THE EFFECTS OF RADIATIONS ON BIOLOGICAL SYSTEMS

I. Influence of High-Frequency X-Ray Radiation upon the Duration of the Prepupal Period of Drosophilae

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Some results of investigations conducted in this laboratory concerned with the influence of x-ray irradiation upon the duration of the prepupal period of *Drosophila melanogaster* have been reported[1,2] previously. In every instance an extension of the larval period resulted. In the case of high-frequency radiation, investigations of the course of such effects could not be extended as much as was desired, on account of the low power of sources then available. However, over the early part of the course it was definitely indicated[1] that the duration of the prepupal period was an increasing function of the time of irradiation under the given conditions. In the absence of more powerful sources investigations of effects of more extensive irradiation were conducted[2] with the inclusion of radiations of lower frequency in order to increase the available power. The demonstration of a maximum point of effectiveness of prolonged irradiation (in extension of the mean prepupal period, ϕ) was a striking result. It is the purpose of the present communication to give the results of similar experiments employing high-frequency radiation from a more powerful source.[3]

Preparation and Maintenance of Larvae

The *Drosophilae* employed in the present and former work[1,2] were raised in distinct generations from an original culture obtained from Dr. J. H. Northrop

[1] Hussey, R., Thompson, W. R., and Calhoun, E. T., *Science*, 1927, **66**, 65.

[2] Tennant, R., *Science*, 1931, **73**, 567.

[3] A Coolidge water-cooled tube kindly supplied by the General Electric Co. (General Electric X-Ray Corp.).

who had grown the strain for many generations under aseptic conditions, and have been maintained under similar conditions at 25°C. (approximately within 1°) in this laboratory. The stock food consisted of a mixture of 1 part of thoroughly ripened banana to 3 parts of yeast[4] by weight to which was added 2 ml. of glacial acetic acid for each 100 gm. of yeast used, the whole being autoclaved for 45 minutes on each of 2 successive days at approximately 121°C. This autoclaving technique was employed in preparing all foods, water, flasks or voile filters for use in the present work. Flasks for seeding and generation were prepared with side tubes for delivery of imagos *via* an aseptic rubber tube ordinarily fitted with a glass plug; those for stock containing a sufficient amount of the food described above for maintenance upon absorbent cotton, and those for preparation of larvae for experiment containing 6 ml. of a food consisting of 15 gm. of yeast to 20 ml. of beef infusion agar (pH 7.0). The flasks were ordinarily Pyrex Erlenmeyer flasks of 125 to 200 ml. capacity; the neck being fitted with a plug of non-absorbent cotton wrapped in cotton gauze at its base and covered with a paper cap. Seeding flies (of age not exceeding 9.5 days) were used in every instance for an interval between 23.5 and 24.5 hours in a given flask.

In the case of stock flasks additional food was added 24 hours after removal of the parent flies. This food consisted of a mixture of 454 gm. of yeast and 400 ml. of distilled water plus 1 part to 50 by volume of glacial acetic acid, autoclaved in the usual way. 10 ml. of this food were used (added as stated above through the delivery tube) for each stock flask, and in the case of the yeast-agar flasks (for experimental larvae) 4 ml. of this food were added to each flask immediately after removal of the parent flies, and the flask left tilted at an angle of about 60° from the normal vertical position for 24 hours, after which it was returned to the vertical position.

Ordinarily, 4 days after the commencement of generation the larvae of the yeast-agar flasks were ready for experimentation. The median age of larvae at any given instant is taken as the time elapsed since the mid-point of the seeding interval, α. Thus, ordinarily, irradiations were started at a time when $\alpha = 3.5$ days, approximately, which was the same in earlier work so far reported.[1,2] The larvae were washed with sterile tap water onto sterile voile filters and freed from food and extraneous matter with further washings and the aid of camel's hair brushes. Then the larvae were distributed in random samples in wells in blocks made of stearin-paraffin (1 part by weight of stearic acid to 3 parts of paraffin—the melting point of the latter being 52–54°C.). The wells were cut in a plane surface, and were cylindrical in shape (diameter = 25 mm. and 5 mm. deep). They had three pieces of filter paper (Whatman No. 2) fitted in the bottom upon which the larvae were placed. Then 0.1 ml. of sterile tap water was added to each well and a cover of filter paper permeated with stearin-paraffin sealed over its top. Unless otherwise specified, the cover was then perforated with a needle in a uniform manner so as to permit ventilation. Aseptic technique was employed throughout.

[4] The yeast was supplied by the Fleischmann Yeast Co.

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All stearin-paraffin blocks were of standardized thickness, 25 mm., and those used in irradiations were arranged so that alone or fitted together as the case might be they formed a rectangular prism 250 × 250 × 25 mm. with the plane surface (250 mm. square) uppermost in horizontal position. In the case of all but the depth experiments the wells were cut in this surface. In the depth experiment a slight difference existed in order to permit ventilation even when such blocks were stacked as will be described later.

In all irradiations a stack of blocks of this type was set so as to be inscribed in a rectangular prism with horizontal cross-section 250 mm. square and what will be called its *axis of reference*, through the centers of these cross sectional squares, vertical and passing through the center of the target of the x-ray tube. In describing the situation of wells the distance between the axis of a given well and this axis of reference when in position for irradiation will be called d, and the vertical distance (difference in elevation) between the target center and well top will be called D. The axis of any well in arrangements for irradiation was always in a plane through the ideal circumscribing prism's diagonally opposite vertical edges, and the prism arranged so that such a plane made an angle θ, with the axis of the x-ray tube (which was always in the horizontal position customary for direction of radiations for use vertically beneath the tube), θ being 45° in the case that will be called *Phantom Form 1* and being equal to either 0 or 90° in *Phantom Form 2.* Furthermore, in order to avoid circumlocution in describing irradiations, in addition to the above definitions let Z be the distance of a given point below the uppermost horizontal plane of the above mentioned circumscribing prism; and in particular, let the value of Z for the points on the corresponding lowest plane be called the *phantom depth*, and the value of Z for the uppermost points of a given well be called its *depth in the phantom*, λ. Thus for wells situated at the upper plane surface of the phantom, $\lambda = 0$, which was the case in most of the experiments to be described below.

The details of irradiation technique will be given separately in description of particular experiments using the present outline as a foundation in order to save repetition where treatment was uniform as was the case following irradiations in that control or irradiated larvae were transferred to small wide-mouthed flasks (as nearly at the same time as practicable) containing enough yeast food on cotton for their maintenance while under observation, and subsequently observed at definite intervals (about $\frac{1}{2}$ day) for pupation, all pupae formed being removed, counted, and the number for each class recorded together with the time of observation. Thus, in an obvious manner, the mean age of pupation, ϕ (estimated from the mean time of seeding as is α) or the corresponding median time of pupation, ϕ', could be calculated.

As in preceding work, the greatest care practicable was exercised to prevent contamination. In the experiments reported, transfers were made of flask contents at the end of pupation observations to beef infusion broth and kept at 37.5°C. for 48 hours, during which time they were observed at intervals without ever detecting growth.

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Instead of continuing observation until all larvae had either pupated or died, the alternative of cessation in accord with an arbitrary criterion was adopted; namely, if an interval of more than 1.9 days elapse in which it be observed that less pupae were formed than one-twentieth of the total formed in a given lot, then further observations on this lot were excluded in calculation. This instituted much saving in time and space required for the work without altering the averages greatly (mean or median) and affected increments in such averages as the result of irradiation or other treatment even less.

EXPERIMENTAL

Experiments were carried out in a manner similar to those reported[1, 2] previously to trace the course of variation of the median prepupal period, ϕ', with duration of irradiation, t, under otherwise fixed conditions. In terms of the variables defined above these were as follows:

Radiations generated by a Coolidge water-cooled tube impressed by 191 kv. (peak) with current of 30 ma., were passed through a horizontal circular aperture 380 mm. below the target center and 180 mm. in diameter and through filters immediately beneath this of 0.5 mm. of copper and 1.0 mm. of aluminum to the phantom in Form 2 (phantom depth = 175 mm., $\lambda = 0$, $D = 500$ mm., and $d = 60$ mm.). As in all previous work the top layer of the phantom consisted at all times (in course curve investigation) of four blocks, 25 by 125^2 in millimeters, with well of standard type drilled in the upper square surface 60 mm. along a diagonal from a corner placed in touch with the similar corners of the companion blocks. Eight such blocks were used in each experiment; No. 8 being the unirradiated control, No. 7 being irradiated for the full irradiation time of all, and the pairs, 1 and 6, 2 and 5, and 3 and 4 having the sum of irradiation intervals for each pair equal to that of No. 7. Thus all irradiations in a given experiment were performed during the irradiation of No. 7 by successive replacements with slight interruption; the chronological order of the replacements being reversed in alternate experiments of this sort and the orientation of the pairs in irradiation being varied so as to minimize effect upon the means of several experiments which might be involved. The results of four experiments of this type are given in Table I and represented graphically in Text-fig. 1, where the presence of a maximum point is obvious as is the subsequent decline in effective-

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ness from the point of view of increasing the larval period to an almost level plateau, the curve being much like that already presented[2] in the case of unfiltered radiation. The mean and median prepupal periods give essentially the same form of curve (both are given in the case of the first experiment of Table I), but the median alone will be used in work described below because of greater simplicity in the making of necessary observations and calculations.

In connection with these course curves (Text-fig. 1) it should be stated that in the first report[1] on such work any attempt to approximate the mean prepupal period, ϕ, by an asymptotic logarithmic curve

TABLE I

Influence of the Period of Irradiation upon the Duration of the Prepupal Period

Experiment............	1	1	2		3	4
t	Mean ϕ	Median ϕ	Median ϕ	t	Median ϕ	Median ϕ
0	(5.82)	5.73	5.65	0	5.16	5.21
40	(7.13)	6.90	7.10	50	7.33	7.27
80	(8.96)	8.84	9.19	65	8.18	8.40
120	(8.49)	8.41	8.69	80	8.76	9.00
160	(7.84)	7.72	7.82	95	9.62	9.36
200	(7.66)	7.61	7.70	110	9.69	9.41
240	(7.75)	7.54	7.71	125	9.32	8.77
280	(7.77)	7.69	7.72	175	8.27	7.96

as an increasing function of the period of irradiation, t, was definitely avoided; as was any attempt to explain the results upon the basis of destruction of enzymes in irradiation, which subject has been studied, however, as another means of approach to an understanding of the influence of radiations upon biological systems.[5] Indeed, evidence already at hand but which was considered unready for publication at that time led to the work previously mentioned[2] in which more extensive x-ray irradiations were obtained by eliminating the copper and aluminum filters used in the present work. The form of the curves thus obtained,[2] together with those of the present experiments (Text-fig. 1) definitely contradict the proposition of fit of ϕ to an increasing

[5] Hussey, R., and Thompson, W. R., *J. Gen. Physiol.*, 1922–23, **5**, 647; 1923–24, **6**, 1, 7; 1925–26, **9**, 211, 217, 309, 315; 1931–32, **15**, 9.

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exponential function of *t*. However, another author[6] has employed the data of our earlier work[1] for this purpose and in his presentation

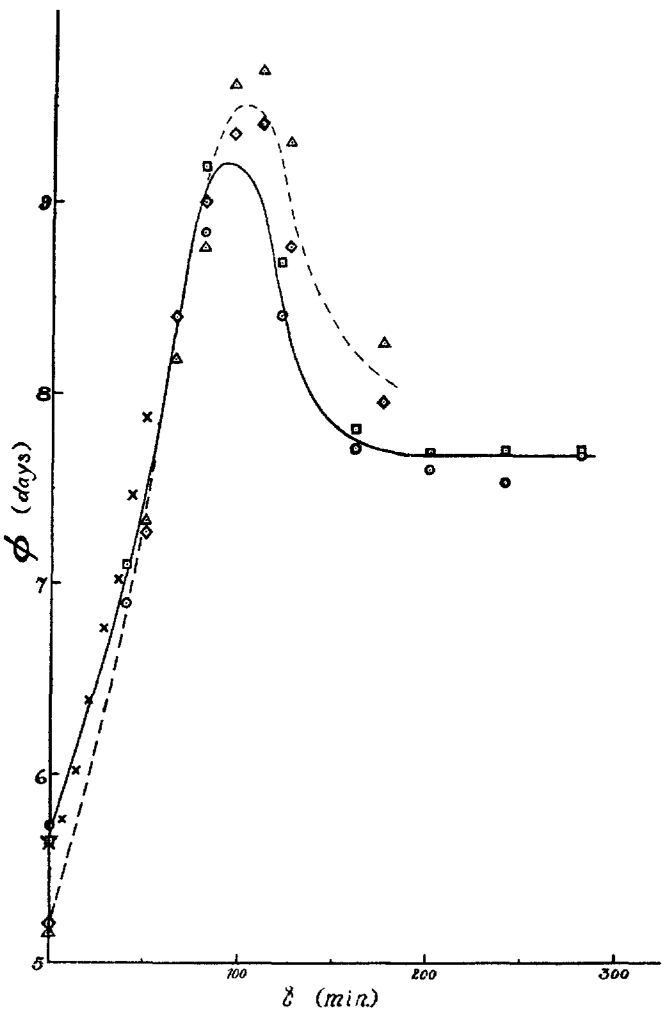

FIG. 1

the approximation appears fairly close. It happens only too often in presentation of biological data that attempts are made to represent

[6] Koidsumi, K., *J. Soc. Trop. Agric.*, 1930, **2**, 258.

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the observations by simple logarithmic curves, apparently as a result of the success with which they have been employed in physical chemistry. Brooks[7] and others have emphasized this fact. In some instances cited by Brooks even the exclusion of the early part of a series of observations as a *period of incubation* has been undertaken in order to fit the data to the favored curve, but in the present instance the difficulty presents itself in the form of a *maximum point* in the middle of the experience.

In Text-fig. 1 the data of the former experience[1] (with approximately the same quality of radiation as at present employed) in the study of the course of effect of irradiation upon the prepupal period are given with the abscissae equal to the time of irradiation times the factor, $\frac{5\ (50)^2}{30\ (54)^2}$, which is the estimated relative intensity of radiation in the former work. The points are indicated by the cross (X) in the figure, where the approximation to the present results on this basis in spite of technical differences is obvious particularly as to the slopes of the curves. This indicates the possibility that within wide limits a close approximation of the same results may be had when the intensity and time of irradiation are varied inversely under otherwise fixed conditions as to quality of radiations and technical handling. Experiments dealing with such relations have been planned for future work.

Another series of experiments were carried out with wells in a phantom of the same dimensions as that for the course curve but with a well in the center ($d = 0$) of the square upper face as well as the four at $d = 60$ mm. and with the phantom in the *Form 1*. The well-block was made as a single piece (25×250^2 mm.) containing the five wells as all irradiations were planned to be equal and simultaneous. These wells and the larvae lots irradiated in them will be designated as follows:

A—coaxial with the phantom and radiation cone, B and D having their line of centers parallel with the axis of the x-ray tube, B on the cathodal side (North), and D on the anodal side (South) relative to A, and C and E being in the relative *West* and *East* positions, respectively. Thus the four wells lay (relative to centers) on a circle of radius equal

[7] Brooks, S. C., *J. Gen. Physiol.*, 1918–19, **1**, 61.

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to 60 mm. in quadrant positions and with A at the center vertically beneath the target centroid. Aside from the period of irradiation which was 60 minutes in each instance, other conditions were as described for the course curve experiments above, including a control, S. For the sake of brevity, the values of ϕ, the median prepupal period, observed in four such experiments are not given in Table II but rather the corresponding values of the ratio (R) of the excess of these values over those for S to the mean of such excess for the four symmetrically placed wells (B, C, D, and E) in each experiment, which furnishes a more satisfactory basis of comparison. Indeed, in view of the results

TABLE II

Relative Effectiveness of Radiations at Given Points on the Upper Surface of the Phantom

Position	A (central)	60 mm. from the center			
		B (cathodal) north	C (medial) west	D (anodal) south	E (medial) east
Experiment	*R*	*R*	*R*	*R*	*R*
1	1.16	0.98	1.04	1.01	0.98
2	1.09	1.04	0.97	0.96	1.03
3	1.06	0.98	1.03	0.92	1.07
4	1.09	0.98	1.00	0.98	1.05
Mean R......	1.10	1.00	1.01	0.97	1.03
A. D.........	0.015	0.013	0.013	0.014	0.014

given for the course curve (in this region being approximately linear) R may be taken as an indication of relative effectiveness. No significant difference appears in the case of the four symmetrical wells, but about 10 per cent greater effectiveness at the center position, A, is indicated with an A. D. of approximately 1.5 per cent in each instance.

An unusually interesting result was observed in a companion to the above experiments, the same arrangement being used except that Well A was left empty and C and B were not provided with ventilation as in the case of an additional control. The period of simultaneous irradiation was 50 minutes under conditions otherwise essentially the same as in the above mentioned experiments of Table II, with the following results:

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	Control (ϕ_o)	Irradiated (ϕ)	Mean ($\phi-\phi_o$)
Ventilated	5.81	7.74, 7.75	1.94
Unventilated	5.92	6.87, 6.86	0.95

The mean number of pupae removed per well was about 450 in the above experience, being 528 for the regularly ventilated control and 395 for the other, and for B, C, D, and E being 428, 429, 472, and 458, respectively. The approximate halving of the effect by omission of ventilation during irradiation appears significant. A qualitative difference in the distribution of larvae in the wells when they were unsealed was so marked that it seems worthy of mention that independently of irradiation (control and irradiated lots alike) in the wells whose covers were unperforated the larvae were massed upon the bottom, almost all being in one contiguous heap, whereas in those wells having covers perforated in the usual manner, the larvae were greatly dispersed about the cover, sides, and bottom.

Another experiment was performed (in duplicate) employing the same conditions of irradiation as above, all well covers being perforated as will be implied unless otherwise stated hereafter. The larvae in the four wells simultaneously irradiated, however, differed in median age by successive intervals of 0.5 day, having parents from the same generation allowed to seed flasks at intervals in advance fixed so as to make this possible, a control well corresponding to each well irradiated being maintained. The results are given in Table III, where a correlation of increase in both ϕ and ϕ_o with age at the time of the confinement in the wells is apparent, ϕ_o being the median prepupal period for the control and ϕ that for the irradiated lot of larvae (here in one to one correspondence).

In the previous report[1] of effects of high-frequency x-ray radiation on ϕ it was pointed out that the larval system in paraffin or other phantom wells might be used as a means of estimating the effectiveness of radiations from a given source at different depths in such phantoms. The need of uniform ventilation in such studies is obvious in view of the results of the ventilation experiment described above. If stacks of blocks (each 25×250^2 mm.) are to be used as in the phantoms so far employed in this laboratory, a convenient form for the well-blocks (which may be used for either depth or upper surface irradiations) is the following:

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The regular size (25×250^2 mm.) phantom unit block of stearin-paraffin mixture with a cylindrical well (3 mm. deep and 50 mm. in diameter) cut in the center of the upper square face with another well cut coaxially in this well of the usual type for holding the larvae to be irradiated (5 mm. deep and 25 mm. in diameter) and a rectangular groove cut in the same face (3 mm. deep and 10 mm. in width) with sides parallel to plane faces of the block.

Obviously, larvae may be sealed in the small well in the usual manner, the cover perforated, and the block placed in any desired position

TABLE III

Variations in effects of a uniform treatment of larvae in different stages of development, comparing correlated effects in control and irradiated lots.

Experiment	Median age at time of irradiation (α)	Control (ϕ_0)	Irradiated (ϕ)	($\phi - \phi_0$)
1	3.0	5.36	7.36	2.00
2		5.43	7.22	1.79
1	3.5	5.61	7.48	1.87
2		5.67	7.19	1.52
1	4.0	5.71	7.68	1.97
2		5.84	7.68	1.84
1	4.5	5.84	8.09	2.25
2		5.89	7.79	1.90

in the stack (*e.g.*, in the seven-block phantom) with the groove in a conventional position. In the experiments to be described this groove was always parallel to the x-ray tube axis, Phantom Form 2 being used. Thus ventilation could be provided by means of a fan, the groove and large well forming a ventilation system when a block was placed above it as all block bottoms are plane in the system described. This arrangement was more satisfactory than that used in the experiments reported previously[1] and has been employed uniformly in investigations of effectiveness of radiations at depths in this phantom.

After certain preliminary investigation it appeared as if the effectiveness of radiations of the quality and intensity used in the work

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described above might be approximately 0.82 times as effective at a depth of 25 mm. as on the upper surface in the phantom (Form 2) 50 cm. from the source. Accordingly, data were collected in four experiments in which two lots of larvae were irradiated in the top block position for 40 and 60 minutes respectively as in the course curve work, but in the standard central-vent well as employed at other positions and intermediately a lot of larvae was irradiated in the position of second block (25 mm. lower) for 61 minutes, all larvae in a given experiment being random samples from the same supply of larvae prepared as in the course curve experiments, approximately 3.5 days old. All larvae

TABLE IV

Relative Effectiveness of Radiation at Top and at Depth of 25 Mm. in the Phantom

Lot	A	B	C	S		
λ	3 mm.	28 mm.	3 mm.			
t	40	61	60	0	R	Deviation
Experiment	ϕ	ϕ	ϕ	ϕ_o		
1	7.46	8.04	8.14	(5.97)	0.936	+.066
2	6.85	7.92	8.32	(5.56)	0.875	+.005
3	7.07	7.38	8.07	(5.59)	0.757	−.113
4	6.82	7.87	8.16	(5.63)	0.913	+.043
Mean	7.05	7.80	8.17	(5.69)	0.870 (A. D. = .028)	

and a control were placed in and removed from the wells at as nearly the same time as practicable and pupation observed as in previous work. The lots irradiated were designated A, B, and C in the order of irradiation, B being at the depth 25 mm. below that of A and C. The value of λ is, of course, 3 mm. greater, due to the cutting of the groove and large well system, but the results are treated as measures of approximate relative effectiveness of radiations at the depth 3 mm. higher.

This relative effectiveness (R) may be estimated by means of a linear interpolation approximation of the time that would be required at the surface for the effect observed in B, R being the ratio of this time to the time actually required in the case of B. The results are given in Table IV, the mean value of R being 0.870 (A. D. = .028).

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The value obtained by similar interpolation using the mean of the four values for each type of irradiation is 0.875, but this method of estimation is not as generally applicable as the former which does not require uniformity in the irradiation scheme.

The results so far presented (in Tables II and IV) obviously represent only a small portion of the phantom explorable in this manner, but are given as an illustration of a method of studying relative effectiveness of radiations in different portions of a phantom employing a living system as indicator. The contrast of such results with those similarly obtained with gaseous ionization chambers should be interesting in view of the controversial character the subject has assumed.

DISCUSSION

It is important to note that the shift in the average prepupal periods with irradiation in the above experiments is not accountable on the basis of selective mortality alone; and, indeed, the rôle of selective mortality in such effects seems to be insignificant. This is clearly brought out in the diagram of the distribution of pupation observed in the first course curve experiment (Table I) which is presented in Text-fig. 2. In the form of class-frequency diagrams (the height of the black rectangles being proportional to the number of pupae formed in the given observational interval, taken as $\frac{1}{2}$ day) the distribution of ϕ is given with the median value indicated by a white vertical line in each instance; the base line of each diagram being placed at the ordinate height relative to the period of irradiation, t. The representation is convertible to the conventional form in a three dimensional Cartesian coordinate system by the simple device of rotating each frequency diagram (blackened region) about its base line through an angle of 90° in a given direction.

Approximately the same number of larvae (about 300) were taken for each well (as stated previously), hence the approximation of the blackened areas to equality indicates the lack of great difference in the fraction of larvae reaching the pupal stage in these experiments. Thus it is obvious that the diagram would discredit any attempt to account for the observed shifts in the position of the median ϕ upon the basis of selective mortality alone. The total pupae formed for the different lots (in order of increasing t) were 287, 308, 313, 335, 268,

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272, 265, and 270, respectively, in this experience. The value of the pupation-rate ordinate is not given in the figure, but the *distance* corresponding to 40 minute difference in t was taken to represent a pupa-

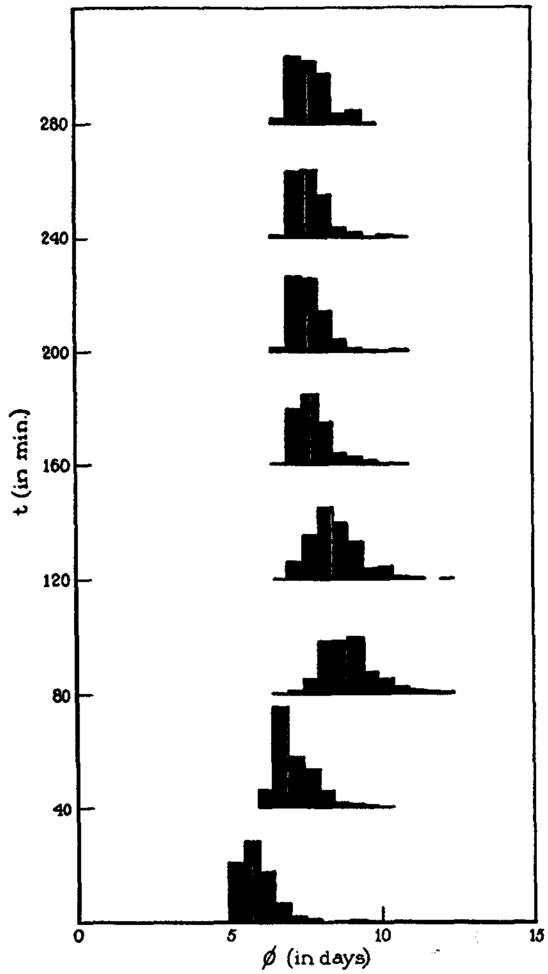

FIG. 2

tion rate of 150 per half day. This was done to avoid overlapping in the diagrams, the greatest rate observed being one of 133 pupae in a half day in the case of the lot irradiated for 40 minutes.

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It is interesting to note the apparent decrease in skewness in the neighborhood of the maximum displacement of the average ϕ (about an irradiation time of 80 minutes).

SUMMARY

The effect of high-frequency x-ray irradiation in prolongation of the larval stage of *Drosophila melanogaster* has been studied further, and evidence presented of the attainment of a maximum effect followed by a decrease to an almost level plateau in the course curve of average (median) prepupal period (ϕ) as a function of the period of irradiation (t) under otherwise fixed conditions. The variation of effects of the experimental treatment with age of the larvae at the time of irradiation has been demonstrated in both control and irradiated lots, and a strikingly decreased effect observed when ventilation was not supplied as usual.

Means of employment of a living system of this type as an indicator of effectiveness of radiation as in phantom depth or other distributional experiments have been presented and their use illustrated.

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