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THE INDUCTION OF MUTATIONS IN PARAMECIUM AURELIA BY BETA RADIATION

R.F. KIMBALL

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R. F. Kimball

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THE INDUCTION OF MUTATIONS IN PARAMECIUM AURELIA BY BETA RADIATION

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For the study of mutation induction by radiation, Paramecium aurelia has some advantages. The animals can be made completely homozygous by the process of autogamy (Sonneborn, 1939), and so any mutations produced in the diploid micronuclei can be brought to light easily. Lethal mutations can be kept in the heterozygous state and so subject to further genetic analysis. In addition, there is available in fission rate a character which would be expected to be affected by many different genes. Thus, in theory at least, it should be possible to obtain a measure of the overall mutation rate including mutations with individually small effects.

Counterbalancing these advantages, certain drawbacks must be mentioned. The detection of chromosomal aberrations by cytological methods is rendered difficult if not impossible by the small size of the nuclei and chromosomes. There are few known genes in Paramecium so that a refined genetic analysis of the individual changes would be difficult. Finally, each exautogamous clone, the equivalent of an individual in higher organisms, must be kept in a separate container thus considerably increasing the labor and decreasing the numbers that can be handled.

Nevertheless, it has been possible to carry out an analysis of the induction of mutations by beta radiation, to obtain some information about the kind of mutational changes involved, and to make calculations of the number of mutations induced on the basis of certain theoretical considerations. In addition to the information which this organism can give about the mutation process, such studies form a necessary background for a general investigation

1. Valuable assistance was given in the planning and carrying out of the experiments by Nenita Gaither, Roma G. Eisenstark, and Margaret S. Griess.

of the effects of radiation upon Paramecium, especially effects on the cytoplasm, for the study of which Paramecium is especially suited.

Kimball (1947) has given a preliminary account of the work done with an external source of beta radiation using stock 90 of variety 1. Powers (1948) in an independent investigation has reported somewhat similar results using stock 51 of variety 4 and beta emitters in the culture medium. For solutions of equal activity, he found that P^{32} was more effective in inducing mutational changes than $Sr^{89,90}$, Y^{90}. The exact interpretation to put upon this result is not clear, but it may well yield important information about the mutation process on further analysis.

Materials and Methods

The work to be reported was carried out with stock 90 of variety 1 which was obtained originally from Prof. T. M. Sonneborn.

The procedure used to detect mutational changes was as follows. A number of animals of a single clone were exposed to the radiation. Immediately after the exposure, individual rayed animals were isolated, allowed to multiply vegetatively, and a part of the descendants caused to go into autogamy by allowing them to rapidly deplete the supply of food. When it was believed that a culture was in autogamy, a small sample was stained with aceto-orcein and examined. Only those cultures in which at least 90% of the sample was in autogamy were ordinarily used. The method is subject to some error in that a small fraction of the animals isolated probably were not in autogamy. For each original rayed animal 25 autogamous animals were ordinarily obtained. A set of unrayed animals from the same clone was carried through this same procedure as a control.

Each autogamous animal was placed in a limited quantity of culture medium and allowed to multiply for four days. At the end of this period, all were observed for survival and amount of growth. Control animals by this time

ordinarily had produced a maximum population which was distinctly starved. The exautogamous descendants of rayed animals frequently showed less growth. The amounts of growth ranged in continuous series from death within the four days through various amounts of growth less than the control to maximum, starved populations just like the controls. For purposes of recording, several qualitative classes were set up, and each exautogamous clone was recorded as belonging to one of these. In the final compilations, all clones in classes which differed distinctly from a maximum, starved population were grouped together as clones of reduced vigor. This included those which failed to survive the four day period; and, at first thought, it might appear that these should be considered as a distinctly separate class. However, many clones which lived for four days proved incapable of surviving further culture. Thus death in four days is not an adequate criterion of ability to live indefinitely and can only be looked upon as one extreme in a continuous series of reduction in vigor.

It is obvious that this method would not be sufficiently accurate to detect very small changes in vigor. A more refined technique using the daily isolation method with daily counting of the number of animals would be expected to detect more changes; and indeed, as will be shown below, this is so. However, the latter method is so laborious that little work has been done with it. The effect of the relative crudeness of the method of detection upon the results will be discussed later in the paper.

As a source of beta radiation, plaques of P^{32} produced in the Oak Ridge pile were used in accordance with the technique described by Anderson et al (1947). The animals were placed in 0.5cc of culture fluid in a thin aluminum dish, a thin aluminum cover was placed over the dish, and it was suspended between two phosphorus plaques in a specially designed lucite plaque holder.

The holder contained three pairs of plaques so that three exposures could be made simultaneously. Dosage calculations were made from measurements of the radiation from the plaques prior to their insertion into the holder. The measurements were made with a plane source ionization chamber calibrated against an extrapolation chamber. The dose per unit time was calculated in rep from these measurements taking into account the decay of the P^{32}, the absorption by the dish and fluid, and the geometry of the system. There are still some features of this calculation that are not too certain, and so it is possible that the rep as used in this paper is not the true roentgen equivalent physical. However, some recent work with x-rays on Paramecium has suggested that it is not too badly in error in so far as its biological equivalence to the roentgen is concerned. The two plaques taken together delivered to the animals approximately 13,000 rep per hour though this figure varied somewhat from time to time. A small amount of gamma radiation was always present as a contaminant.

General Remarks Upon the Effect of Radiation

Total doses up to about 13,000 rep were employed. No noticeable effects of even this highest dose were found as long as the animals multiplied vegetatively. It is possible that there was a slight reduction in fission rate in the first day, but the effect, if any, was so slight that it has not yet been clearly established.

However, when the progeny of rayed animals were sent through autogamy some of the exautogamous clones proved to be of reduced vigor as defined above. This is at least suggestive evidence that mutational changes were induced in the micronucleus by the radiation. Evidence from inheritance studies which supports this belief will be presented in another paper. For present purposes, it will be assumed that reduced vigor after autogamy

is evidence for mutational changes in the micronucleus.

The Theory of the Measurement of Mutation in Paramecium

The reduction in vigor after autogamy could be and probably is the result of chromosomal aberrations as well as gene mutation, but detailed genetic analysis of any large amount of material would be difficult if not impossible. The one quantity which can be readily measured on a reasonably large scale is the fraction of exautogamous clones which are of reduced vigor. It becomes necessary, therefore, to find out how this quantity is related to the number of mutational changes on various hypotheses concerning the kind and relative frequency of the changes.

The case of gene mutations with individually detectable effects is the simplest and forms the basis for various modifications. I am indebted to Dr. A. S. Householder of Oak Ridge National Laboratory for carrying out the mathematical analysis of this case.

In *Paramecium aurelia*, there are two diploid micronuclei in either or both of which mutations might occur. At autogamy, both micronuclei undergo two meiotic divisions. One of the eight haploid nuclei produced divides mitotically to form two pronuclei which then fuse to form the diploid syncaryon. From this syncaryon are produced the micronuclei and new macronucleus of the exautogamous clone. The two pronuclei should be of identical genotype since they are formed by the division of a single haploid nucleus, and so the syncaryon which they produce should be completely homozygous.

If a micronucleus is heterozygous for a single mutant gene, the chances that the pronuclei derived from it do not contain the mutant gene should be one-half. If the micronucleus contained *a* independently assorting mutants, the chances that the pronuclei would contain none of them is $(\frac{1}{2})^a$. There is

no reason to believe that one micronucleus is any more likely to give rise to the pronuclei than the other. Thus if there were a mutants in one and b in the other, the chances that a syncaryon derived from this animal would have no mutants would be $p(a,b) = (1/2)^{a+1} + (1/2)^{b+1}$, and the chances that it would contain at least one mutant would be $q(a,b) = 1 - (1/2)^{a+1} - (1/2)^{b+1}$. As the simplest case, let us assume that the presence of a single mutant gene is sufficient to produce a detectable effect upon vigor. Then the probability of ν normal clones out of n exautogamous clones from an animal with a mutants in one micronucleus and b in the other would be

$$P(\nu/n;a,b) = \binom{n}{\nu} p^{\nu}(a,b)\, q^{n-\nu}(a,b)$$

The chances that a mutations will occur in a given micronucleus when the mean number per micronucleus is m should be given by the appropriate term of the Poisson series: $p(a) = e^{-m} m^a/a!$. Similarly, the chance of b mutations is given by $p(b) = e^{-m} m^b/b!$.

The chance for ν normals out of n exautogamous clones for a given dose of radiation characterized by a certain value of m is then:

$$(1) \quad P(\nu/n) = \sum_a \sum_b P(\nu/n;a,b)p(a)p(b).$$

The mean number of normal exautogamous animals is:

$$\bar{\nu} = \sum_{\nu}^{n} \sum_a \sum_b \nu P(\nu/n;a,b)p(a)p(b).$$

Substituting the values of the functions on the left and substituting for

$\nu P(\nu/n;a,b)$ its equivalent $np(a,b)$ we have:

$$\bar{\nu} = n \sum_a p(a) \sum_b p(b)p(a,b).$$

Performing the summations over all values of a and b independently, this simplifies to $\bar{\nu} = n\, e^{-m/2}$. Substituting F, the average fraction of exautogamous clones of reduced vigor, for $1 - \bar{\nu}/n$, this becomes:

(2) $F = 1 - e^{-m/2}$.

The problem then becomes one of finding F and calculating m from equation (2). It would be possible, at least in theory, to calculate F by the maximum likelihood method using equation (1) as the basis. However, this involves some very complex summations, and it has seemed better to approximate it in another way until it can be shown that the data warrant a more exact treatment. Since it appeared that the maximum likelihood method would yield a treatment dependent upon the values of n and ν, this was done by taking an average over the whole group of rayed animals of the fractions obtained from each using the angular transformation for averaging. Each fraction in the group to be averaged was converted to an angle using the table in Snedecor (1946), the arithmetic mean of the angles found, and the resulting mean angle converted back to a fraction. In the rest of the paper, F is calculated in this way.

The expectations for chromosomal aberrations have not been worked out in detail. The expectation of 1/2 for segregation of gene mutations as embodied in the formula for p(a,b) might have to be changed. If this is done, equation (2) becomes $F = 1 - e^{-rm}$ where r is some fraction representing the average probability that a syncaryon derived from a micronucleus having an aberration will determine reduced vigor and m is the mean number of chromosomal aberrations per micronucleus.

For gene mutations, further refinements in equation (2) might be desirable to take into account linkage and crossing-over. However, except at very high doses, this is probably not important. The most important modification concerns the probability of a single mutant gene producing a detectable effect on vigor. If it be assumed that there are mutants which by themselves have too little effect to be detected but in combination

with other similar mutants produce a detectable effect, the following modification has to be made in the development. Let c_0, c_1, c_2, c_3, etc. represent the probabilities that animals having respectively none, one, two, three, etc. homozygous mutants will be of normal vigor. Let p(a,b,c) represent the probability that an exautogamous clone from an animal having a mutations in one micronucleus and b in the other will be normal. Then this function is equal to $c_0 \left[(1/2)^{a+1} + (1/2)^{b+1}\right] + c_1 \left[a(1/2)^{a+1} + b(1/2)^{b+1}\right] + c_2 \left[a(a-1)/2! \quad (1/2)^{a+1} + b(b-1)/2! \quad (1/2)^{b+1}\right] + c_3 \left[a(a-1)(a-2)/3! \quad (1/2)^{a+1} + b(b-1)(b-2)/3! \quad (1/2)^{b+1}\right]$ etc.

Substituting this expression in place of p(a,b) in the above development and q(a,b,c) = 1 - p(a,b,c) for q(a,b) and carrying out the analysis as before, we find:

$$(3) \quad F = 1 - e^{-m/2} \left[c_0 + c_1 m/2 + (c_2/2!) \quad (m/2)^2 + (c_3/3!) \quad (m/2)^3 \text{ etc.}\right]$$

While the solution of this equation is more difficult than that of equation (2), it will be shown how it can be used.

Effect of Stage in the Fission Cycle

Before any adequate data on dosage relations could be obtained, it had to be determined whether radiation was of equal effectiveness at different stages in the life history. So far, no study of this matter has been made for different parts of the interautogamous interval. Most studies have been carried out by radiating on the eleventh day after a preceding autogamy so that any variation from this source has been, for the most part, eliminated. Studies have been made on the effectiveness of radiation given at different stages in the fission cycle, and it has been found that radiation is more effective at certain stages than at others.

The method was as follows: A number of animals of a clone were isolated and allowed to multiply with excess food for a day to furnish a good

supply of dividing animals as needed. During a ten to fifteen minute period, all animals which were dividing were picked out with a micropipette and put together in a small container. This was repeated at one or more later periods so that two or more groups of animals of known time since the preceding fission were available. At an appropriate time, all groups were exposed simultaneously to the same dose of beta radiation.

The results are shown in table 1. In the last column, the average fraction of exautogamous clones of reduced vigor is shown. The number of rayed or control animals from which these came is given in the sixth column. It is obvious that radiation at different stages in the fission cycle was of markedly different effectiveness in producing detectable changes. Animals rayed just prior to the next division (rows 2, 6, 10, 13, 15) produced far fewer clones of reduced viability than did those rayed immediately after fission (4) or one or two hours afterwards (3, 8, 11, 16). A comparison of rows 6 and 7 and of rows 13 and 14 shows that the ineffective period must end rather abruptly just at or just before the beginning of fission. In each case, the two rows represent single groups of animals which divided some four to five hours before radiation. Just before radiation, few, if any, animals in these groups had divided, but during the half hour to hour needed for radiation a part of them did, as indicated by their small size, and also by the dividing animals seen immediately after radiation. On isolation, a record was kept as to whether or not the animal had divided, as judged by its size. On the basis of this record, the divided and undivided groups were considered separately in analyzing the results. Those which had divided showed markedly more effect than those which had not.

The results indicate that radiation given in a period of an hour or

Table 1

Effectiveness of Radiation at Different Stages of the Fission Cycle

Experiment number	Row	Approx. dose in rep	Time from division to beginning of radiation	Notes	Number of rayed or control animals	Average fraction of exautogamous clones of reduced vigor
47.17	1	none	----------	-----	20	.02
	2	13,000	4 hours	(1)	20	.41
	3	13,000	2 hours	-----	20	.88
	4	13,000	10 min.	-----	20	.93
47.22	5	none	----------	-----	10	.005
	6	13,000	4 hours	(2)	12	.41
	7	13,000	4 hours	(3)	18	.96
	8	13,000	1 hour	-----	35	.97
47.26	9	none	----------	-----	11	.02
	10	13,000	4 hours	(4)	13	.21
	11	13,000	1 hour	-----	22	.93
47.35	12	none	----------	-----	68	.06
	13	6,500	$5\frac{1}{2}$ hours	(2)	16	.29
	14	6,500	$5\frac{1}{2}$ hours	(3)	14	.92
	15	6,500	$4\frac{1}{2}$ hours	(4)	30	.10
	16	6,500	2 hours	-----	15	.81

Notes: (1) Both divided and undivided animals present at end of radiation. Not separated.

(2) Both divided and undivided animals present at end of radiation. This group includes only the undivided ones.

(3) Both divided and undivided animals present at the end of radiation. This group includes only the divided ones.

(4) No animals had divided by the end of radiation.

more before fission is relatively ineffective when compared with the same exposure given during fission and for at least two hours afterwards. There is a suggestion of a slight dropping off in effectiveness as the time since fission increases but this cannot be considered as established. All work to be reported below was done with animals one or two hours after fission.

Dosage Dependence

Figure 1 shows the relation between the average fraction of exautogamous clones of reduced vigor, calculated as indicated above, and dose; and figure 2 shows the relation between m as calculated from equation (2) and dose. The points for the individual experiments are based, in most cases, upon the progeny of 20 animals with approximately 25 autogamous clones from each. The open circle points, representing fractionated doses, will be discussed later. Only doses up to 6500 rep are included in figure 2 since the very high values of F obtained at 13,000 rep make it impossible to calculate m with any precision.

On the simple gene mutation hypothesis, m should increase as a linear function of dose. It is obvious that this is not the case. A curve has been fitted to the data by eye, and it appears to approximate an increase of m as some power of the dose.

Fractionated Doses

Since the relation of m to dose is not linear, a re-examination of the simple gene mutation hypothesis is necessary. Two alternatives seem possible. If chromosomal aberrations of the so-called two-hit type make up most of the detectable changes, then m, as the mean number of aberrations, would be expected to increase approximately as the square of the dose. On the other hand, if most of the reduction in vigor is the result of the

Figure 1

The relation between dose of beta radiation in 10^3 rep and the average fraction of exautogamous clones of reduced vigor (F). The solid points represent single exposure experiments; the open circles, fractionated exposures. Each point represents the average for about 20 rayed animals. The curve was calculated from an equation to be explained in the text.

FIGURE 1

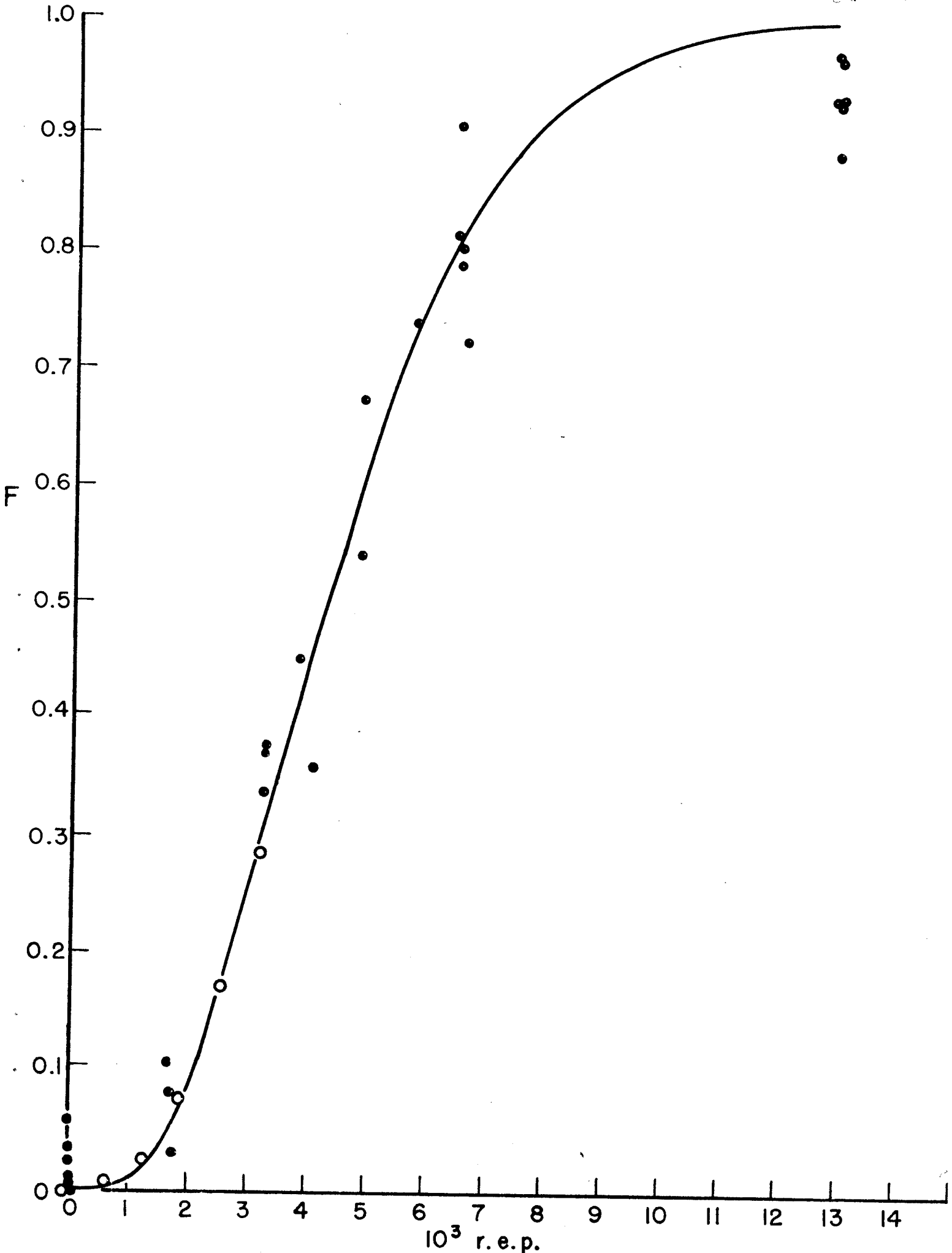
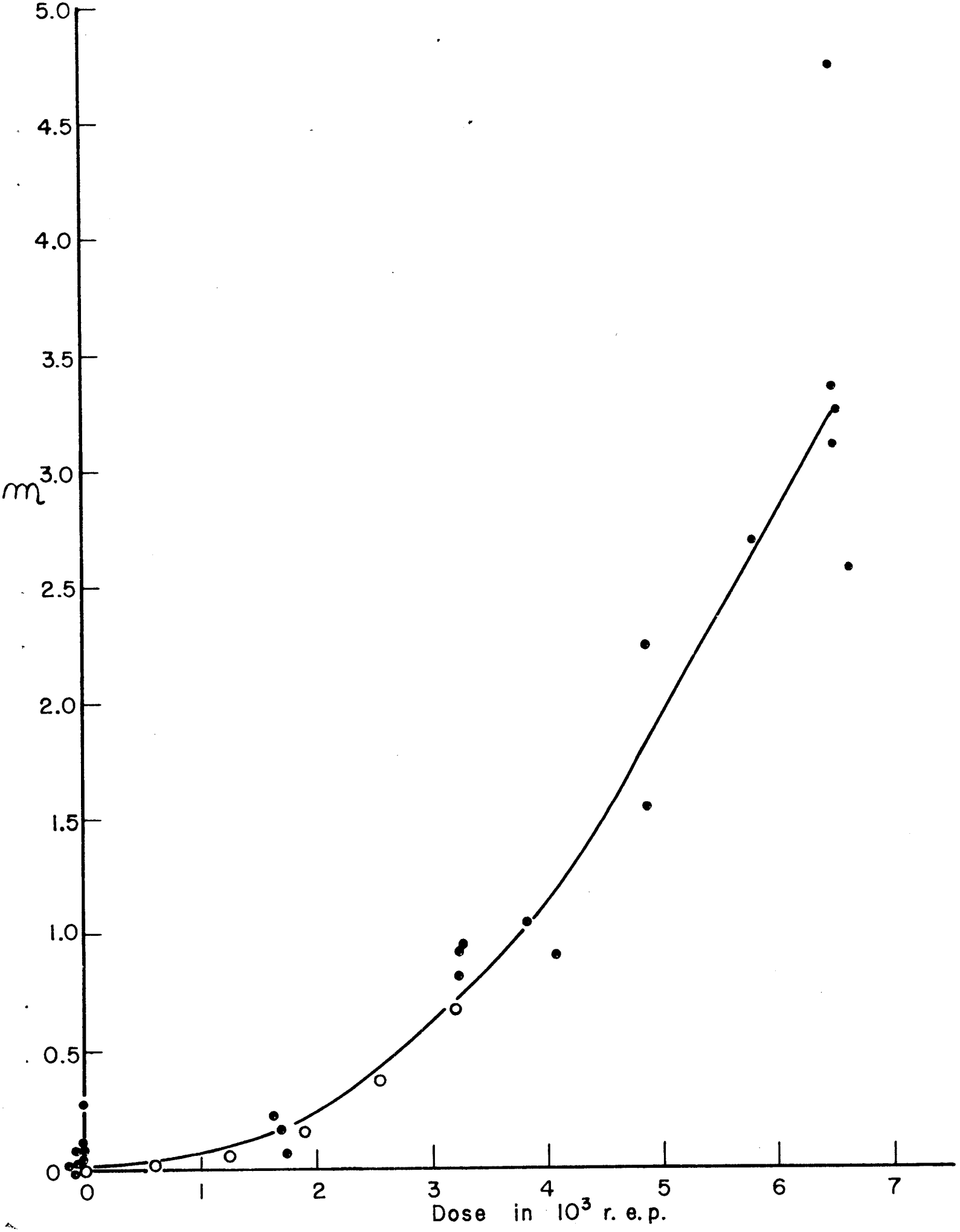


Figure 2

The relation between dose in 10^3 rep and m calculated from the relation $F = 1 - e^{-m/2}$. The individual points are the same as plotted in figure 1.

FIGURE 2



combined action of mutant genes with individually small effects, then m as calculated from equation (2) would be expected to show a non-linear relation to dose even though the true mean number of gene mutations as calculated from an appropriate form of equation (3) should show a linear relation.

It should be possible to distinguish between these two hypotheses by use of fractionated doses. Various workers (see Lea, 1947, for review) have shown for Tradescantia that a given dose of radiation is less effective in producing interchange aberrations if it is given at low intensity over a long time than at high intensity over a short time. Sax (1941) has shown that a dose is less effective when given in fractions with a rest period in between than when given as a single concentrated exposure. These facts have been interpreted to mean that broken ends remain available for recombination for only relatively short periods of time.

For Paramecium, it seems highly improbable that breaks produced on one day would remain available for recombination with breaks produced on the following day after some four to five fissions. Thus if the non-linear relation of m were due to two-hit aberrations, a dose given in small daily fractions would be expected to be much less effective than the same total dose given within a single interfission interval; and a linear relation to dose should be approached. On the other hand, if the non-linear relation is the result of most of the detectable reduction in vigor being due to the combined effect of several genes, fractionation of the dose should have no effect.

The experiment was performed as follows. A number of animals from a single clone were chosen and divided into two groups. One group was kept as a control and the other was exposed to about 300 rep. After radiation,

the animals were isolated and allowed to multiply. One descendant from each was chosen on the next day, exposed to 300 rep and reisolated. On the next day, one descendant of each was isolated and its progeny kept without further radiation for the remainder of the experiment. This group, then, received a total of 600 rep in two fractions. At the same time another descendant of each of the animals which had received 600 rep was chosen, exposed again to 300 rep and reisolated. This procedure was repeated every day for ten days, every second day a group of animals being set aside and kept without further radiation. Thus groups were obtained which had received approximately 600, 1200, 1800, 2400, 3000 rep in 300 rep daily doses. At the end of the ten day period, autogamous animals were obtained from 20 lines of each group, including the control.

The results are plotted in figures 1 and 2 as open circles. It is obvious that fractionation of the dose made little if any difference in the results. It can be concluded that two-hit aberrations could not have contributed much to the effect. This leaves as the most plausible hypothesis that most of the detectable reduction in vigor is due to the combined action of several mutant genes with individual effects too small to be detected by the usual method.

Demonstration of Small Reductions in Vigor

If this is so, a number of clones classified as normal by the usual test should be homozygous for mutant genes with very slight effects upon vigor which might be detected by a more refined method. To find out if this were so, ten animals from a clone were exposed to 6500 r of 250 kv x-rays. Ten animals from the same clone were kept without radiation as controls. Of the 250 exautogamous clones from rayed animals, 50 were classified as normal after the standard four day test. These were distributed

among the progeny of all ten rayed lines. A single animal was isolated from 48 of these apparently normal clones, and at the same time an animal was isolated from 17 normal exautogamous clones from the controls. The descendants of these 65 animals were kept as daily isolation lines with daily counts of the number of animals for five days. Of the 48 clones from rayed animals, 39 had a distinctly lower fission rate than any of the controls. In addition, a number of abnormal animals appeared and 14 of the clones died out before the end of the period. Thus, a more refined technique demonstrates that a large number of changes in vigor occur which are not detected by the standard method. The fact that some of the clones which had appeared normal at four days died within the subsequent five day period suggests that the failure to detect all changes by the usual method may be due in part to lag phenomena. Some of the mutant genotypes may have a lag in their expression lasting beyond the four day period. However, this is not the only cause of the failure to detect all changes; for several of the clones continued through the five day period with a constant fission rate only about one fission per day less than the controls. It is highly improbable that such clones would be detected by a four day total growth test no matter how long after autogamy it was made. In addition, it seems not unlikely that, other things being equal, the more deleterious mutants present, the shorter the lag period.

Mutation Rate

Equation (3) can be solved for $\underline{m}$ if the constants c_0, c_1, c_2, etc. can be found and if the relation $m = bD$; i.e., a linear relation to dose, is assumed. This last assumption seems reasonably justified since $\underline{m}$ is by definition the mean number of gene mutations per micronucleus and, from

the work with other organisms, the number of mutations should be a linear function of dose.

It is necessary, then, to estimate the value of c_0, c_1, c_2, etc. and of b from the data. This could be done by fitting equation (3) to the data by the least squares method, but the complex form of this equation makes this approach very tedious. It is probable that the data are not accurate enough to justify the labor involved. For this reason, less accurate approximation methods were used. Two methods were tried which gave slightly different values for the constants. Quite good fits can be gotten when c_0, c_1, c_2, c_3 are equal to unity; c_4 is either unity or zero; and c_5, c_6, etc. are zero. The value of b is approximately .002. These values mean that some four to five mutants must be present in an exautogamous clone to produce a detectable effect on vigor and that approximately two mutations affecting this character per micronucleus are produced per micronucleus per 1000 rep.

The curve in figure 1 was drawn with c_0, c_1, c_2 and c_3 equal to unity and b equal to .0017. Most of the points fall fairly close to the line except those at about 13,000 rep. In the section on methods, it was pointed out that a small error was inherent in the technique in that occasional non-autogamous animals were isolated and treated as though they were autogamous. Such animals would probably produce a four-day culture which would be classified as normal. This error would have little effect until the fraction of clones of reduced vigor becomes very high. At the upper extreme, it would have the effect of making the experimental value of F approach some value less than unity as an upper asymptote.

Discussion

A high resistance of vegetatively multiplying Paramecia to ionizing

radiation has been reported, among others, by Back and Halberstaedter (1945) and is, perhaps, not too surprising. The presence of the macronucleus, according to Sonneborn (1940) a multiple structure with each gene represented many times, makes it improbable that gene mutations induced in vegetatively reproducing animals would express themselves unless doses capable of causing tremendous numbers of mutations were applied. Thus the animal is well protected against the effects of gene mutations and immediate deleterious effects of radiation would have to be produced through some other mechanism which might well be less readily affected by radiation than are the genes. The fact that, even with relatively very small doses, mutational effects appear after autogamy emphasizes the need to control this process in studies of the effect of radiation on Paramecium.

The difference of effectiveness of radiation applied at different stages of the fission cycle remains at present unexplained. It should be noted that the ineffective period probably includes, if it does not coincide with, the division of the micronuclei which immediately precedes cytoplasmic division; but it is not clear what interpretation to place upon this apparent connection.

It is not possible by the methods used in this work to detect individual mutations. However, by making a few simplifying assumptions, it is possible to arrive at an approximate value for the overall mutation rate including as a major component mutants with individually undetectable effects. The approximate values given in the preceding section for the constants of equation (3) allow for only one class of mutants, those which express themselves when four or more are together and do not express themselves when three or less are present. It is obvious that this is an oversimplification. There are undoubtedly mutants which express themselves

in the absence of any others, and the expression of those that do not probably depend, not only upon the number, but also the kind of other mutants present. Moreover, it seems probable that chromosomal aberrations by their segregation from the heterozygous state add to the total reduced vigor found. The data merely show that two-hit chromosomal aberrations and gene mutations with large effects do not make a very considerable contribution to the changes detected. While the major part of the effect has been attributed to gene mutations, it is obvious that nothing in the data rules out the possibility that very short deletions with small effects on vigor may be involved. It seems improbable that deletions that were not very short could play an important role since they would be expected to have major effects on vigor.

The calculated value of about two mutations per micronucleus per 1,000 rep is based on the assumption that there is only one class of mutants, those which have an effect only when four or more are present. Mutants which express themselves when one, two or three are present will tend to make the calculated rate too high while those which express themselves only when five, six, or more are present will tend to make it too low. Insofar as these opposing errors cancel each other out, the calculation will be correct.

The present investigation confirms studies on other organisms that show that mutations with very slight effect are the commonest kind. Muller (1941) states that about twice as many "detrimental" mutations are produced as lethals in Drosophila. In Paramecium, it would appear that the ratio is considerably greater perhaps because the method used detects, at least in part, mutations with very slight effects.

Summary

1. Beta radiation from an external P^{32} source in doses up to 13,000 rep has no immediate marked effect upon vegetatively multiplying *Paramecium aurelia*.

2. When the progeny of rayed animals are sent through autogamy, many of the exautogamous clones are of reduced vigor as judged by total growth in four days.

3. This reduced vigor is interpreted as the expression of mutational changes in the micronuclei partly because it first appears when the animals are made homozygous by autogamy, partly because data on inheritance to be published in a separate paper support this view.

4. Radiation given in a period of about an hour before cytoplasmic division is less effective than radiation given at other stages of the fission cycle. No adequate explanation is available for this phenomenon.

5. The relation of the percentage of exautogamous clones of reduced vigor to dose shows that most of the mutational changes cannot be gene mutations with individually detectable effects. The relation is consistent either with the hypothesis that most of the effect is due to two-hit chromosomal aberrations or with the hypothesis that most of it is due to the combined action of several mutant genes with individually undetectable effects.

6. Fractionation of the dose has no noticeable effect on dosage relations so two-hit chromosomal aberrations cannot be an important factor.

7. This leaves the hypothesis that most of the detectable reduction in vigor is due to the combined effect of mutant genes with individual effects too small to be detected.

8. This hypothesis gives expectations in agreement with the dosage relations and is further supported by the fact that a number of clones

which appear normal by the usual test for vigor prove to be of reduced vigor when examined by a more refined method.

9. The mathematical relation between the average fraction of exautogamous clones of reduced vigor and the mean number of mutations per micronucleus has been worked out for this hypothesis. By approximation calculations, a value of about two mutations per micronucleus per 1000 rep has been obtained.

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