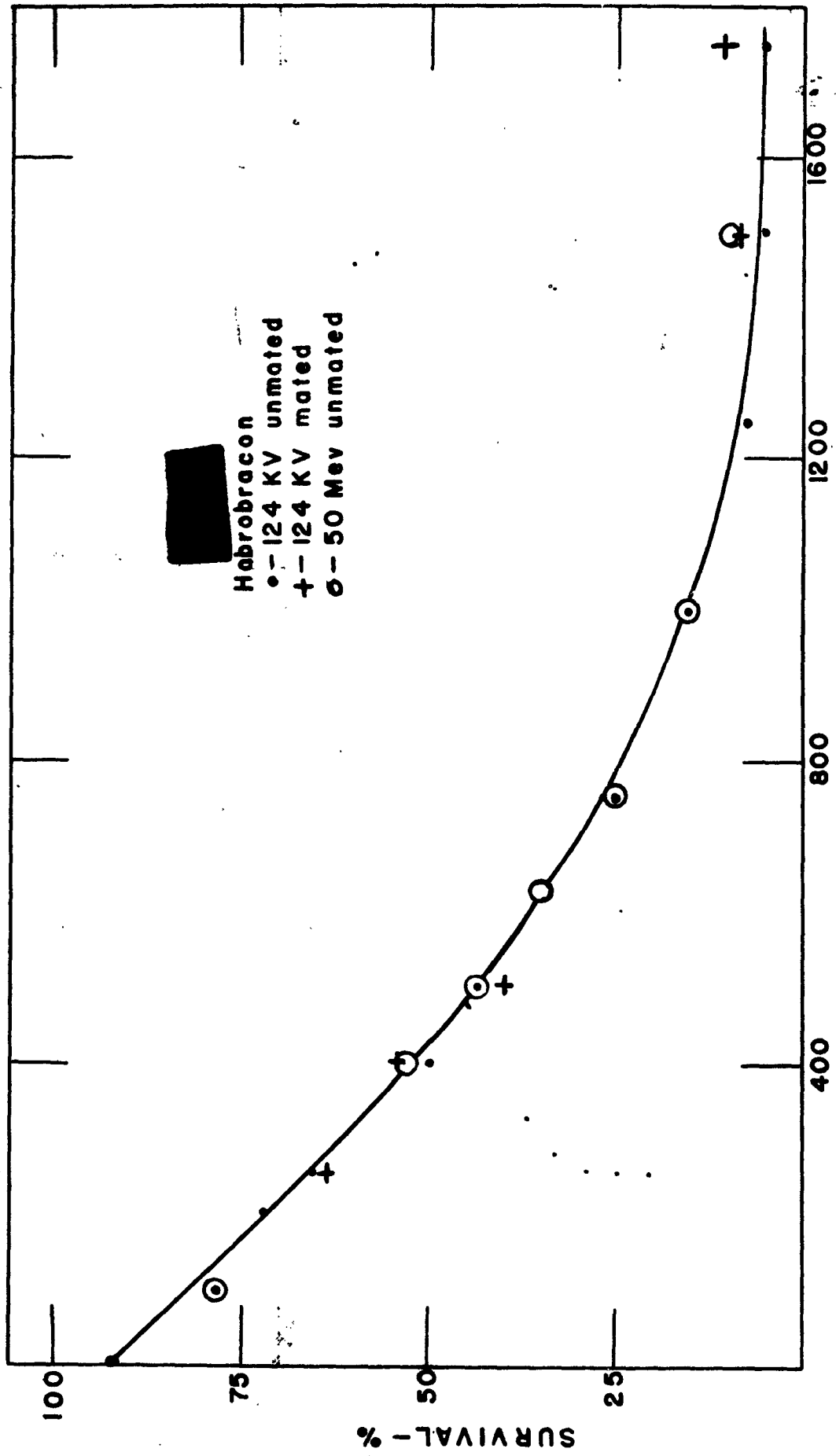


Fig. 3



DOSE - r

FIG. 4

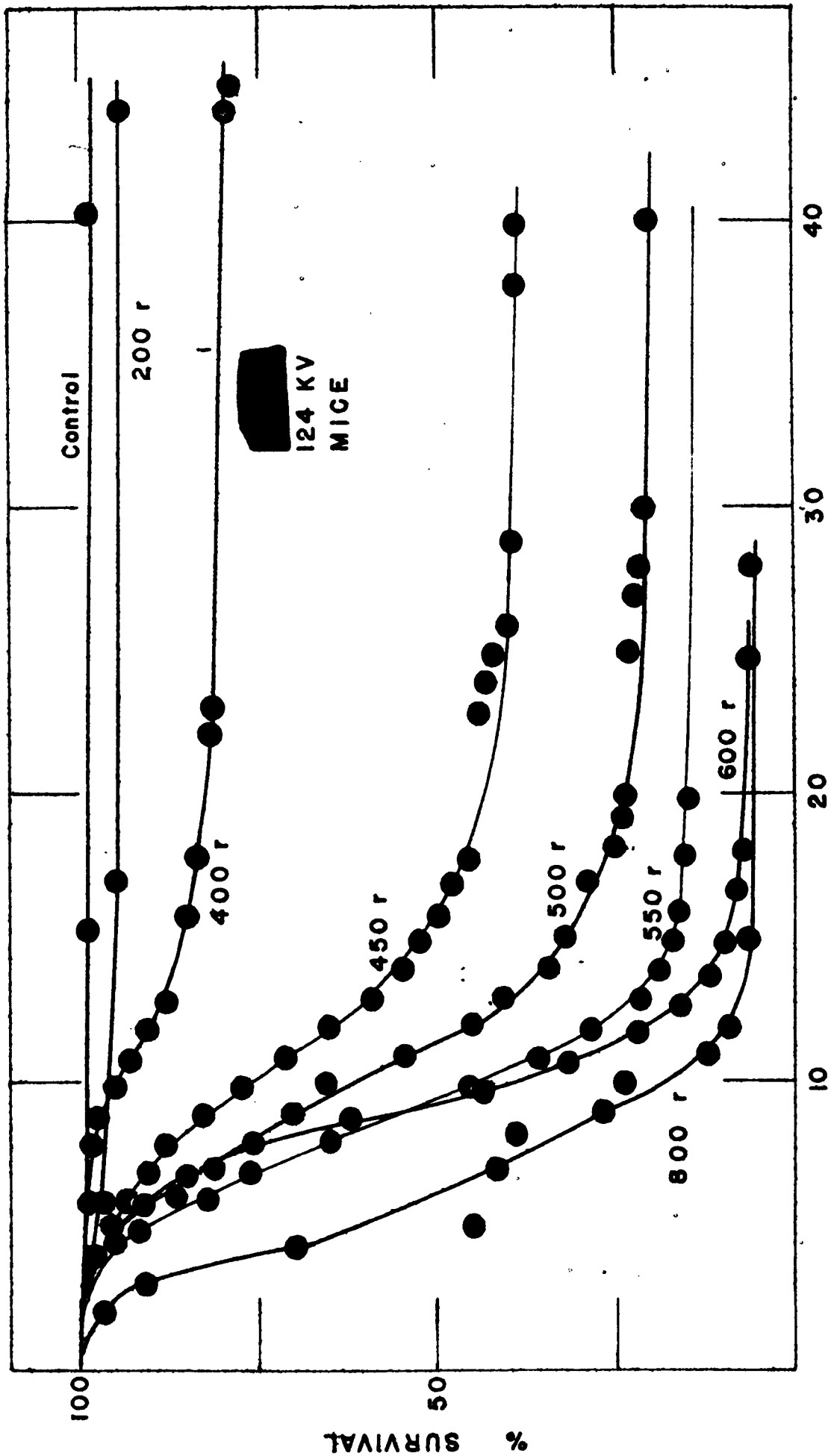
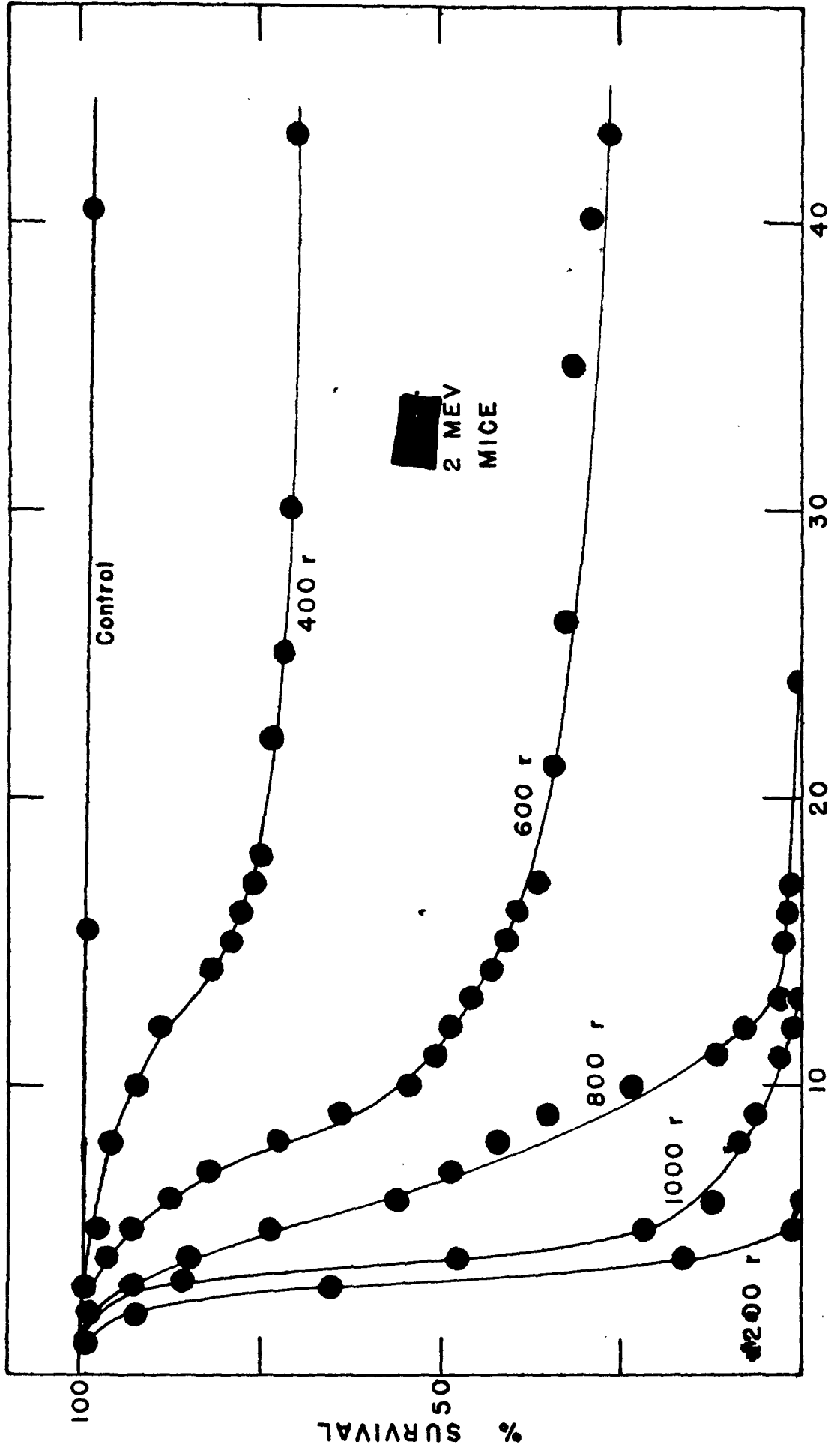


FIG. 5



Days after radiation
Fig. 6

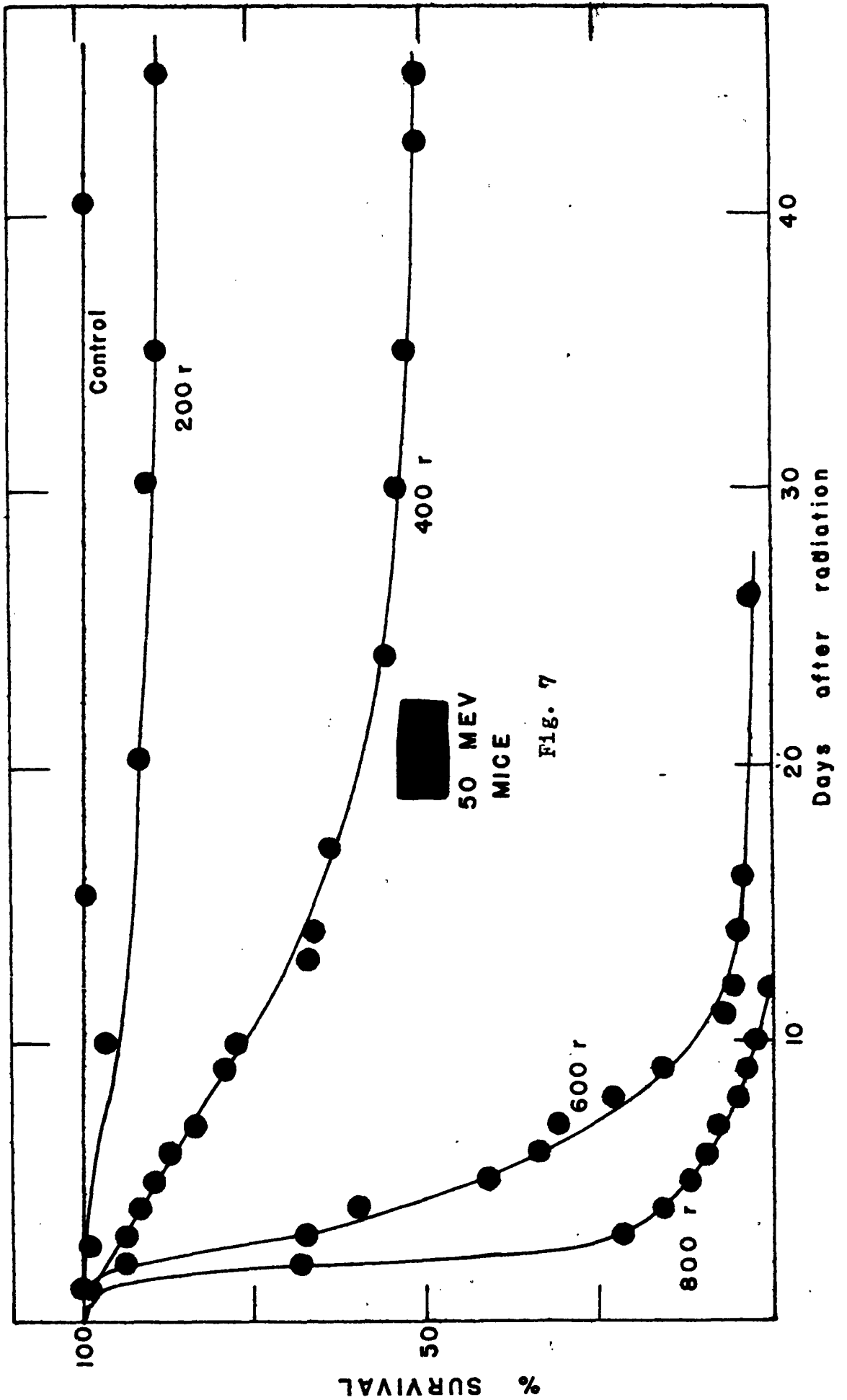


FIG. 7

50 MEV
MICE

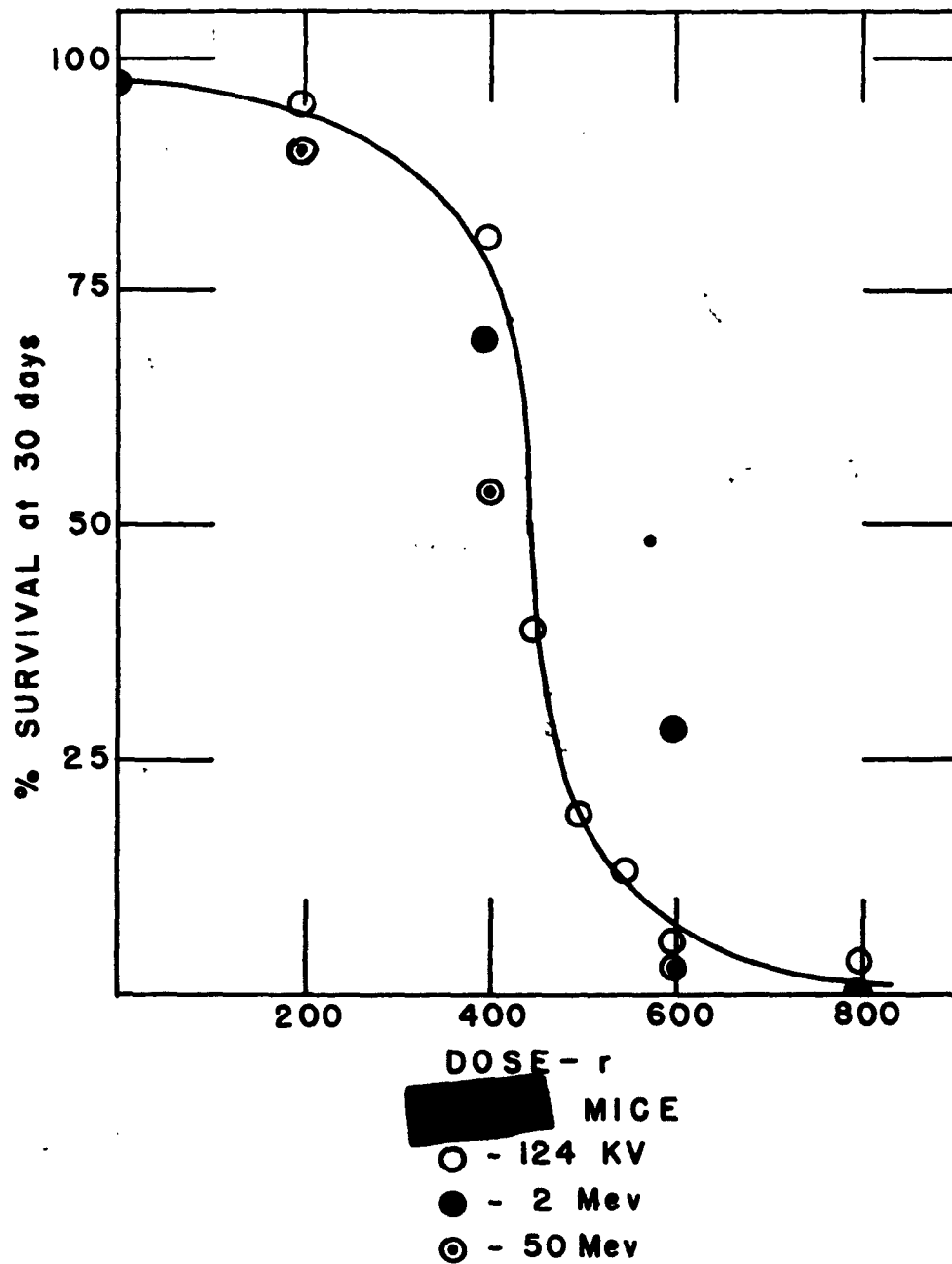
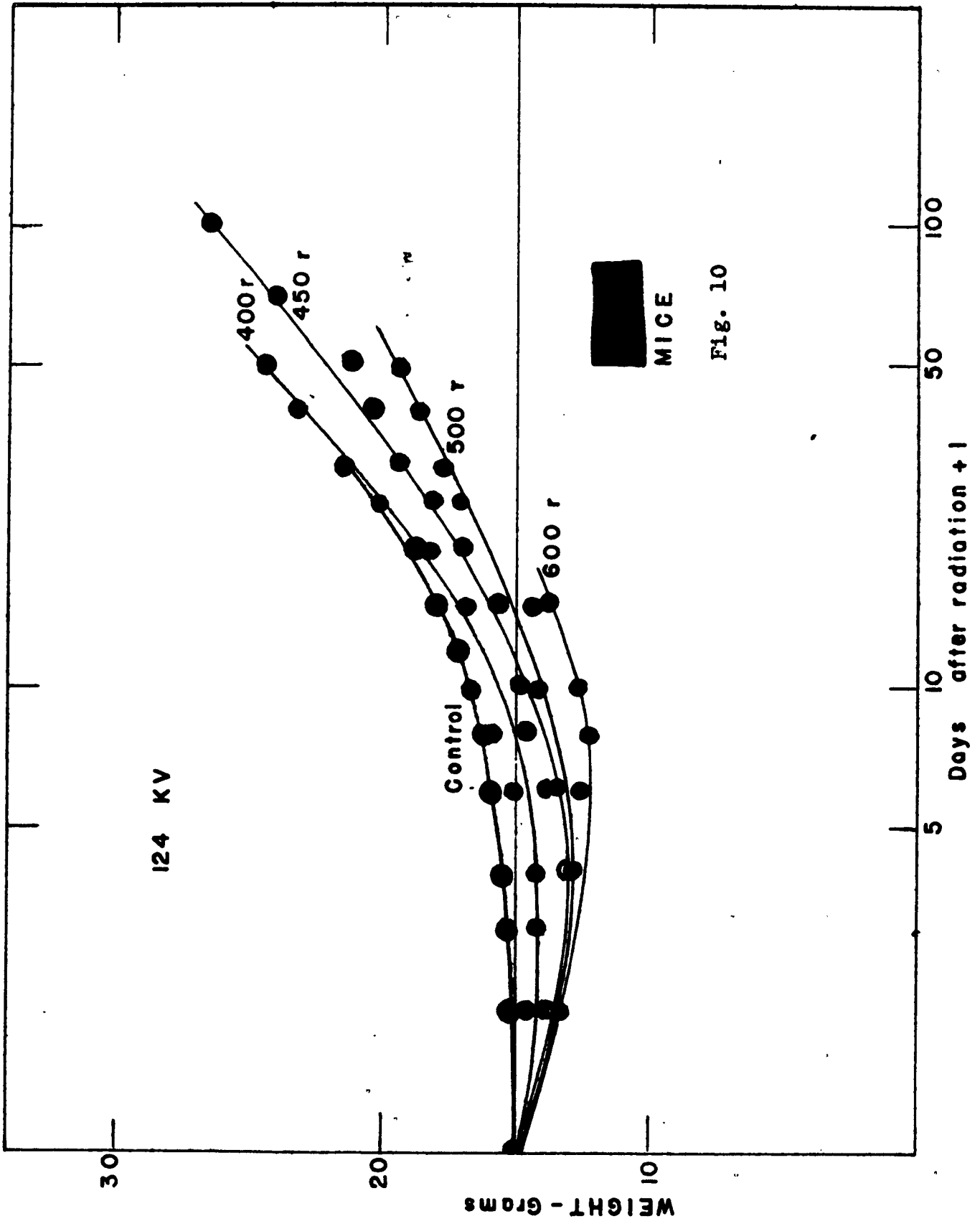


Fig. 9



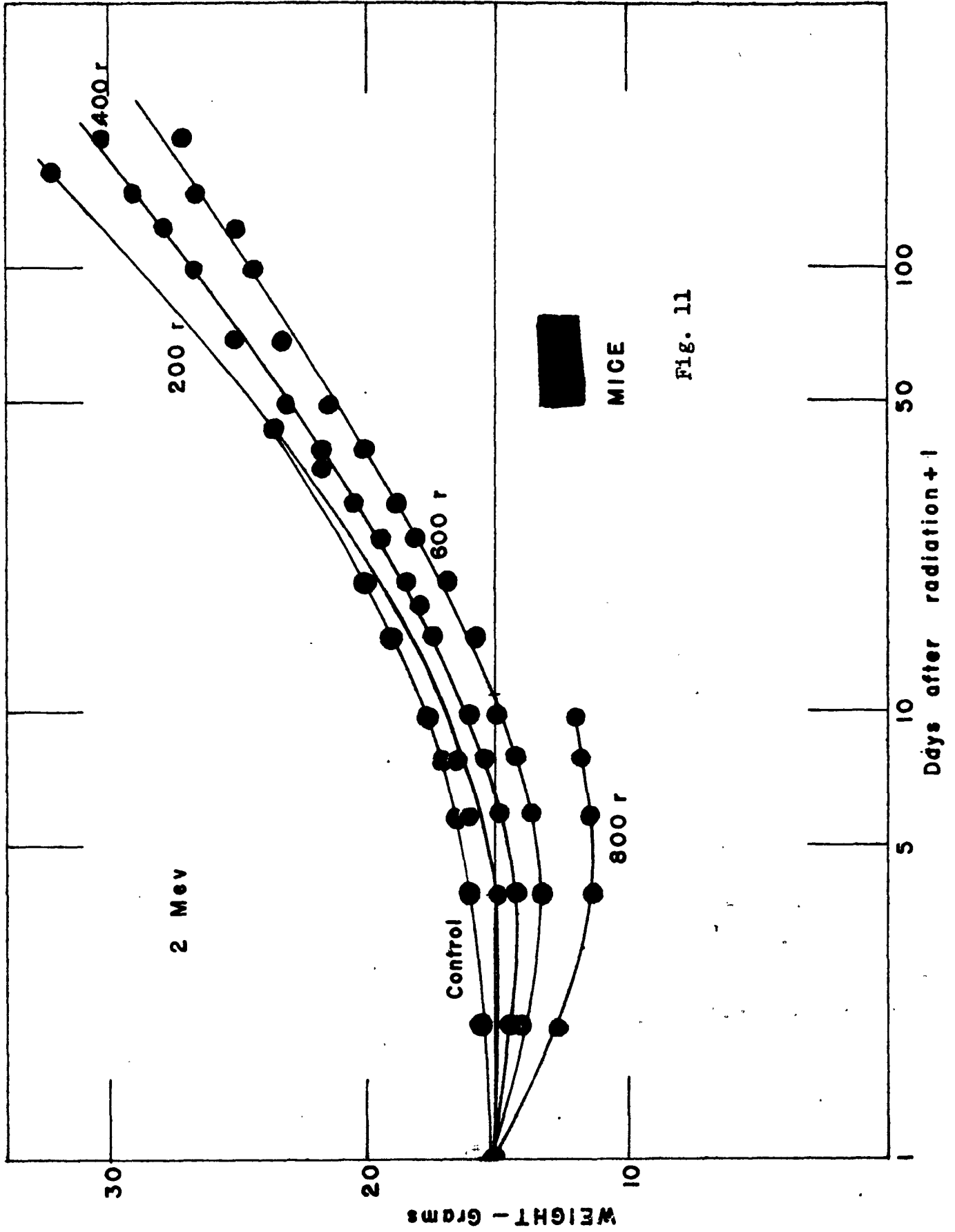
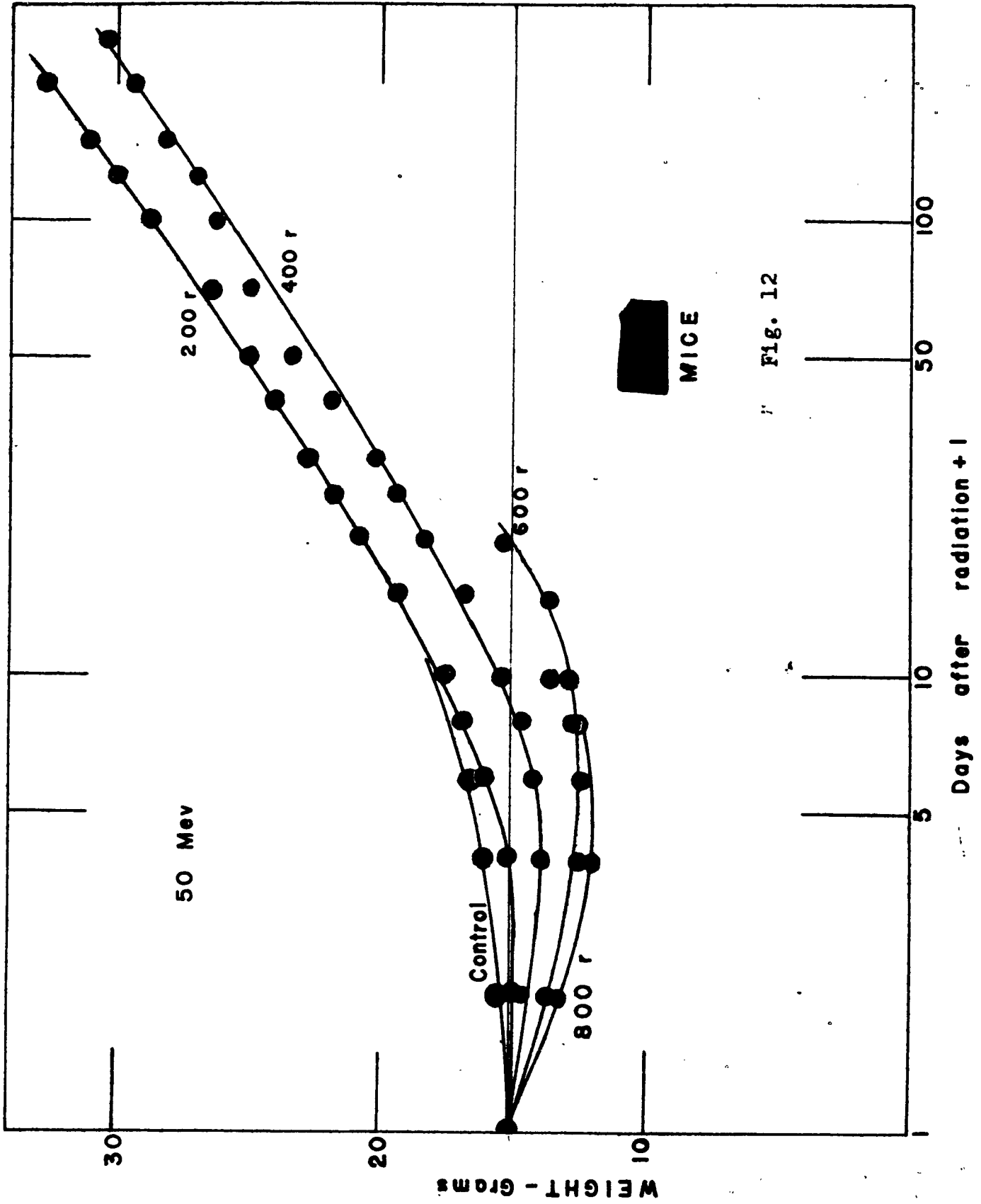


Fig. 11



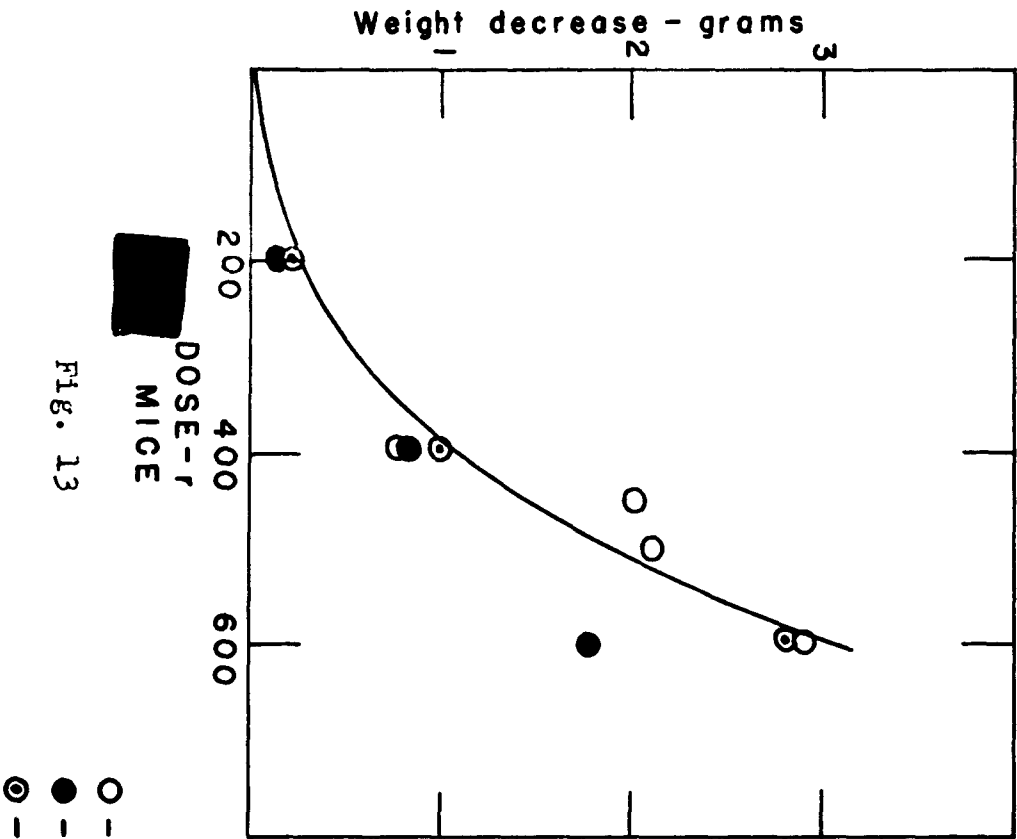


FIG. 13

O - 124 KV
 ● - 2 Mev
 ⊙ - 50 Mev

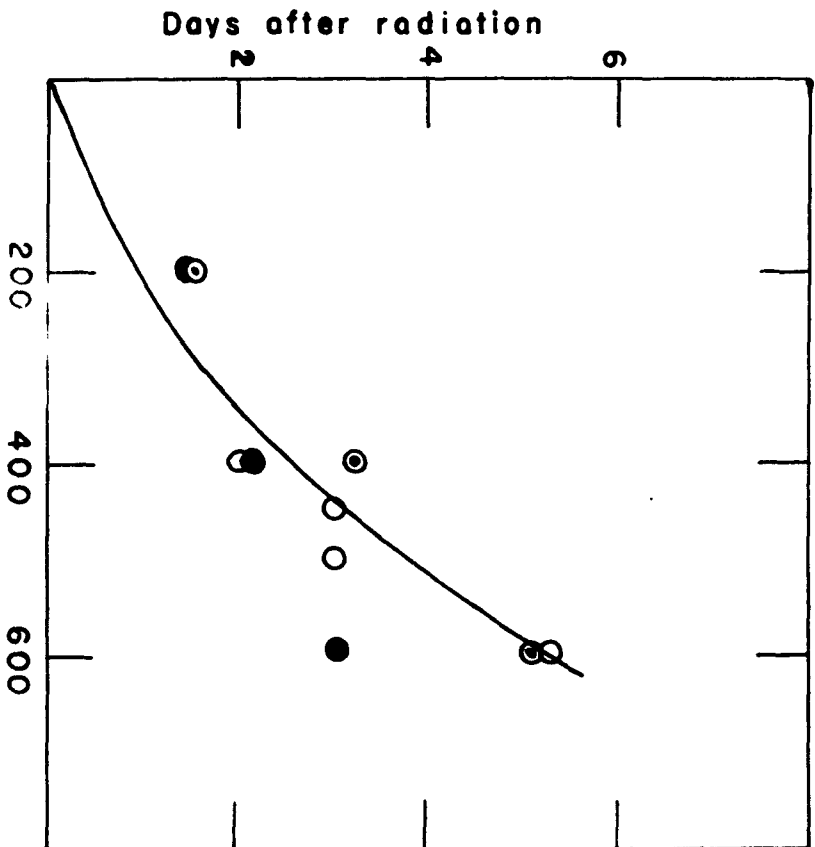


FIG. 14

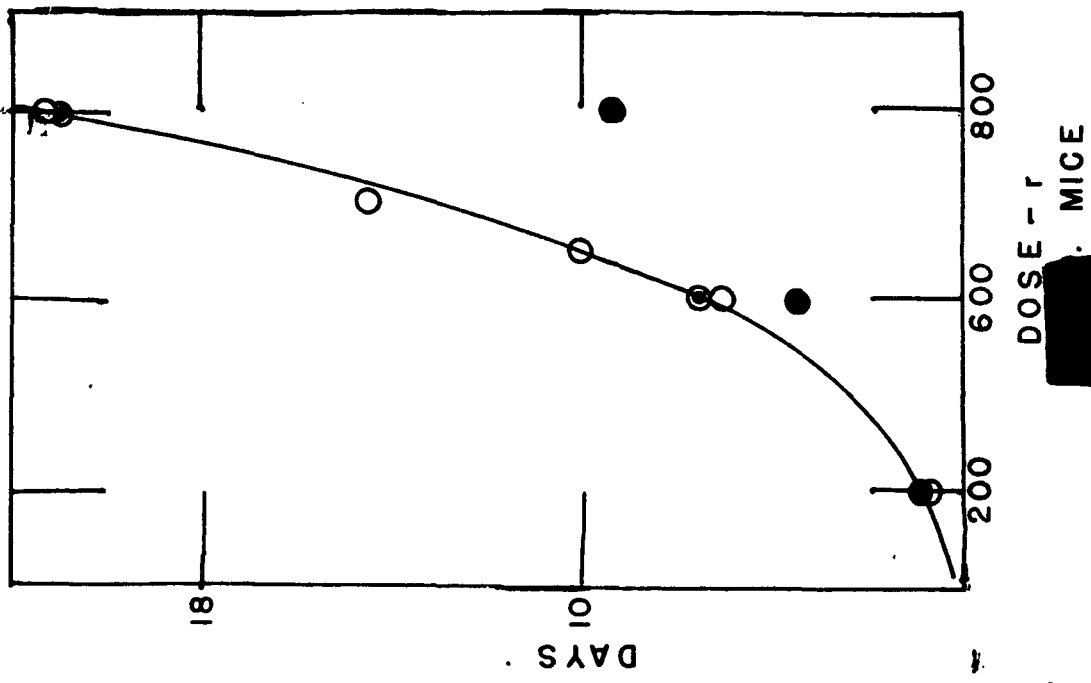


FIG. 15

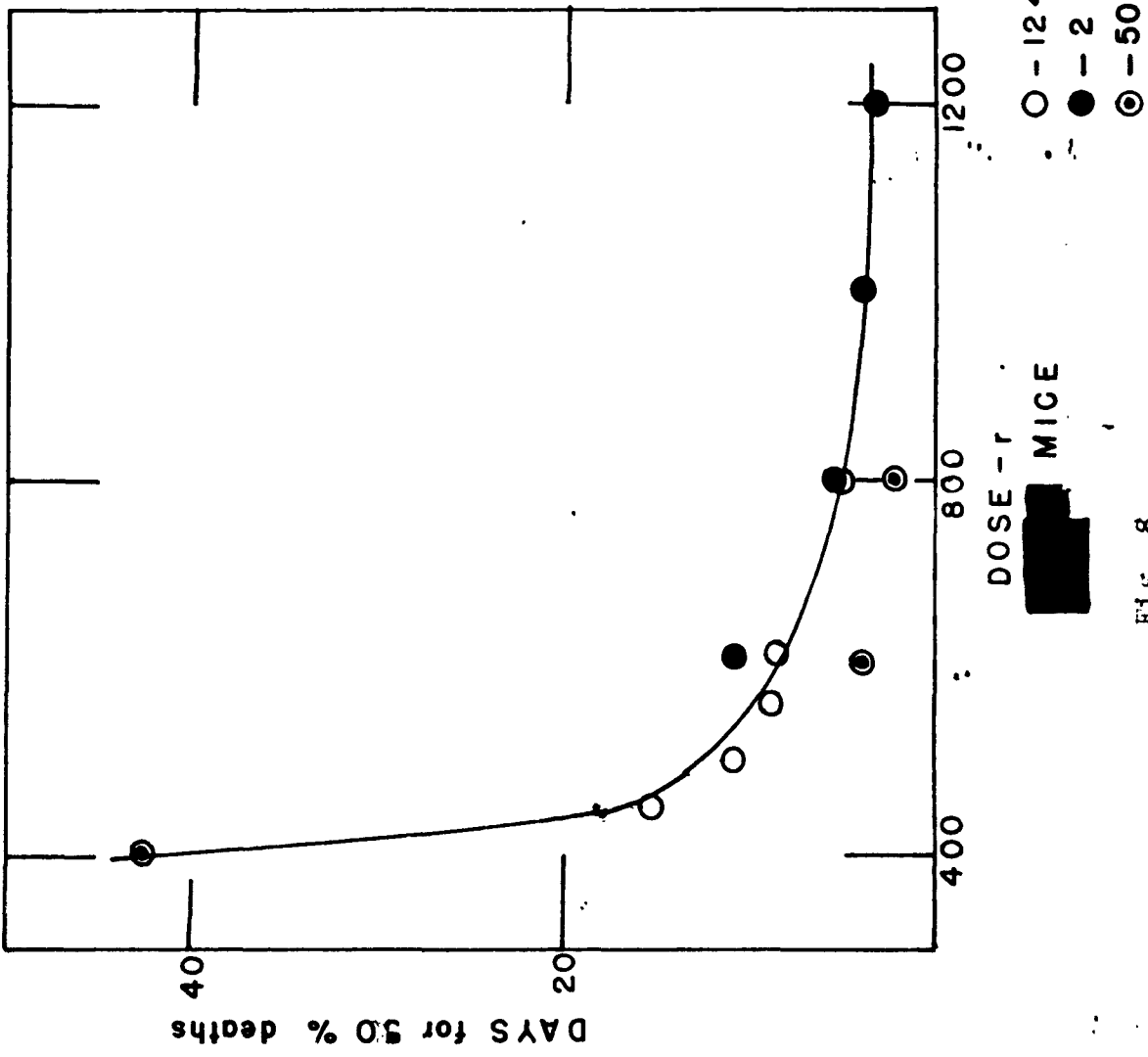


FIG. 8

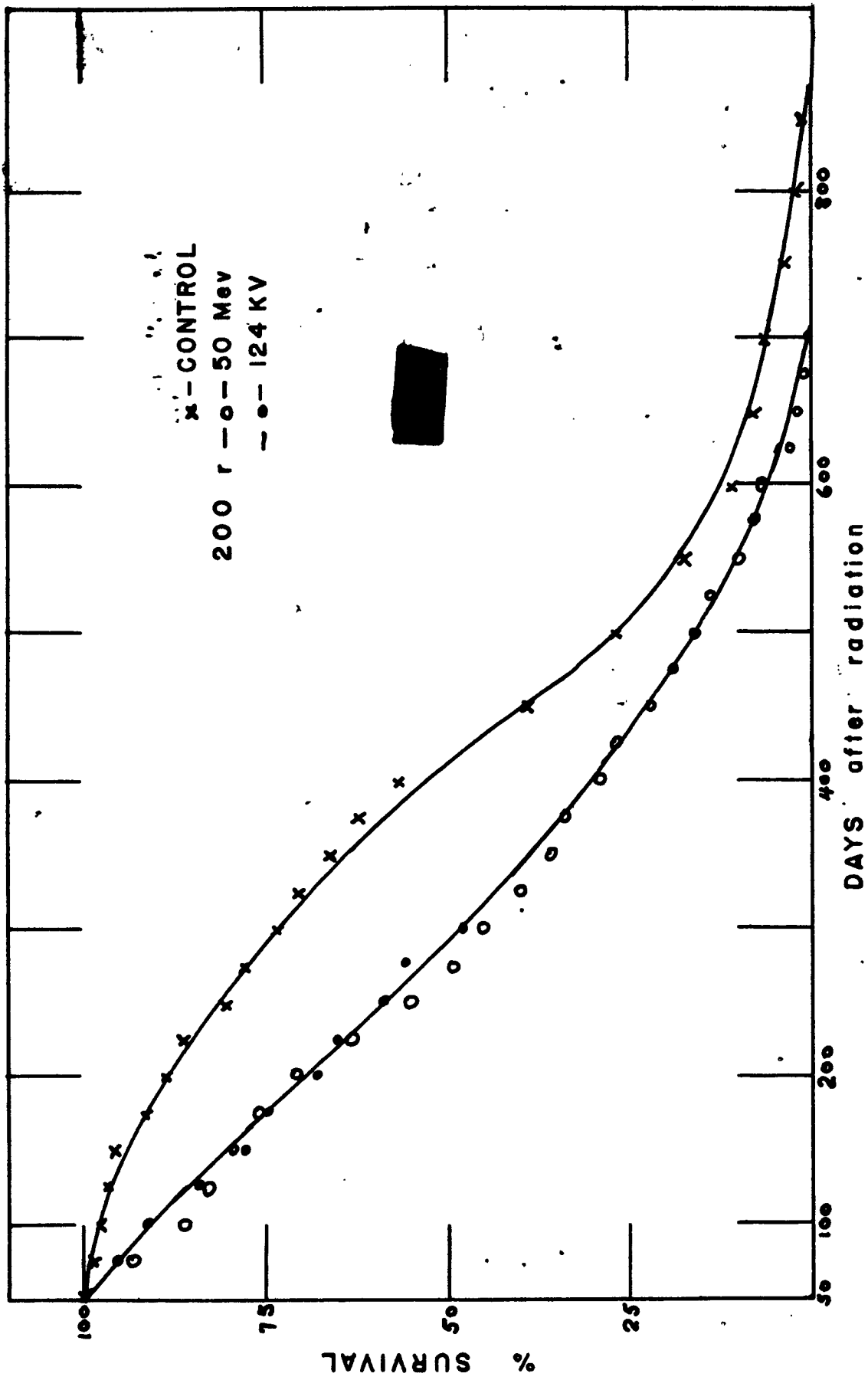


FIG. 16

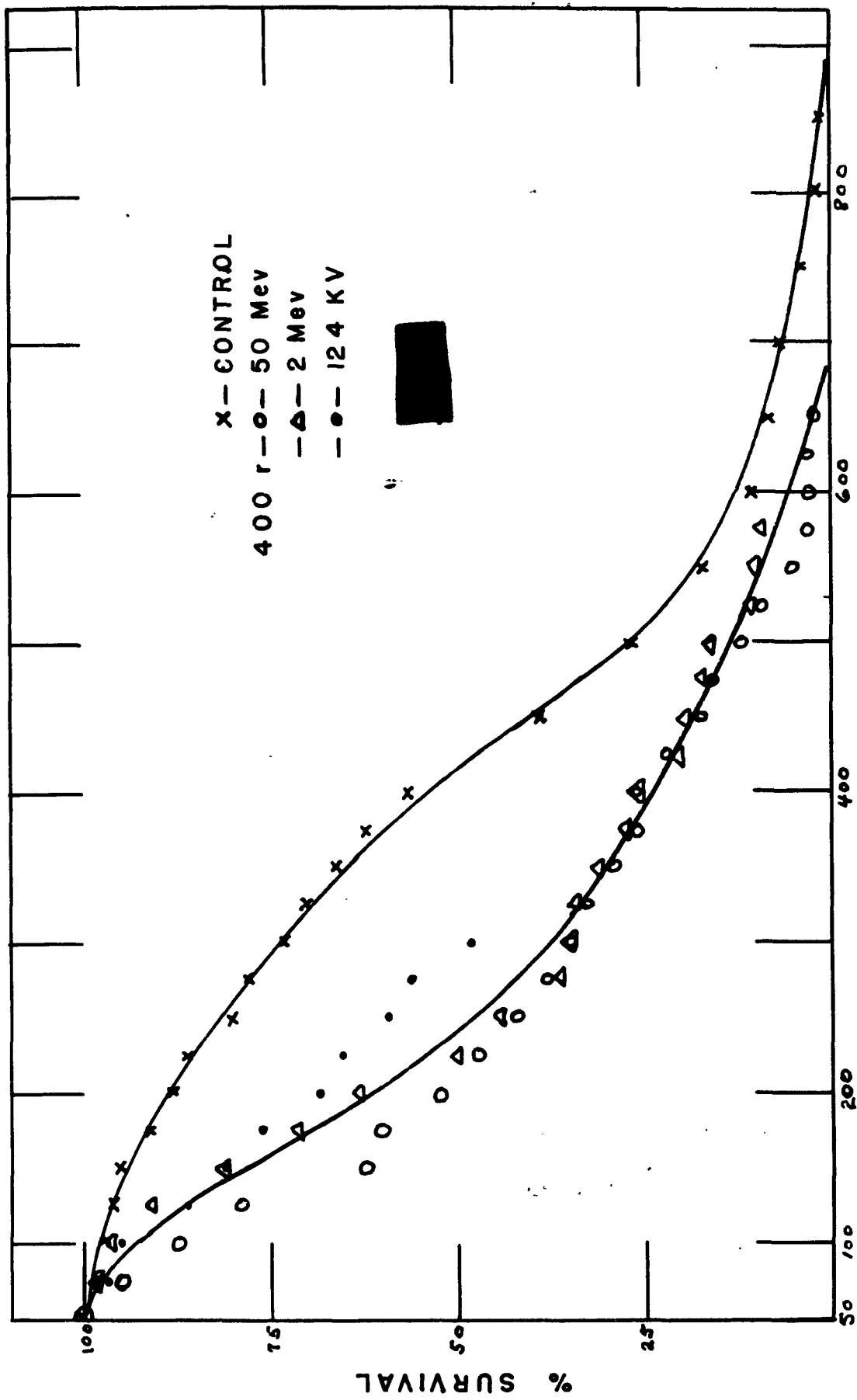


FIG. 17

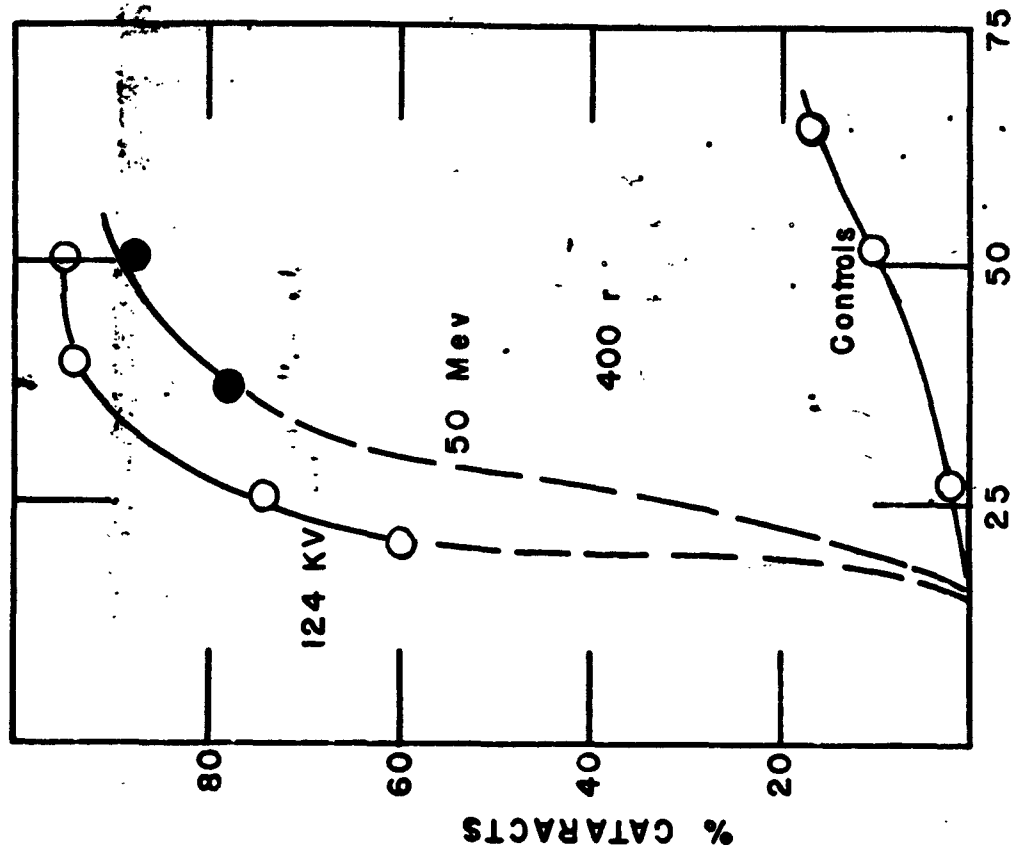


FIG. 18

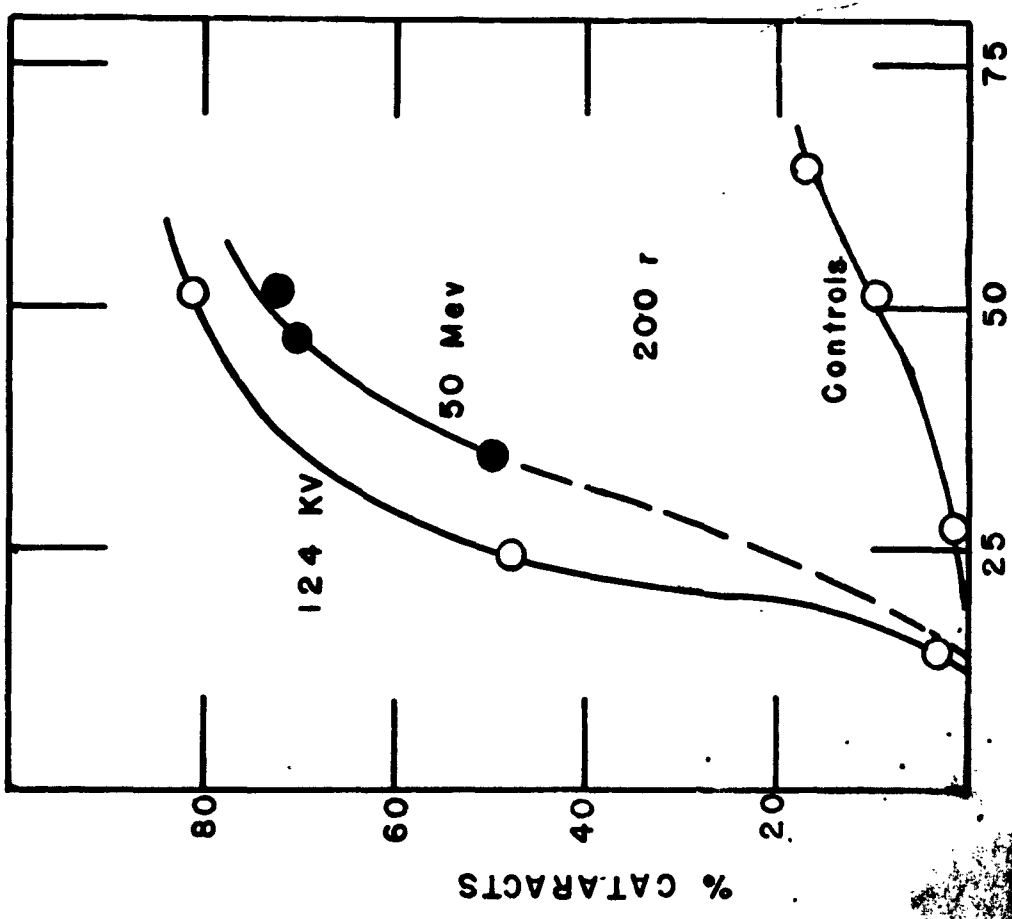


FIG. 19

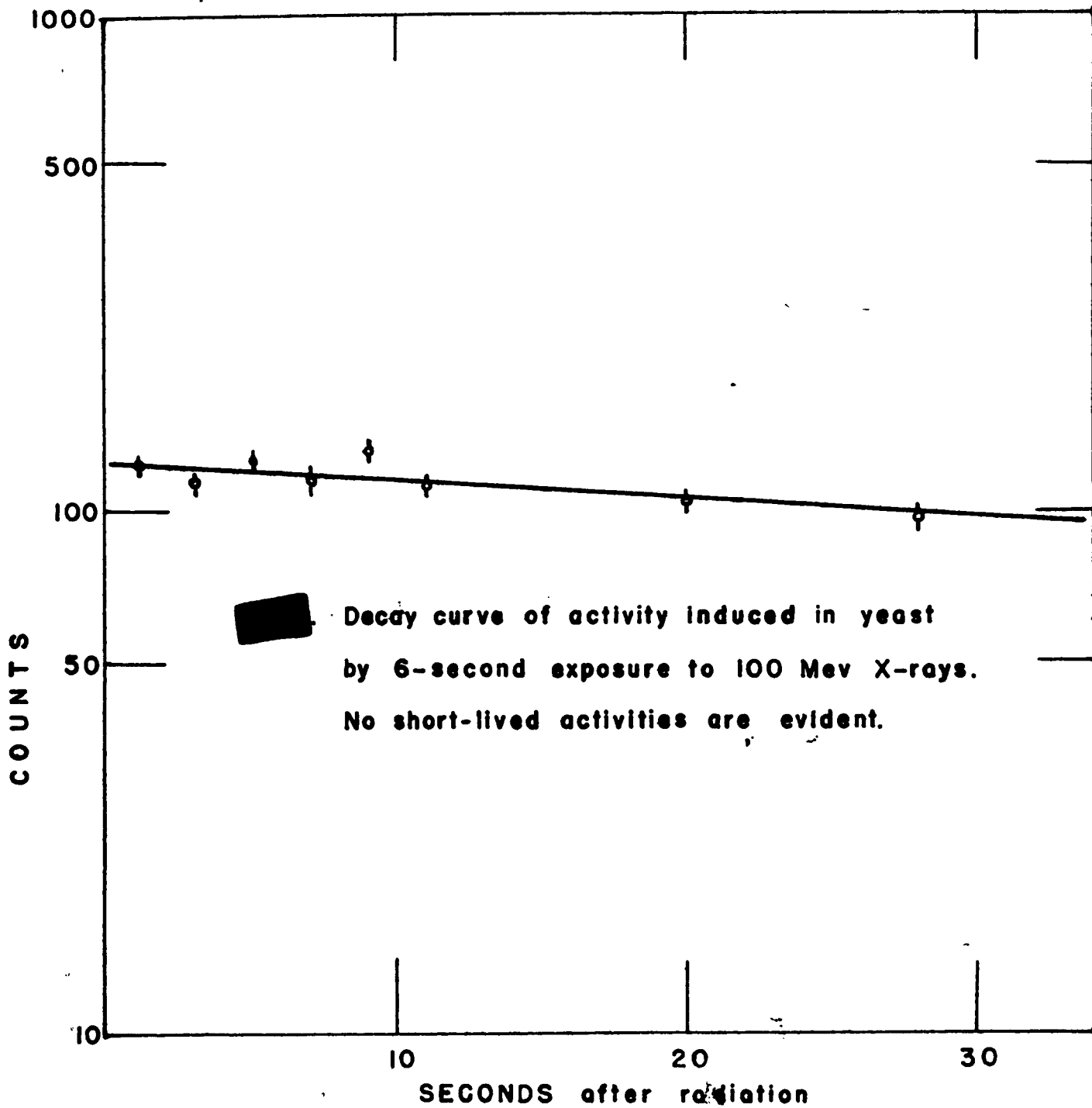
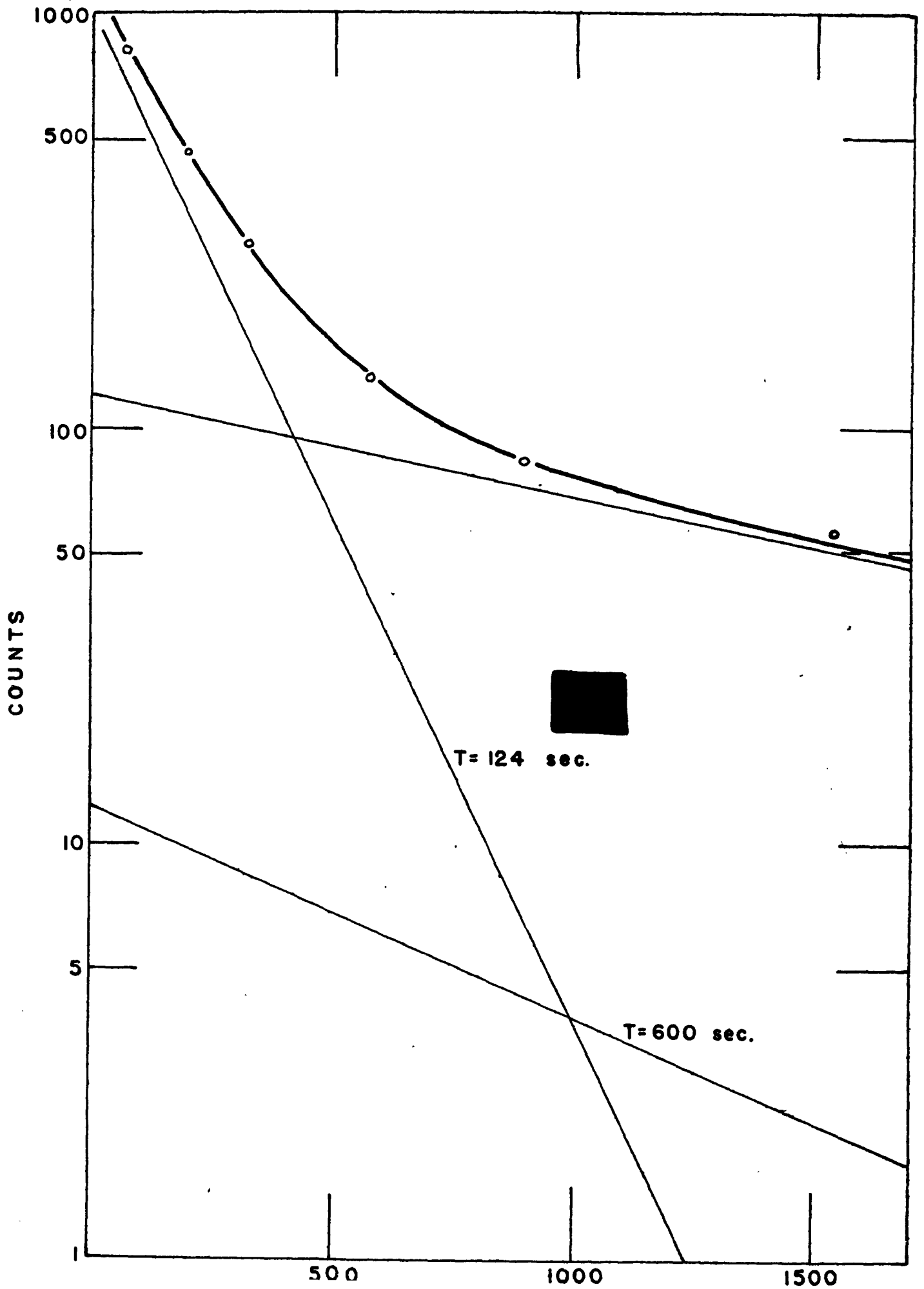
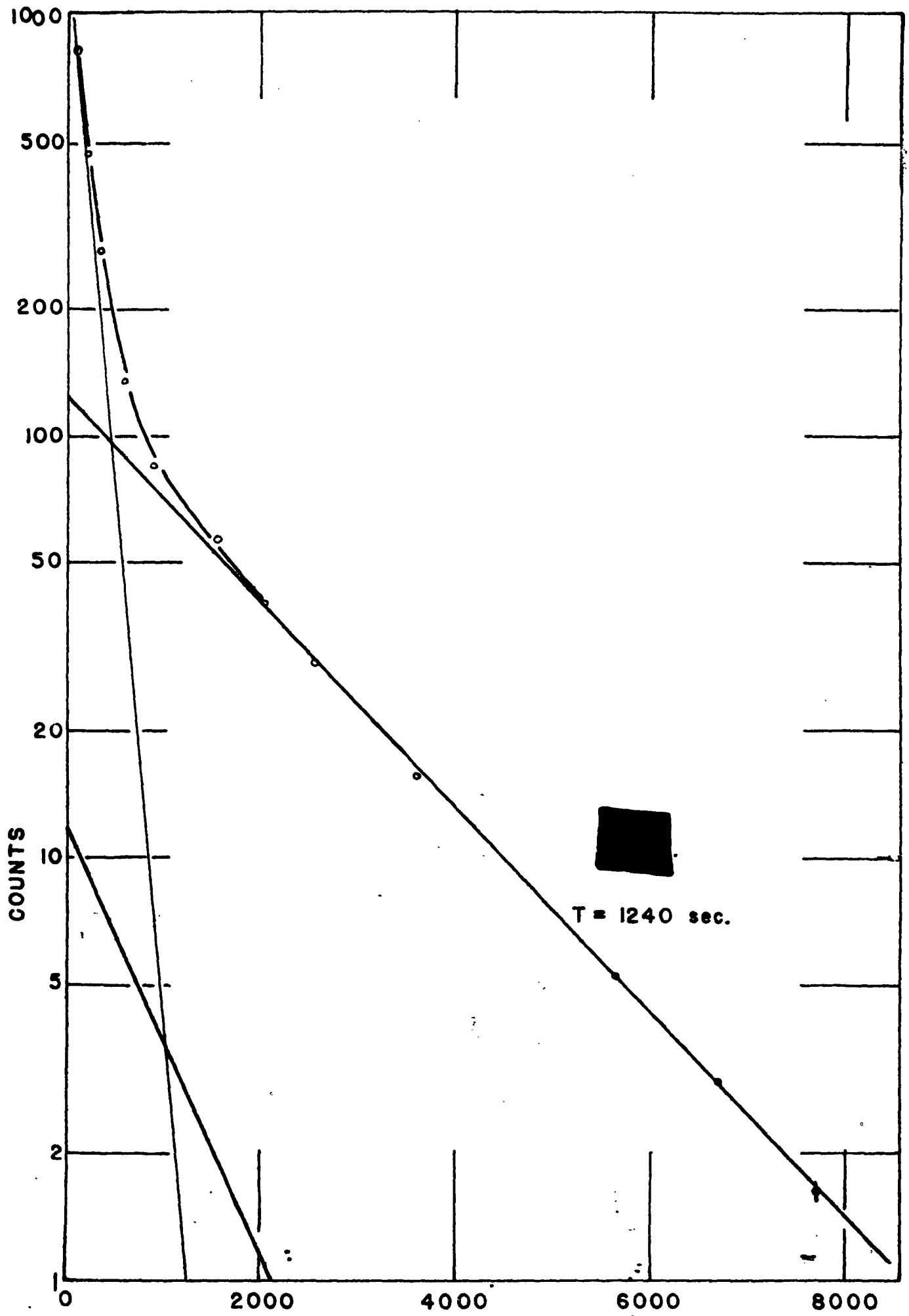


Fig. 20





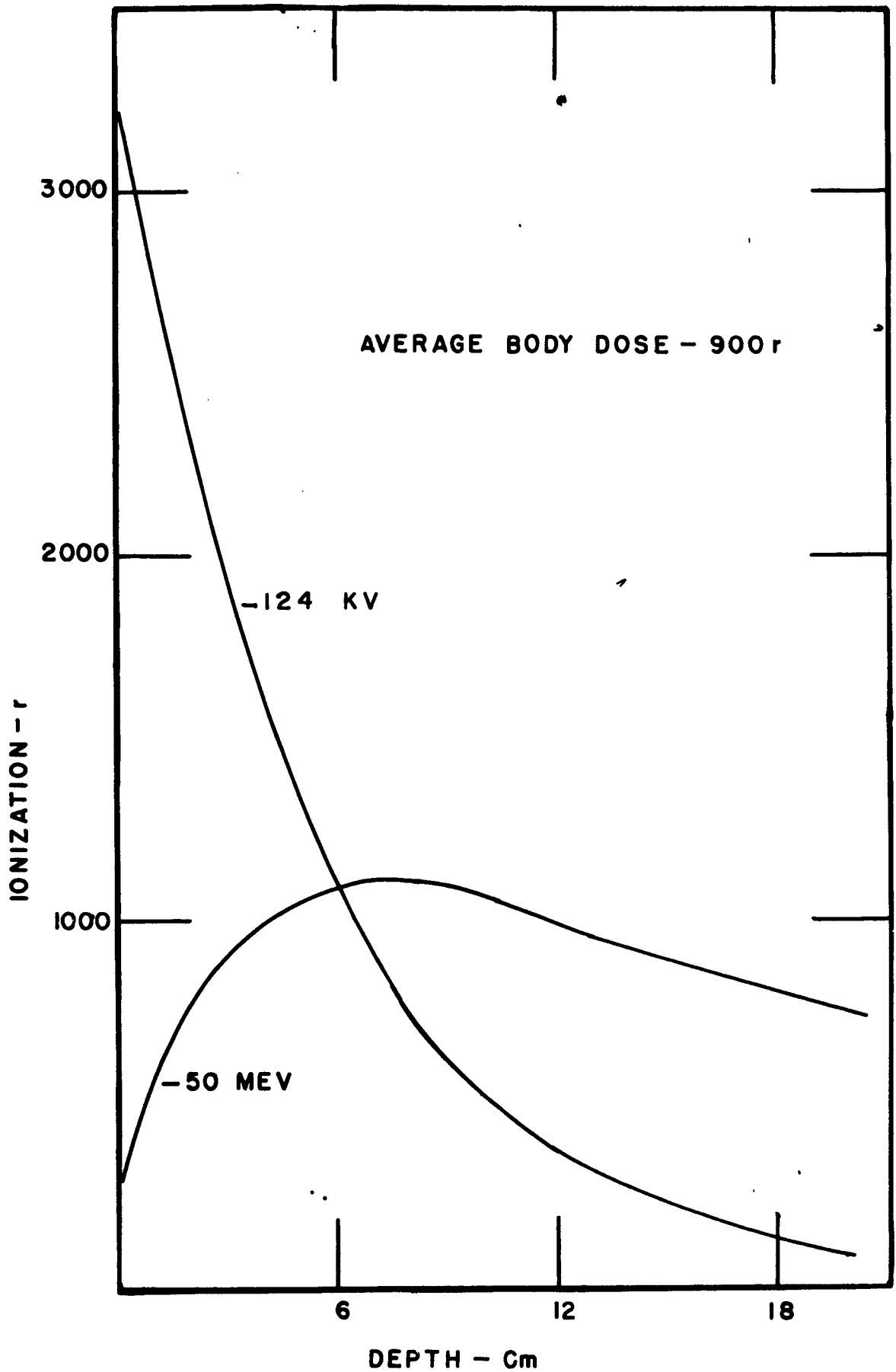


Fig. 2

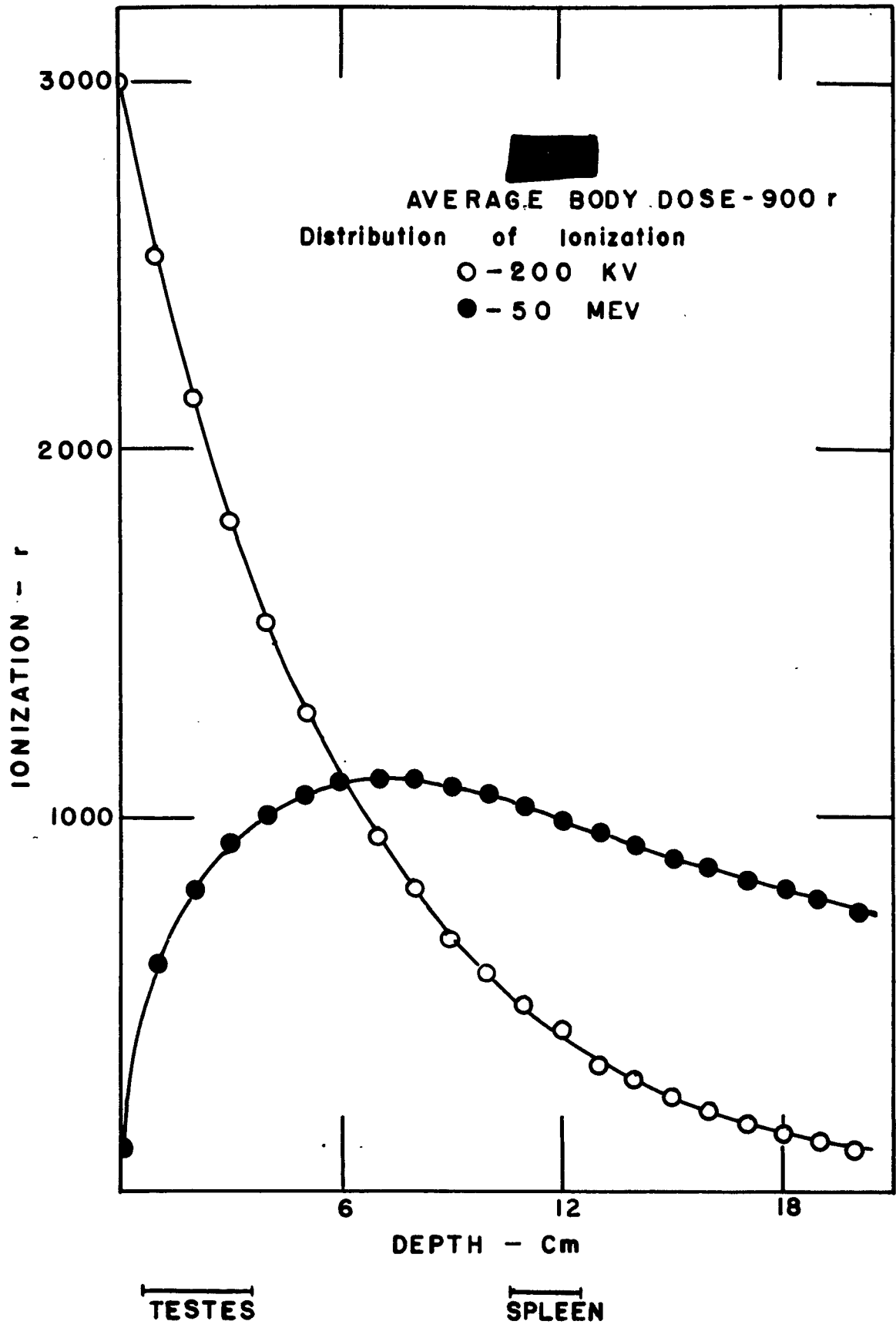


Fig. 23A

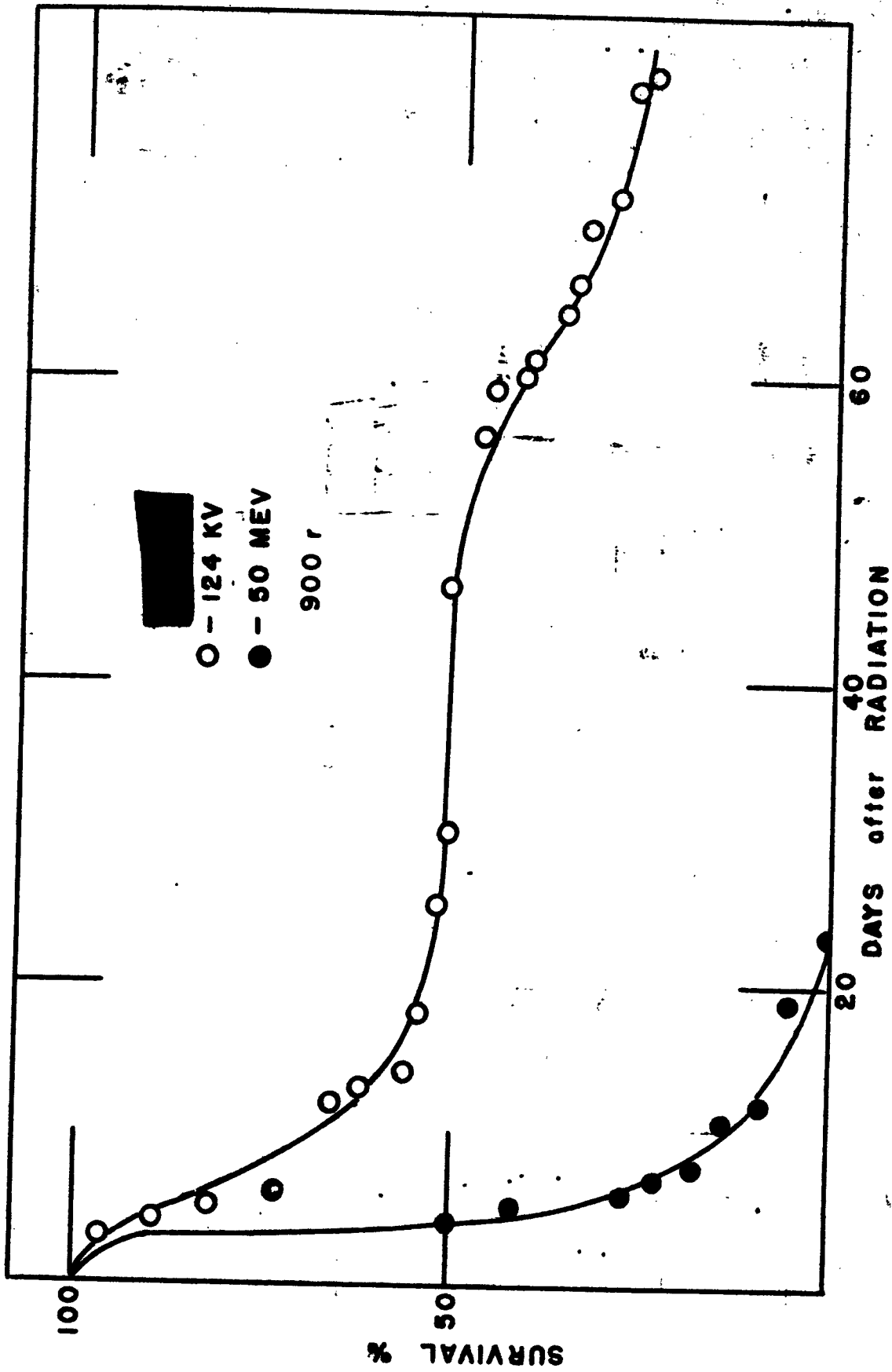
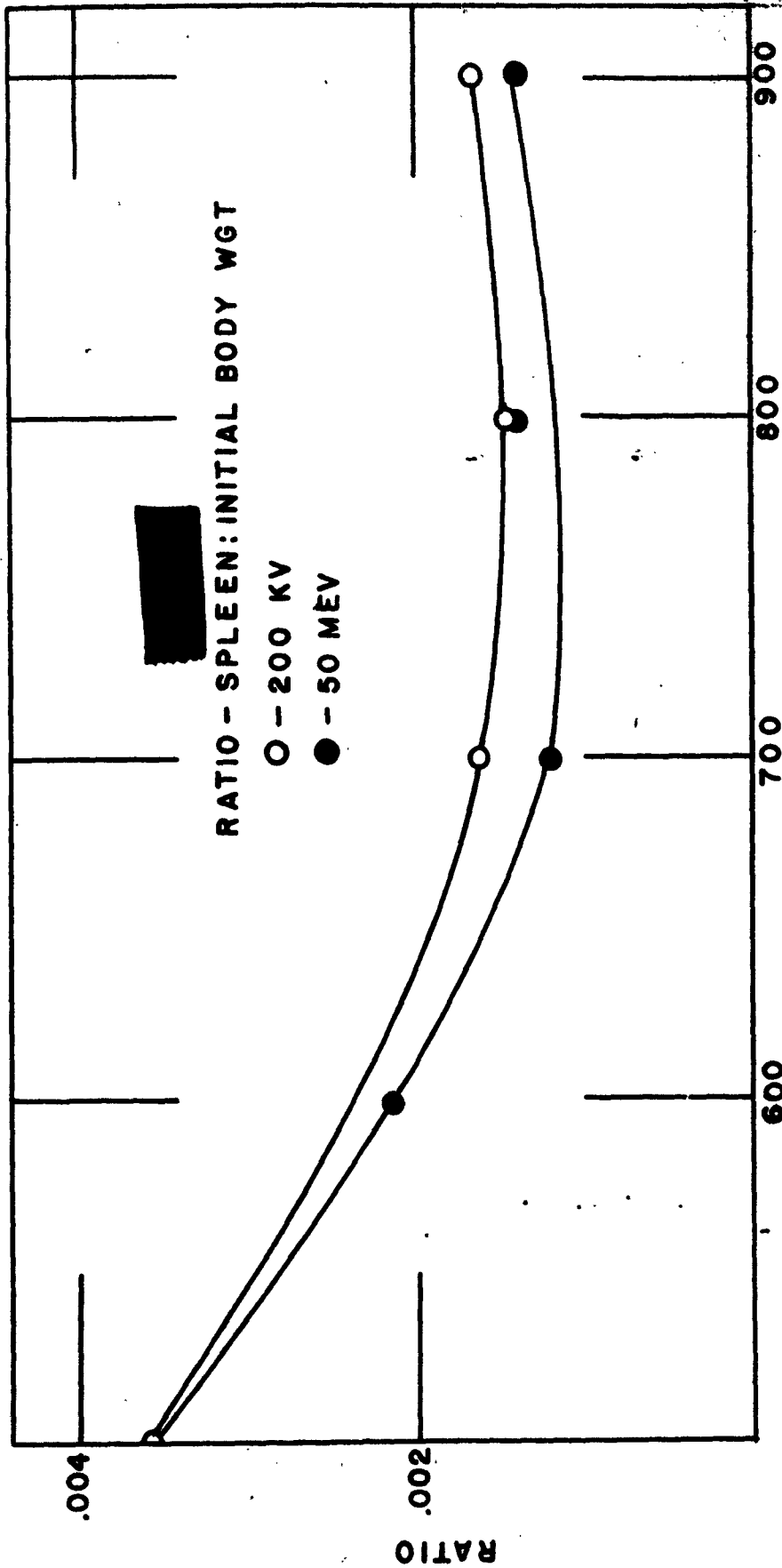
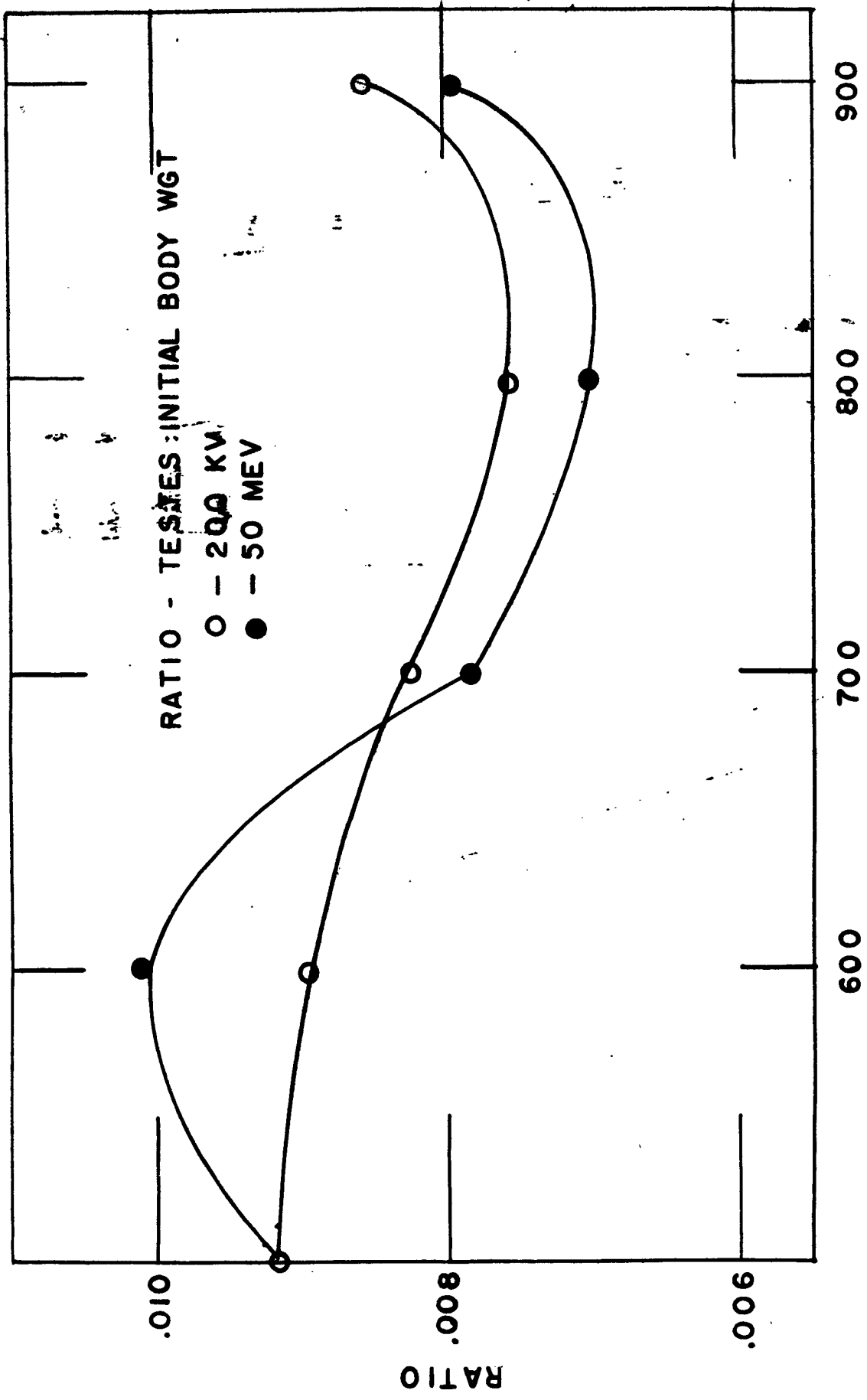


FIG. 24



DOSE - r

Fig. 25



DOSE - r

FIG. 27

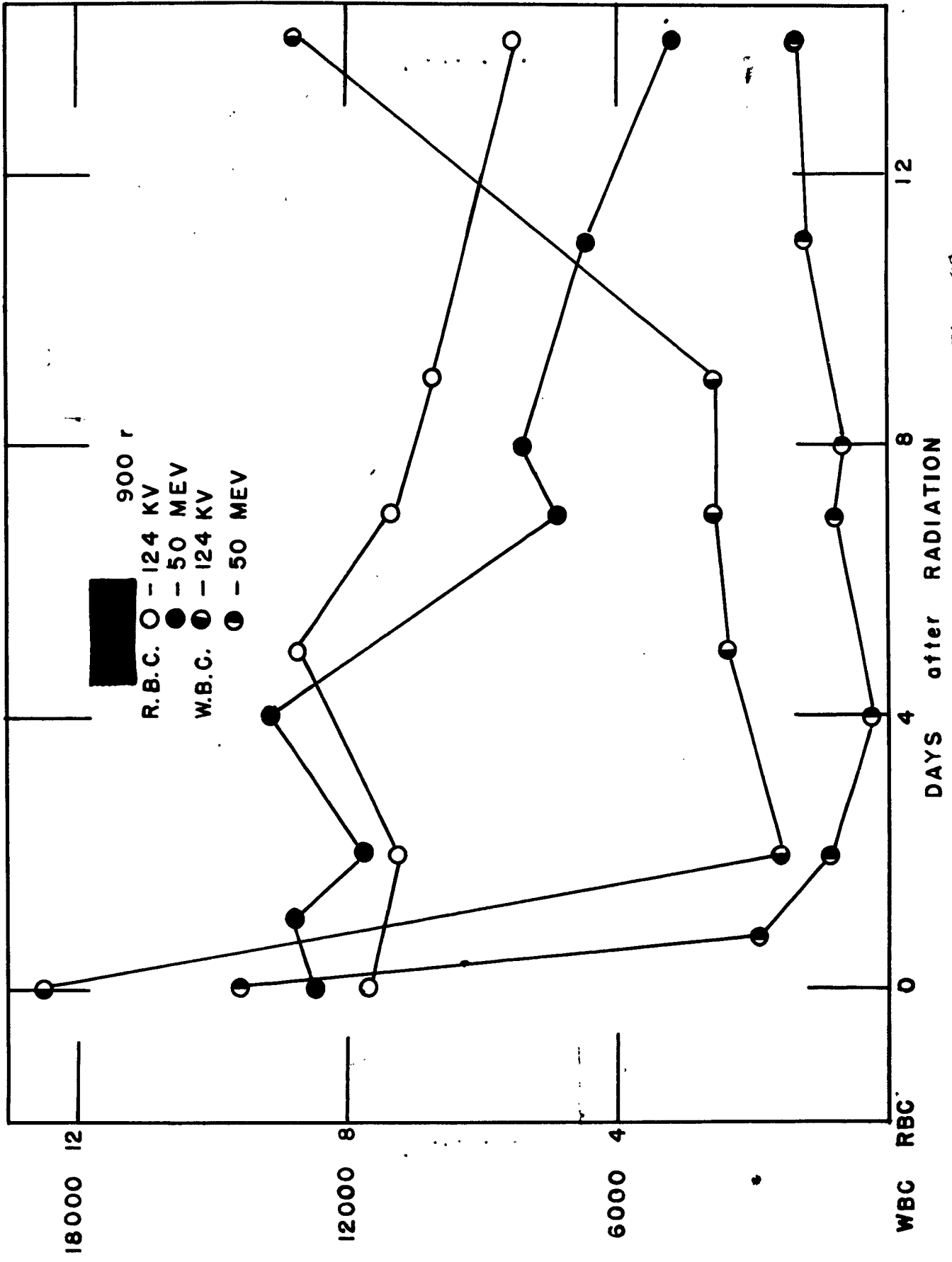


Fig. 28

II. Radiation Genetics of the Parasitic Wasp, Habrobracon.

In the Semiannual Report dated January 1, 1952, page 3, it was shown that X-ray induced recessive lethals could be studied in sperm, metaphase eggs and in prophase eggs.

For the past few months, starting in February, work has been concentrated on the problem of X-ray (200 Kv.) induced recessive lethals. The background for doing this work is briefly, as follows.

During the summers of 1947, 1948, 1949, at Woods Hole, we developed a method for detecting the incidence of recessive lethals induced in the sperm. Males were irradiated and then mated. Viable F_1 females could later be tested for recessive lethals by counting viable larvae and dead eggs and computing the percentage of viable larvae which developed from all eggs laid by a given female. Since the F_1 females were kept virgin, all eggs laid were haploid. Any eggs bearing one or more recessive lethals would then die because of their haploid condition. It should also be noted in this connection that all eggs not bearing lethals would be expected to hatch since Habrobracon eggs normally develop parthenogenetically; and among control F_1 virgins, none has been found which showed a ratio of living eggs to total eggs laid as low as 50%. Figure 1 shows only one female in which the hatchability percentage was low and even that one was 69%. On the basis of these data the total hatchability of eggs laid by control F_1 s is roughly 92%. It would be expected, however, that if a given F_1 virgin had developed from an untreated egg fertilized by a treated sperm bearing one recessive lethal that the ratio of dead eggs: living larvae should be roughly 1:1. If that sperm bore 2 independently assorting recessive lethals, the ratio of dead eggs to viable larvae should be 3 dead eggs: 1 viable larva. Since it has been established that the haploid number of chromosomes is $n = 10$, independent assortment of 2 or more lethals might be expected. Linked lethals also would be found. Data for 10 dosages for the sperm (125r, 250r, 500r, 1000r, 1500r, 2000r, 2500r, 3000r, 3500r, and 4000r) were secured at Woods Hole, but data for only 3 of these are summarized here because these will be used later in this report for comparisons with rates for 1st leiotic metaphase eggs. The table which follows corresponds to figures 2, 3, 4.

TABLE I.

Dose	No. of F ₁ Virgins Tested.	No. of F ₁ * females bearing one or more recessive lethals.	% of sperm bearing one or more lethals.
500r	80	4	5.0 ± 2.4
1000r	84	9	10.7 ± 3.4
1500r	71	11	15.5 ± 4.5

*F₁ females derived from radiated sperm.

It will be noted from the above that the standard errors are large, that many more data are needed. Nevertheless the three values, 5.0, 10.7, 15.50 suggest a straight line relationship.

The above work was done before the author joined the Union College project, but nevertheless is contributed to it. It embraces egg counts totalling 12,744.

Essentially the same techniques as those employed above have been used in studying rates of recessive lethals induced in 1st meiotic metaphase eggs. These eggs, as previously reported, are stored by restraining the virgin females from oviposition. Following such storage of eggs, the females were irradiated and then mated. The cross then is irradiated #33 female x #1 male. All eggs laid during a six hour period following treatment, were irradiated in 1st meiotic metaphase. Only F₁ female (and virgin) progeny derived from these eggs were tested for recessive lethals. The #1 males were untreated. Results of these experiments are given in figures 5, 6, 7 and in the following table:

TABLE II.

Dose	No. of F ₁ virgins tested.	No. of F ₁ females bearing one or more recessive lethals.	% of sperm bearing one or more lethals.
500r	78	4	5.1
1000r	106	15	14.2
1500r	18	5	30.0

Note the comparison and very close similarity between the data for these first meiotic metaphase eggs and for the sperm. The value for 1500r is, of course, very uncertain, although crucial. The reason for the low number is that at 1500r, more than 90% of the 1st meiotic metaphase eggs have dominant lethals. Since one virgin female lays only an average of about 9 eggs and since only 2/3 of these may be expected to develop into females, it is readily seen that a very large number of females will have to be irradiated in order to secure a large number of F₁ virgins. The reasoning is as follows: Suppose 200 females are used in an experiment. Each can lay 9 eggs. But about 95% of these die because they bear dominant lethals. Only 2/3 of the eggs are fertilized and therefore potential females. Therefore $200 \times 9 \times \frac{1}{20} \times \frac{2}{3} =$ expected female progeny. This equals 60. Of these, a large

proportion die because the host caterpillars are likely to "go bad" - or the wasp larvae are likely to be dislodged from the caterpillar. We have in practice been getting about 8-10 F₁ offspring from 50 mothers.

A further interesting point seems to be emerging. If figures 2-7 inclusive are examined at the interval along the abscissa from 40 - 50%, it can be seen that there appears to be a kind of "clustering". Such groupings can be interpreted as representing the number of single recessive lethals. Any at the 25% level can be thought of as representing eggs in which 2 independent lethals were induced. It should be remembered in this connection that extensive work of P.W. Whiting shows that linkage in *Habrobracon* is "very loose" and also that the haploid number of chromosomes is 10. Work of the same author also has shown that one linkage group is between 400 and 500 map units long. As more data on the incidence of recessive lethals are accumulated, it would seem then that the shape of the curves below 50% on the abscissa would be highly interesting and informative. It is perhaps not too optimistic to believe that such curves might be analysed in such a way as to show incidence of single lethals, 2 independently assorting lethals and 2 linked lethals.

The relationship between the incidence of dominant and recessive lethals in sperm and in egg is also of considerable interest. The rates of dominant lethals in the sperm were shown by the author to be roughly 5% at 500r and 30% at 1000r; for the first meiotic eggs, 60% at 500r and about 85% at 1000r. In this connection it should be noted that our earlier work on dominant lethals in 1st meiotic metaphase indicated (by egg counting technique) that the hatchability counts of eggs laid by irradiated females were about the same regardless of whether the mother was mated or not, following radiation. At first sight this would indicate that no recessive lethals were induced. The data at 1500r and 1750r, the highest dosages which can be used, did however indicate a 3 - 5% difference. This suggested that recessive lethals were induced but in terms of the whole gamete sample, in relatively low numbers. It could then well be that a difference of 1 - 2% at say 500r or 1000r would not be easily detected statistically by egg counting techniques. The crucial test as to whether recessive lethals were or were not induced has been presented in this report. It indicates that they are produced. But in what proportion of the total sample of gametes? Suppose that at 500r, there really is a small difference between the hatchability of eggs from mated and from unmated mothers - a difference not readily found by egg counts. This leads to speculation of the sort summarized in Table 3.

TABLE III.

	<u>1st Meiotic Eggs</u>			;	<u>Sperm</u>		
	Dominant Lethals	Recessive Lethals	No Lethals.		Dominant* Lethals	Recessive Lethals	No Lethals
500r	60%	5% x 40% = 2%	38%	:	5%	5% x 95% = 4.75 90.25%	
1000r	85%	14% x 15% = 2%	30%	:	30%	11% x 70% = 7.7 62.3%	

*Heidenthal, Gertrude 1945. The occurrence of X-ray induced dominant lethal mutations in *Habrobracon*. Genetics 30:197-205. Rates for eggs are taken from the data previously reported. (Semiannual Report, June 30, 1950). No claim is made for a high degree of accuracy for the recessive rates because the number of data is still too small. Nevertheless there is reason to believe that orders of magnitude are reasonably represented.

The possibility that in *Habrobracon* we might have not 2 but 3 gametic stages which could be studied for recessive lethals was intriguing. We, therefore, have gathered data on just one dose for the prophase. It has long been known, as a result of the work of A.R. Whiting, that this stage is by far the least sensitive to radiation (LD₅₀ dose = 12,000r). A dosage of 12000r was therefore used as a kind of trial. Previous experience had indicated that the Lawton cathode tube might be useful here. Tests had shown that the metaphase dominant lethals are induced by this type of radiation at approximately the same rate per dose as by low voltage X-radiation. Studies on prophase eggs at 28,000r also gave comparable survival rates. We therefore used the cathode tube with dosage at the rate of 12000r per second. The results are shown in Table 8. We are not, in this connection, claiming that the Lawton cathode tube radiation gives exactly the same effect in our material as low voltage radiation, although at present, we know no evidence to the contrary. However this comparison may turn out to be, it is interesting to note that the rate of recessive lethals for prophase eggs treated with 12,000r (the LD₅₀ dose) is about 7% while that for 1st meiotic metaphase eggs treated with 500 r (LD₆₀ dose) is about 5%.

It is now clear that recessive lethal rates can be secured for each of 3 gametic stages. The plan is to complete the work on 1st meiotic metaphase first; that on prophase will be postponed until after the work on the sperm has also been finished.

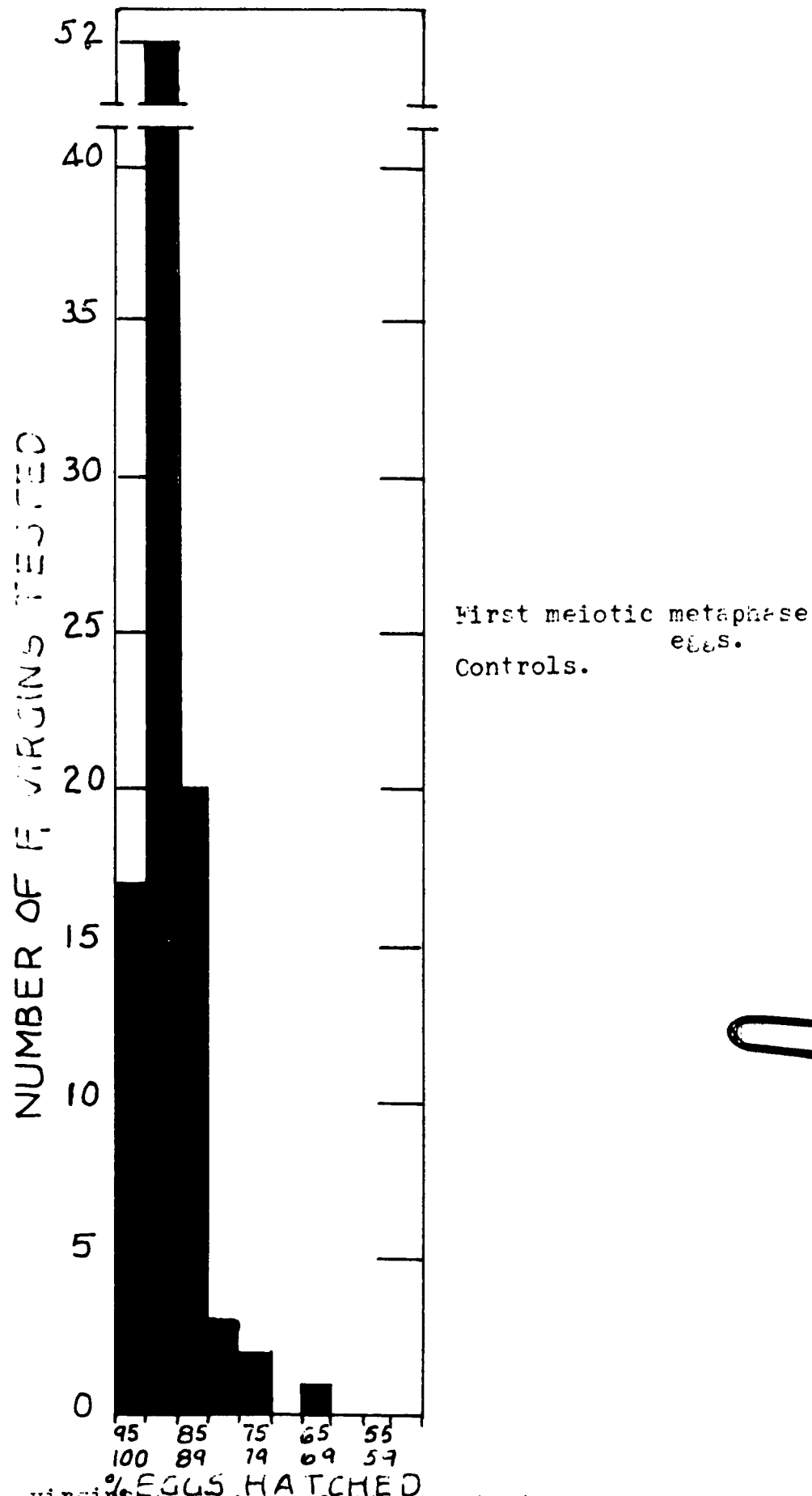


Fig. 1. The number of F₁ virgins tested is plotted against $\frac{\text{number of eggs hatched}}{\text{number of eggs laid}} \times 100$. F₁s developed from stored but untreated first metaphase eggs fertilized by untreated sperm. Percentage of eggs hatched = 91.4%.

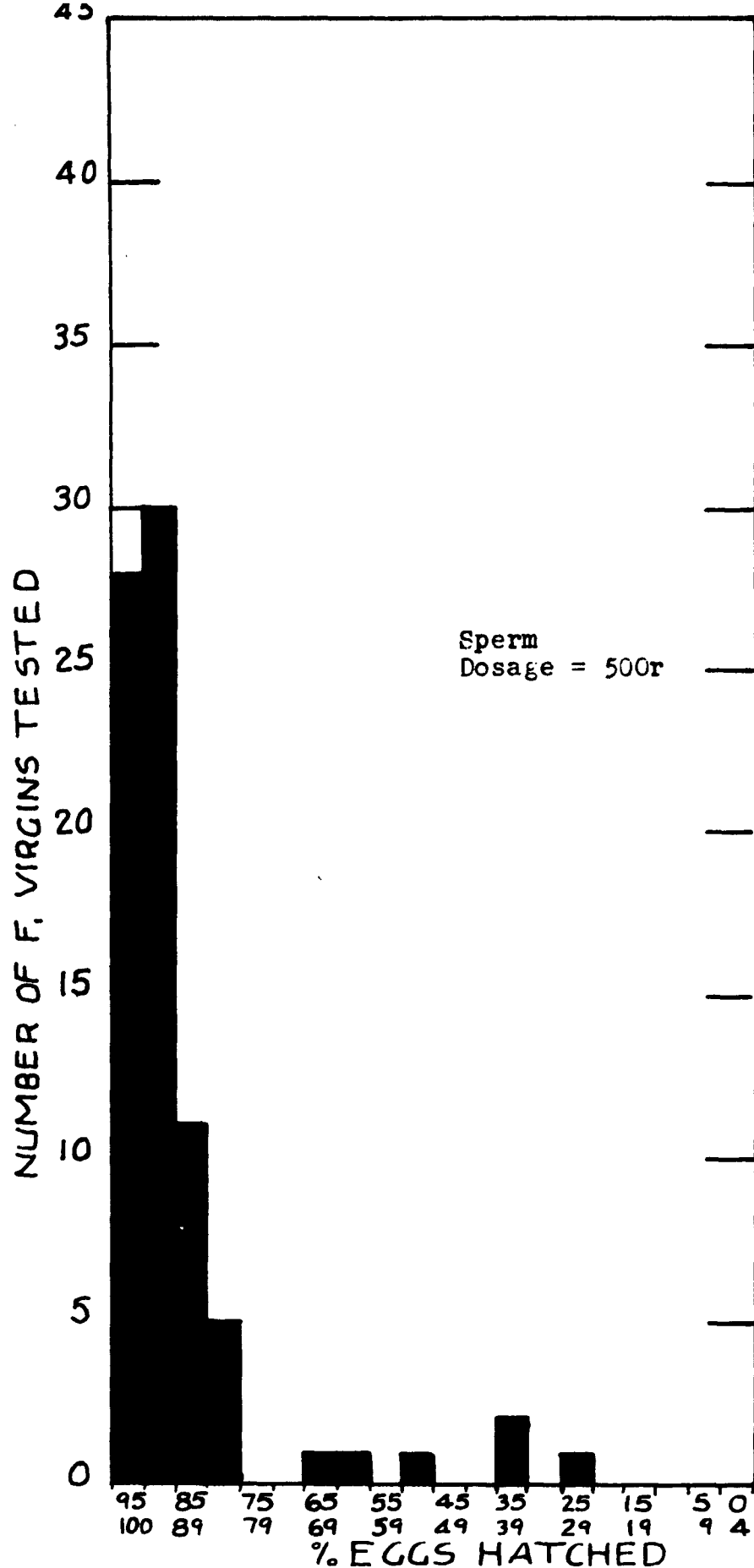


Fig. 2. The number of F₁ virgins tested is plotted against $\frac{\text{number of eggs hatched}}{\text{number of eggs laid}} \times 100$. F₁s developed from untreated eggs fertilized by treated sperm. Percentage of sperm bearing one or more recessive lethals is $\frac{4}{8} = 5\%$

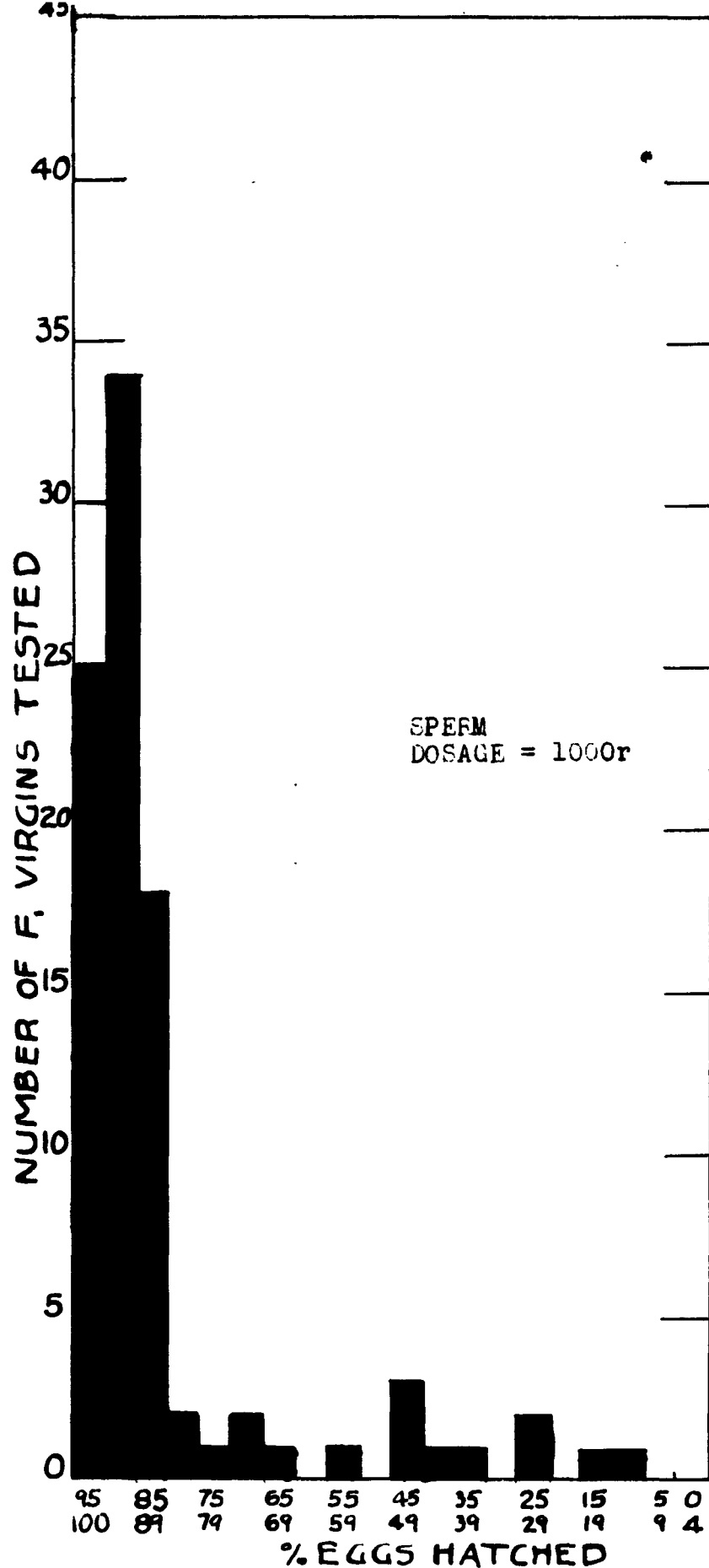


FIGURE 3. The number of F₁ virgins is plotted against number of eggs hatched.
number of eggs laid
 F₁ virgins developed from untreated eggs fertilized by treated sperm.

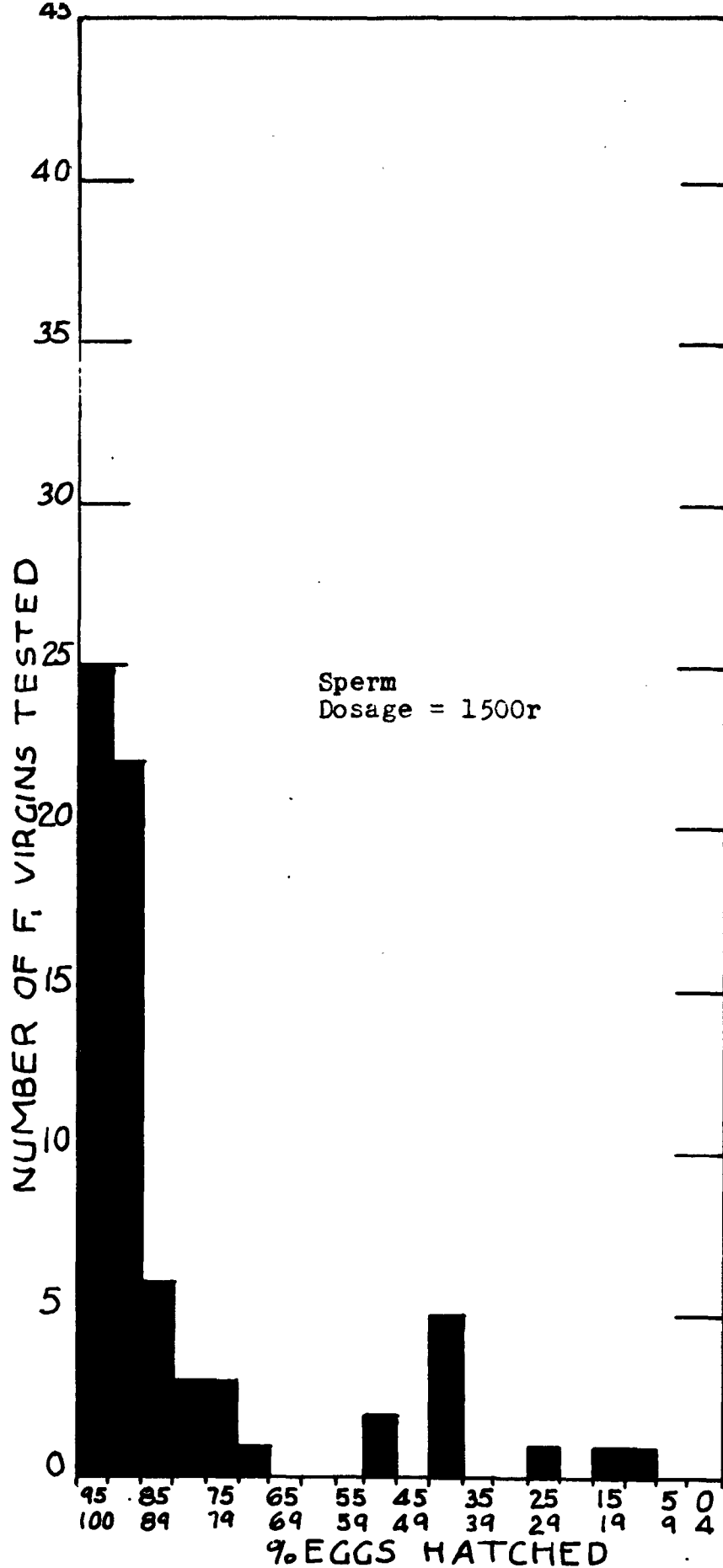


Fig. 4. The number of F_1 virgins plotted against $\frac{\text{number of eggs hatched}}{\text{number of eggs laid}} \times 100$. F_1 s developed from untreated eggs fertilized by treated sperm. Percentage of sperm bearing one or more recessive lethals is 11 - 15 %

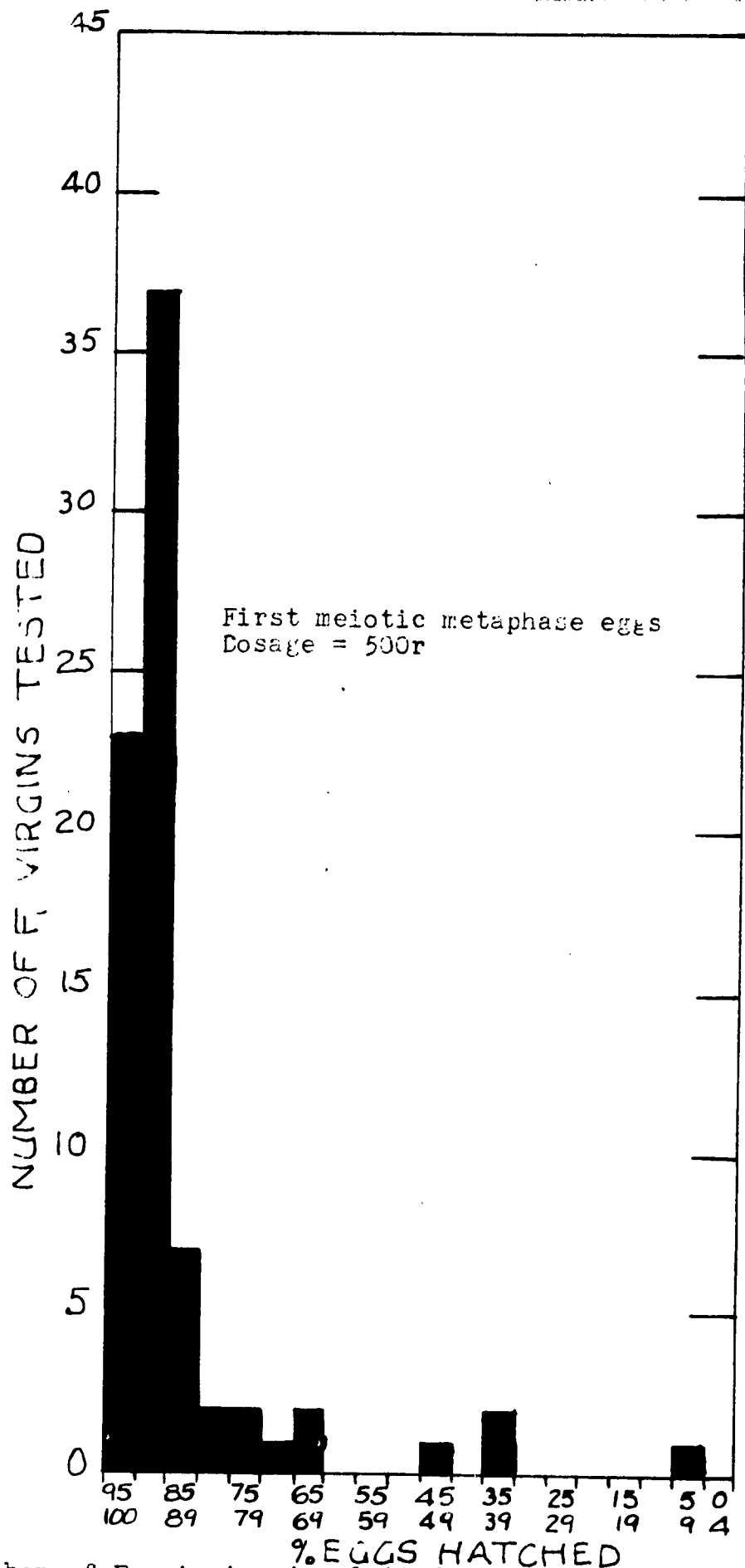


Fig. 5. The number of F_1 virgins is plotted against $\frac{\text{number of eggs hatched}}{\text{number of eggs laid}} \times 100$. Percentage of eggs bearing one or more recessive lethals is $\frac{4}{78} = 5.1\%$

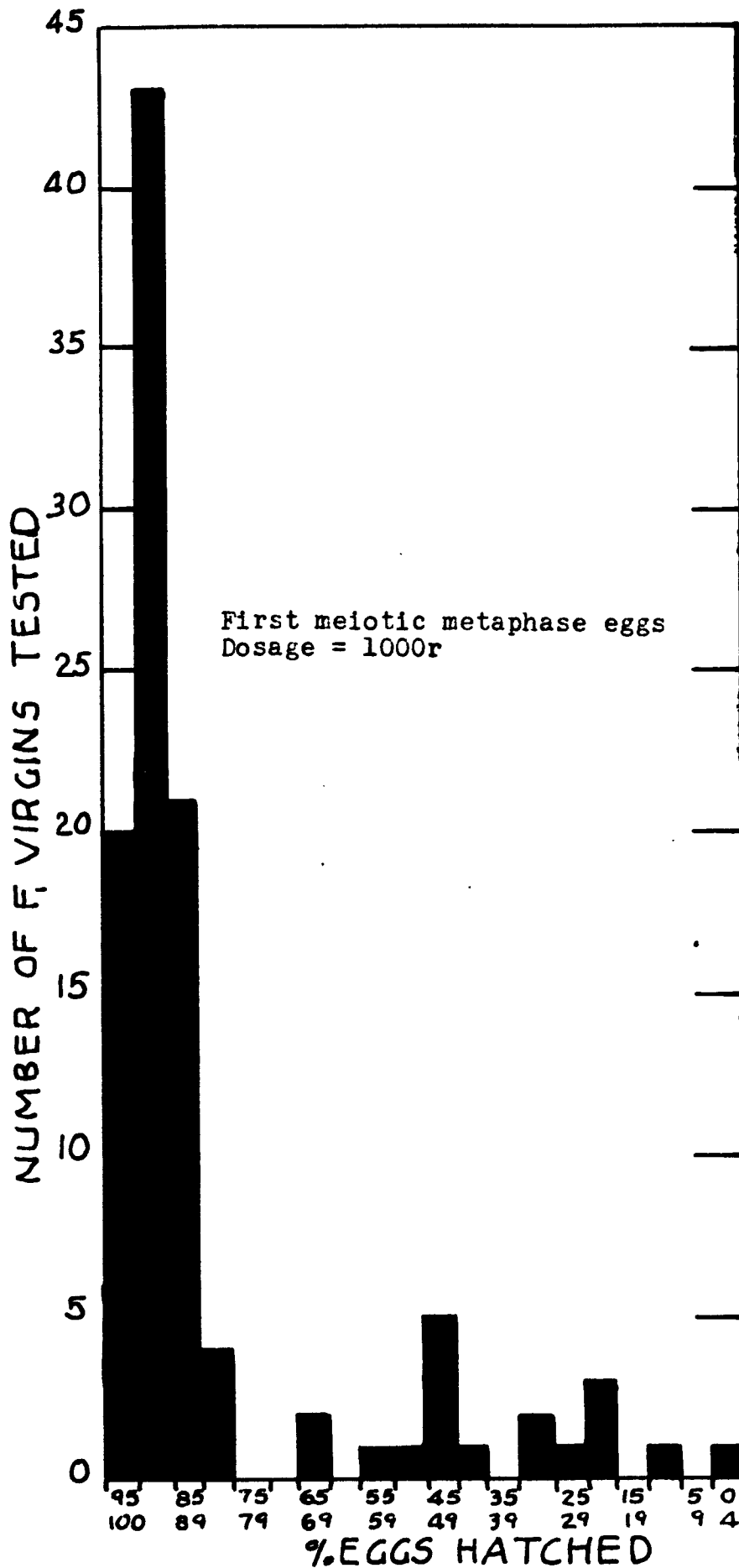


Fig. 6. The number of F₁ virgins tested is plotted against $\frac{\text{number of eggs hatched}}{\text{NUMBER OF eggs laid}} \times 100$. F₁s developed from eggs treated in first meiotic metaphase and fertilized by untreated sperm. Percentage of eggs bearing one or more recessive lethals is

$$\frac{15}{106} = 14.2\%$$

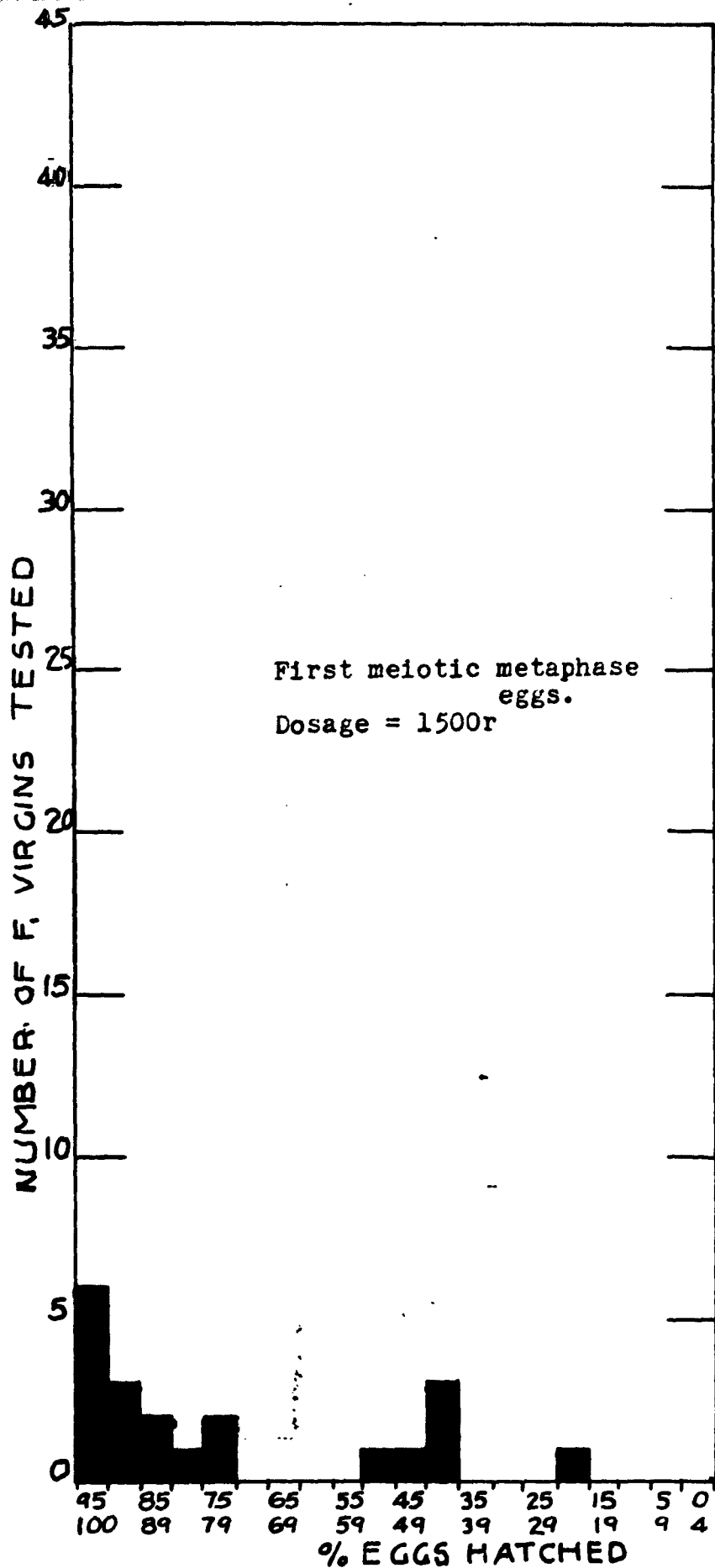


Fig. 7. The number of F₁ virgins tested is plotted against $\frac{\text{number of eggs hatched}}{\text{number of eggs laid}} \times 100$. F₁ virgins developed from eggs treated in first meiotic phase. Percentage of F₁s bearing one or more recessive lethals is $\frac{6}{20} = 30\%$.

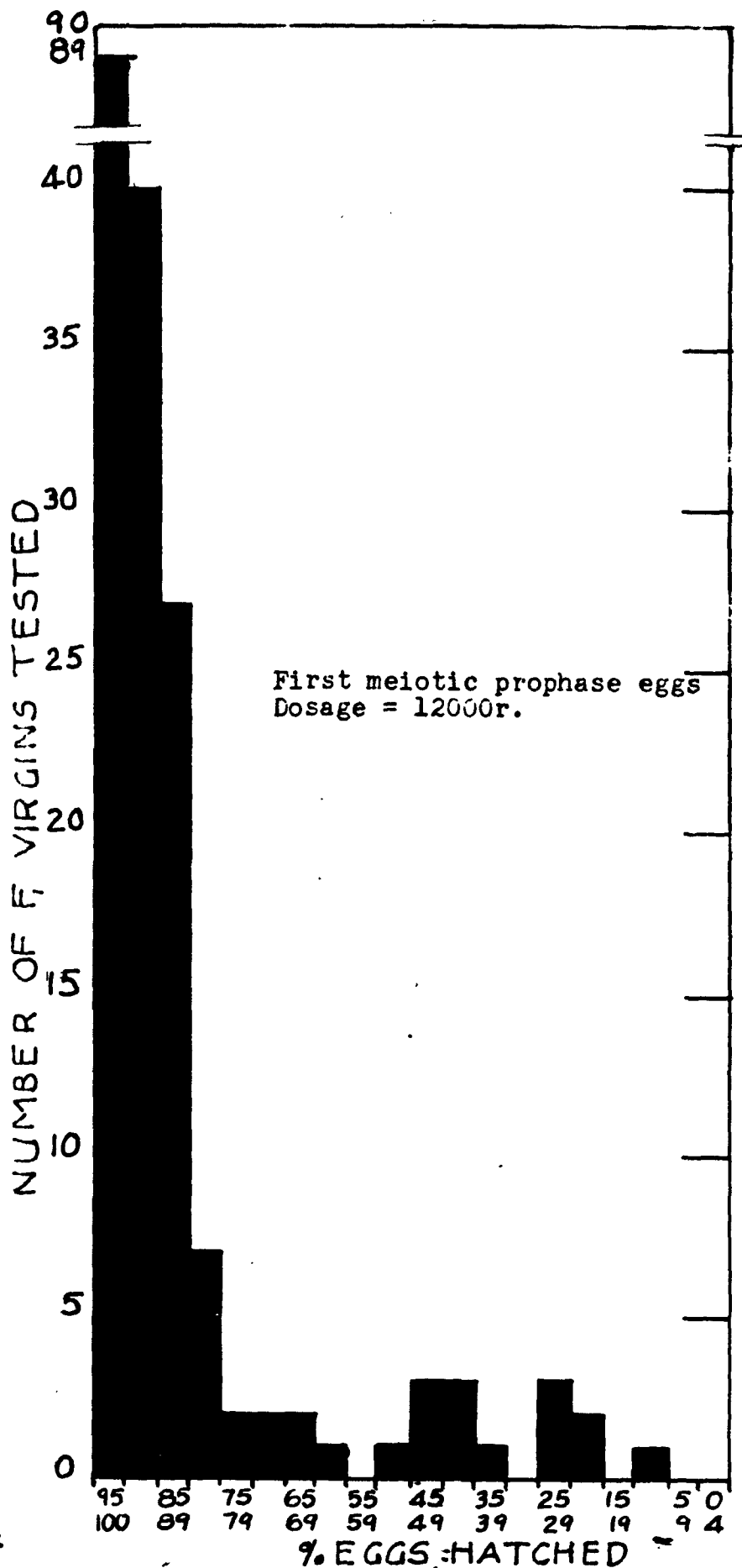


Fig. 8. The number of F_1 virgins is plotted against $\frac{\text{number of eggs hatched}}{\text{NUMBER of eggs laid}} \times 100$. F_1 s developed from eggs treated in first meiotic prophase with 12000r (cathode tube) and fertilized by untreated sperm. Percentage of one or more recessive lethals = $\frac{14}{189} = 7.4\%$

III. The Biochemical Activities of X-Ray Induced Yeast Mutations.

Irradiation of yeast samples of bakers' yeast, *S. cerevisiae* yielded numerous mutants differing from the parent strain in growth characteristics and metabolic behavior.

Studies were initiated on one mutant (46 Y) which lacks the power of utilizing atmospheric oxygen to determine the character of the physiological block.

It has been shown in previous reports that:

- a. Absence of cytochrome could not be the block in atmospheric oxidation. Cytochrome was demonstrated spectrophotometrically.
- b. Poisoning by H_2O_2 products of oxidation could not be assigned as the cause of lack of atmospheric oxidation as the presence of catalase was demonstrated.
- c. The mutant strain is cyanide resistant at pH levels of 6.0 and 7.2 using final cyanide concentrations of 0.005M and 0.01M (KCN). Using CO_2 production from glucose (0.0.M) as a criterion, it was found that cyanide did not inhibit glycolysis.

Present Studies.

- a. Using ribose concentration as an index, the relative concentrations of nucleic acids in the parent and mutant strains were determined (Table 1). Ribose was determined colorimetrically by a modified Mejbaum-orcinol procedure. The data show that at least on a dry weight basis there is virtually no difference between the ribose (nucleic acid?) concentrations of both strains.
- b. From the earliest stages of these investigations, it had been noted that aqueous suspensions of the parent strain were yellow in color, while, in contrast, suspensions of 46 Y were colorless. A microbiological assay of the hydrolyzed cells was performed. Lactobacillus casei E was the test organism, and the methods were those described in the Sixth Edition of the "A.O.A.C., Methods of Analysis."

The data are presented in Table 2, and indicate that there is a distinct difference in riboflavin concentrations of the two strains:

46 Y contains only 85% of the riboflavin in *S. cerevisiae*, control.

The implications of this remain to be investigated. It is planned to study some of the respiratory

systems intermediated by the flavin enzymes to determine whether the found riboflavin difference is evinced in some metabolic process.

- c. One of the theories which may be employed to explain the non-oxygen-utilizing nature of 46 Y is that during the course of radiation a gene enzyme-precursor was eliminated or suppressed; that this enzyme may represent a break in the normal terminal respiratory process; that if this break be 'repaired' by a substitute redox system oxygen utilization could be restored.

To test this hypothesis, different dyes were employed; (Table IV). These dyes ranged from E'_{\circ} (7.0) 0.06 to -0.3.

Although not a dye, the tripeptide glutathione was included. The mutant cells were incubated in the presence of each of the dyes, with glucose as the substrate. Oxygen uptake was measured manometrically.

The data in Table III are of some interest. Glutathione had some stimulatory effect on the oxygen uptake by the mutant cells. The other dyes (with the exception of Nile blue, BXA, National Aniline) tested had no effect. Nile blue was very pronounced in its stimulation. After three hours, some 140 μ l of oxygen had been utilized in the presence of this dye, in comparison with 15 μ l utilized in the dye's absence. This represents an increase of 125 μ l above the dye-less glucose metabolism. When the cells were incubated with a mixture of all dyes, the apparent stimulation can be shown to be due to the action of Nile blue alone (Table III). At the cessation of measurements, the mutant cells plus Nile blue were still steadily utilizing oxygen. (Figure 1). It should be noted that the dye also stimulated the endogenous oxygen uptake.

The implications of this experiment cannot be evaluated as yet. It does appear that a redox system may be added to the metabolising cells, which system may act to bridge the gap between one enzyme system and another (terminal line of respiration?) The E'_{\circ} of Nile blue is -0.10 at pH 7.0. This is likewise the approximate E'_{\circ} of the systems 3 phosphoglycerate - phosphopyruvate.

Whether Nile blue will stimulate oxygen uptake in the presence of 3-P.G.A. or pyruvate remains to be seen.

Finally, it should be stressed that the cell permeability of the non-acting dyes must be tested. It may be that some one, or more, of the dyes

would be active, could they penetrate the cell. Work along this line will be performed with cell-free systems.

The theory presented in (3) of this report appears to have some validity. One would not expect an enzyme-substituted dye to have the same activity or efficiency as the normal enzyme system. This is borne out by the data.

Table I

Comparison of ribose content of mutant and normal yeast.

	46 Y	C
Micrograms ribose per mg dry wt, cells	28.2	29.8

Table II

Comparison of Riboflavin content of mutant and normal yeast.

	run #	46 Y	C
Micrograms RF per microgram dry wt., cells, X 10 ⁵	1	4.38	5.19
	2	4.79	5.64
	3	4.57	5.18
	average	4.58	5.37

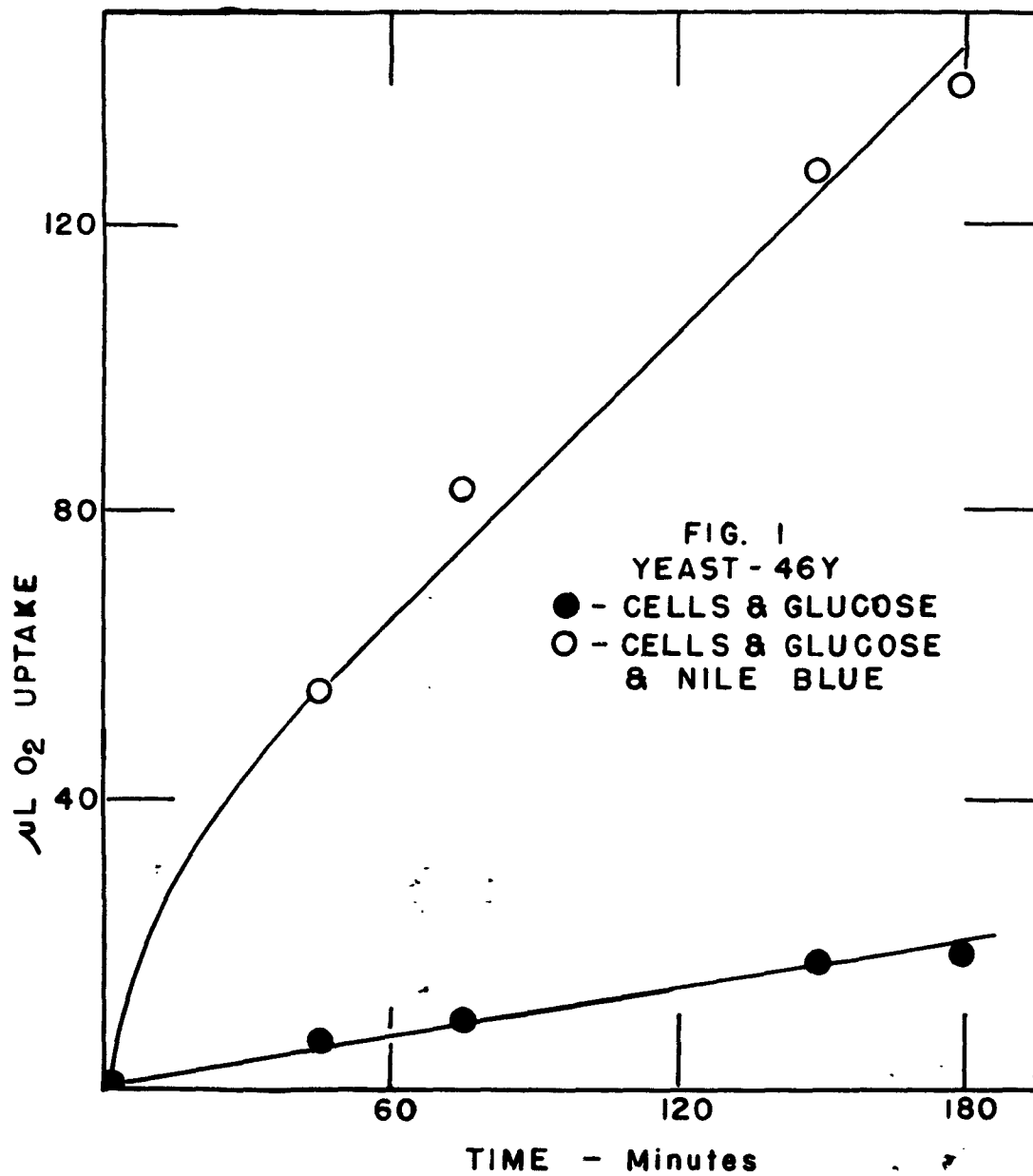
$$\text{Ratio } \frac{\% \text{ RF}_{46Y}}{\% \text{ RF}_C} = 0.854$$

TABLE III

Effect of Dyes on Oxygen Utilization by 46 Y

Dye (1/25,000, aqueous)	μ l O ₂ utilized in presence of glucose (corrected for endogenous).	O ₂ utilized, corrected for glucose.
-	15	0
Glutathione	54	39
Methylene blue	18	3
Thionone	15	0
Neutral red	24	9
Indigodisulfonate	10	-5
Gallocyanin	19	4
Brom-cresyl-blue	36	21
Nile blue	139	124
All dyes in mixture	158	143
All dyes in mixture, but without glutathione	119	104

Cells, 10%; dye in final con. of 1/25,000; glucose 0.05 M,
pH = 7.0. Temp = 30, Time = 3 hrs.



PUBLICATIONS

The status of publications of the material may be summarized as follows:

- Heidenthal, G. & L.B. Clark -
Survival Rates of Habrobraccon eggs treated in first meiotic metaphase with low and high voltage x-radiation. Records of the Genetics. Soc. Amer. 1951, #20, p. 102.
- Clark, L. B. and P. Sykowski.
A Comparison of the Effect of Low (124 KV.) and of High (50 Mev) x-radiation on the Incidence of Lens Opacities in Mice. 1951. Amer. Jour. Opthal. 35, pp. 848-851.
- Paretsky, D. & L. B. Clark.
Studies on the biochemistry of x-rayed yeast. Paper read - Meetings of American Society of Bacteriologists - April 1952.
- Baldwin, G. & L. B. Clark -
Radioactivity induced in yeast by 100 Mev. x-radiation (in Press).
- Clark, L. B.
Comparison of the Biological Effects of low and high voltage x-rays. I.
The effects on yeast. (in preparation)
- Clark, L. B.
Ibid. II.
The effects on growth of wheat seedlings. (in preparation).
- Clark, L. B.
Ibid. III.
The effects on survival time, weight changes, longevity and blood picture in white mice. (In preparation).
- Gorham, L.V., L. B. Clark, D. Remp and K. Olson.
Comparative effect of high and low voltage x-radiation on biological materials. Paper read - Meetings of Association Cancer Res. April 11-13, 1952. (This was a progress report on comparison of ionization distribution of low and high voltage x-rays on survival, liver and spleen catalase, blood picture and organ weights in rats.