STUDIES ON THE REFRACTORY PERIOD FOR THE INDUCTION OF RECESSIVE LETHAL MUTATION BY X RAYS IN PARAMECIUM

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WE have reported (KIMBALL 1949, 1961; KIMBALL, GAITHER and WILSON 1957) that very few recessive lethal and slow growth mutations can be induced in *Paramecium aurelia* by irradiation in postduplication interphase (G2) or early prophase, although mutations are easily induced by irradiation in preduplication interphase (G1) and in mitotic stages after midprophase. The previous reports have assumed, without proof, that this large difference in the amount of detectable mutation resulted from a decreased amount of induced mutation and not from decreased detection. The first purpose of the present paper is to establish this view on a firm basis; the second is to consider in more detail the implications of the refractory period for ideas about mutation and its induction.

MATERIALS AND METHODS

Paramecium aurelia, syngen 4, stock 51 free of kappa was used except in one experiment in which a kappa containing Kk heterozygote derived from a cross between stock 51 (KK) and stock d4–8 (kk) was used. The paramecia were cultured in lettuce infusion with *Aerobacter aerogenes* as food.

For cytological observation the paramecia were air-dried on slides, fixed in three parts absolute alcohol and one part glacial acetic acid, hydrolyzed in 1 N HCl at 60°C for 12 minutes, and stained with 0.25 percent azure A to which a few drops of thionyl chloride had been added. Feulgen microspectrophotometry was carried out on Feulgen-stained preparations by the two wavelength method with a Canalco microspectrophotometer.

Dividing paramecia were collected from small log phase cultures and irradiated at known times after collection with X rays from a General Electric Maxitron 250 operated at 250 kvp and 30 ma with 3 mm Al added filtration (half-value layer, 0.44 mm Cu). The dose rate was approximately 1.5 kr/minute.

Recessive lethal and slow growth mutations in the micronucleus were detected by the same method as in our previous work by using autogamy to produce complete homozygosis. The procedure will be described more fully than previously because the details are important for understanding experiments designed to test for selection artifacts.

A group of paramecia of known age since division were irradiated. As soon after irradiation as possible but always before the first postirradiation division,

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each paramecium was isolated into a separate container to establish a line of descent, hereafter called a *line*. Each line was maintained by daily reisolation of a single specimen and the leftover specimens from each daily reisolation after the first two were kept two days longer. By this time the food was exhausted, and usually the leftover paramecia had undergone autogamy and progressed to an early postzygotic stage, the two-anlagen stage, at which further progress stopped until after transfer to fresh medium.

The daily reisolations were continued long enough to provide a new set of autogamous cultures on each of four or five successive days, and on each day some of these were used as a source of autogamous isolates. A sample was stained and checked for autogamy and the culture was used as a source only if at least 18 out of 20 specimens were in autogamy; usually all 20 were. Enough extra lines were established originally to insure that autogamy could be obtained from a predetermined number from each treatment each day without using any line twice despite some loss through death before autogamy or rejection because of a low frequency of autogamy. Thus, estimates of the amount of preautogamous selection could be made from the records of the fate of each line.

From each leftover culture used as a source, 25 specimens were isolated one at a time into fresh culture medium; and these 25 cultures were observed four days later for survival and amount of growth. The isolation of this number of autogamous specimens per line insures nearly full detection of recessive lethal and slow growth mutations anywhere in the four genomes in the two diploid micronuclei of the original treated cells. Each mutation will segregate to two of the eight meiotic products and thus will be made homozygous in one quarter of the exautogamous clones, if each meiotic product has an equal chance of becoming the progenitor by mitosis and fusion of sister nuclei of the synkaryon. Therefore there is a large chance that any mutation will become homozygous in at least one of the 25 clones. This nearly full utilization of the original four genomes keeps to a minimum the number of initial cells needed to attain a given accuracy in the mutation rate. This circumstance greatly facilitates the precise ascertainment of the preirradiation and postirradiation history of these cells.

On the other hand, the method does not yield an unambiguous estimate of the number (m) of mutations per genome but only a quantity that is proportional to this number over a fairly wide but limited dose range. On simplest theory, m is related to the mean frequency (p) of normal clones by the exponential relation, $p = e^{-m}$ (KIMBALL 1949); but several factors, including gene interactions (KIMBALL 1949) and selection of meiotic products free of mutation (KIMBALL *et al.* 1957), make the actual relation more complicated. Expressions relating m and p with these factors taken into account can be derived, but they are too complicated and contain too many unknown parameters to be of much practical value.

In more recent work, we have chosen instead to use a variance equalizing transformation developed for us by DR. A. W. KIMBALL (unpublished). This transformation converts p to a quantity γ by the formula

$$\gamma = \sin^{-1}\frac{n-1}{n+3} + \sin^{-1}\frac{2p^{0.5}(n+1)-(n-1)}{n+3}$$

where n is the number of autogamous isolations per line, in this case 25, and p is the frequency of normal clones among the n clones (KIMBALL *et al.* 1959a). For $n = 25$, γ varies from 2.6 to 0 as p varies from 1 to 0. This transformation equalizes the variance very satisfactorily as long as the mean frequency of normal exautogamous clones lies between ten and 90 percent.

To obtain a quantity more or less proportional to m, the mean value of γ for each experimental group is subtracted from the mean value of γ, usually about 2.4, for the unirradiated controls. This difference, M, increases nearly linearly with dose over a fairly wide range of values and can therefore be treated as proportional to m over this range though with an unknown constant of proportionality. The approximation is most reliable for comparisons between values of M that are not too different from each other and for values of M well above zero and well below the upper limit of about 2.4.

RESULTS

Estimation of the relative amount of mutation inducible in G1 and G2: It has been estimated (KIMBALL 1961) that at least a tenfold difference in the amount of detectable mutation exists between G1 and G2. This conclusion was based on comparisons at single doses at which the value of M for the G2 group was very low. It is possible that such very low values of M underestimate mutation because of departures from proportionality to m in the initial part of the dose curve.

Two kinds of experiments have shown that this is not a serious problem. Dose curves for paramecia irradiated at one (G1) and 3.3 (presumed G2) hours after division are shown in Figure 1. On the basis of more recent microspectrophoto-

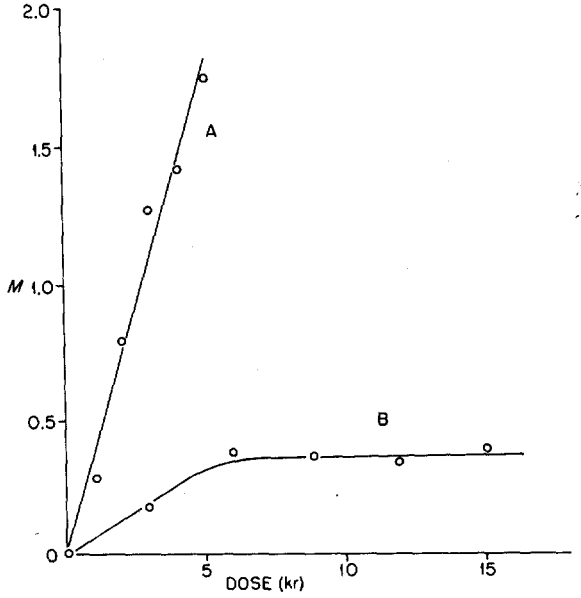

FIGURE 1.—X-ray dose curves for paramecia irradiated at 1.0 hr (A) and 3.3 hr (B) after division. The data for curve A were replotted from KIMBALL *et al.* (1959a).

metric studies, it is apparent that the latter group probably contained, because of imperfect synchrony, some paramecia in late G1 and a few in the very short duplication period (S) as well as those in G2. Thus, the whole curve may be nothing more than the dose curve for the more sensitive G1 individuals with mutation reaching a saturation level between 6 and 7kr because M for the G1 individuals approaches its upper limit at about this dose. This suggestion is borne out by the observation at the higher doses that any one line of descent produced nearly all normal or all not normal exautogamous clones with rare intermediates. Presumably the former were descended from G2 individuals, the latter from G1. Thus, this experiment could be interpreted to mean that no mutations are produced in the truly resistant stages up to at least 15kr.

Although this explanation of the plateau is plausible, other explanations involving some kind of balance between mutation induction and side effects of the radiation on the establishment or detection of mutations cannot be entirely ignored. Therefore another type of experiment in which M was increased without using a very large dose was devised. Half of a group of paramecia were irradiated in G2 (four hours after division) with 5kr and half were not irradiated. Dividing specimens were collected about three hours later when divisions were occurring in both groups (for unknown reasons the generation time in both groups was seven hours instead of the usual five to six); and half from each group was given 2kr at 1.25 hours after division when the cells were in the succeeding G1. The results are summarized in Table 1. The amount of mutation induced by irradiation in G2 was estimated from Group 2 to be 0.04 ± 0.05 M units; from the difference between Groups 3 and 4 to be 0.17 ± 0.10 M units. The difference between these two estimates is not statistically significant but is in the expected direction if mutation were underestimated by low values of M. The amount of mutation to be expected in G1 by irradiation with 5kr is roughly 2.5 times the value for Group 3, i.e., 1.85 M units. Thus irradiation in G2 probably produces no more than one tenth (0.17 compared to 1.85) the amount produced in G1 and any underestimation of G2 mutation by low values of M is not serious. The data do not exclude the possibility that no mutation is induced in G2.

Tests for loss of mutations through selection and for failure of expression because of macronuclear regeneration: Mutations might fail to be detected in Paramecium for any of the following reasons: (1) Asexual lines of descent with high numbers of mutations might die or fail to undergo autogamy; (2) mutant

TABLE 1

Combined irradiation in G2 and the succeeding G1

Group	Irradiation (kr)		Number of lines	Mutations		Number of micronuclei
	G2	G1		Percentage frequency of normal clones	$M \pm$ SE	Mean $\pm$ SE
1	0	0	60	95	0.00 ± 0.03	1.81 ± 0.06
2	5	0	60	92	0.04 ± 0.05	1.80 ± 0.06
3	0	2	60	60	0.74 ± 0.07	1.63 ± 0.05
4	5	2	60	50	0.91 ± 0.07	1.62 ± 0.05

micronuclei might be lost through failure to divide or through degeneration of the nucleus without concomitant death of the line of descent; (3) meiotic products without mutations might be selected to produce the synkaryon; (4) old, nonmutant genotypes might be retained by regeneration of the macronucleus from a fragment of the old macronucleus at autogamy.

Since fewer mutations are induced in G2, it is possible that they might be more subject to loss by one of these mechanisms either because they were of different kind from those induced in G1 or because of side effects of the radiation on the selective mechanisms, side effects that differ with the stage irradiated. The data in Table 1 make this latter alternative improbable. The side effects in the doubly irradiated group should be some combination of the individual effects and should influence the G1 and G2 increments of mutation equally since most forms of selection, except possibly (2) would occur after both increments of dose had been given. The fact that the doubly irradiated group had nearly the sum of the mutation in the two singly irradiated ones would be surprising if the difference in mutation were largely due to side effects causing the loss of mutation. In any case, all these alternatives, whether attributable to side effects or to the mutations themselves, can be excluded by fairly direct tests.

One set of tests is shown in Table 2. It summarizes data from an experiment in which paramecia were irradiated with 4.5kr at 1.0 (G1) and 3.5 (G2) hours after division, daily isolation lines were established, samples were stained at various times to check for micronuclear loss and macronuclear regeneration, and the amount of mutation was measured. Sixty lines were set up for each treatment group; and 40 lines from the controls, 44 from the one-hour group, and 42 from the 3.5-hour group had to be checked to obtain autogamy from 40 lines of each group. The percentage frequencies of normal clones in Table 2 would have been changed to 98, 21, and 87, respectively, if the six lines from which autogamy could not be obtained had yielded instead autogamous progeny all of which died or grew poorly. Clearly alternative 1 (selection through loss of lines) cannot account for the G1–G2 difference.

Alternative 2 also cannot explain the data. The number of micronuclei was counted in ten specimens per line from samples fixed and stained one day following irradiation after some four to five divisions had occurred. Only lines

TABLE 2

Tests for loss of micronuclei and for macronuclear regeneration

			Stained samples of autogamous cultures				
			Number of lines with some individuals			Mutation	
Dose kr	Age hours	Number of lines	Number of micronuclei Mean ± SE	with no anlagen	in macronuclear regeneration	Percentage frequency of normal clones	$M \pm SE$
0	1 & 3.5	40	1.75 ± 0.08	2	3	98	0.00 ± 0.03
4.5	1	40	1.41 ± 0.07	20	23	22	1.52 ± 0.06
4.5	3.5	40	1.53 ± 0.07	12	8	92	0.15 ± 0.06

from which autogamy was later obtained were included in the mean counts. Loss of micronuclei at any time during the first day should decrease the average number per paramecium since the number, once changed, tends to remain constant at the new value (KIMBALL *et al.* 1957). Table 2 shows that the average number in the controls was somewhat below two, the number characteristic for this species, but such variation in number is not uncommon in our experience. Similar data on micronuclear number are also shown in Table 1. Radiation reduces the number, contrary to some earlier evidence (GECKLER and KIMBALL 1953), but if anything less in the 3.5-hour than in the one-hour group. There should have been a greater loss of micronuclei from the old than the young groups if loss of mutant micronuclei were responsible for the G1–G2 difference. Furthermore, the amount of loss is too small to explain large differences in the number of mutations.

The correlation coefficients between micronuclear number and mutation provide a test for alternative 3 (selection against mutant meiotic products). The smaller the number of micronuclei the less the chance that at least one meiotic product in a given specimen will lack mutations and thus allow survival of the exautogamous clone. This effect might be balanced to some extent by selective loss of micronuclei during vegetative reproduction, which would tend to make lines with fewer micronuclei after irradiation contain fewer mutations. Nonetheless, a major difference between G1 and G2 in selection of meiotic products should appear as a difference in the correlation coefficients. Thus, the correlation coefficients should be negative and greater for the G2 groups than the G1 groups, if this form of selection were responsible for the G1–G2 difference in mutation. Correlation coefficients were calculated for the experiments in Tables 1 and 2. The coefficients were negative for the three irradiated groups in Table 1, not significantly different from each other, and significantly different from zero when taken together. The rates of change of M with increase in number of nuclei were –0.27, –0.32, and –0.25 M per micronucleus for groups 2, 3, and 4, respectively. Selection of meiotic products may have occurred, but if so it was not more effective in the G2 groups. The correlation coefficients for the two irradiated groups in Table 2 were positive instead of negative but not significantly different from zero, giving no evidence at all for selection of meiotic products. Consequently, alternative 3 can be excluded as a major contribution to the G1–G2 difference.

A check for alternative 4 (macronuclear regeneration) was made on two samples from the same autogamous cultures from which isolations were made for mutation determination (Table 2). One sample from each was fixed immediately to look for the anlagen of the new macronuclei. Normally two anlagen are present at this stage and are clearly distinguishable from the fragments of the old macronucleus. The other samples were transferred *en masse* to fresh culture medium, allowed to grow for one day (two to four divisions), and then sampled. At this time, a few small fragments of the old macronucleus were visible in most specimens together with one large macronucleus. Individuals

with fragments and no distinctly larger macronucleus were scored as macronuclear regenerates.

Table 2 shows that a number of autogamous cultures, especially in the irradiated groups, contained specimens without anlagen, but the frequency of such specimens was usually no more than a few percent per culture with a maximum of 20 percent. Clearly the great majority of autogamous individuals of all lines were able to form normal appearing macronuclear anlagen. This observation shows that all lines used for autogamy must have retained at least one micronucleus since there is no known way in which macronuclear anlagen can arise except from a preexisting micronucleus. Therefore, complete loss of micronuclei followed by survival of apparent "exautogamous clones" through macronuclear regeneration can be excluded.

Table 2 also shows some evidence for macronuclear regeneration in the sample stained a day after autogamy, but again the frequency per culture was low, usually only a few percent. Macronuclear regeneration is appreciably more frequent in the irradiated than the control lines but is more frequent in the one hour than in the 3.5 hour group, the wrong direction to explain the difference in detectable mutation.

A genetic test for macronuclear regeneration was also made using the killer character (BEALE 1954). A clone with the genotype *Kk* containing the intracellular organism kappa was irradiated and sent through autogamy in the usual way. True autogamy should produce *KK* (killer) and *kk* (sensitive) clones in equal numbers; macronuclear regeneration should preserve the *Kk* (killer) genotype. Radiation itself inactivates kappa (PREER 1948) but not sufficiently at this dose to make any paramecia sensitive by elimination of kappa. Groups of 25 autogamous clones were obtained as usual from 40 lines for each treatment group, and ten clones with normal growth, or as many as possible if ten were not available, were tested for the killer phenotype. A few clones reacted as mixtures of killers and sensitives or gave other equivocal tests.

The results are summarized in Table 3, which shows the number of lines tested, the number giving evidence of true autogamy by producing at least one sensitive clone, and the overall frequency of killer and sensitive progeny. The

TABLE 3

Segregation of k

Dose kr	Age hours	Mutation: Percentage frequency of normal clones	Mutation: $M \pm SE$	Killer tests: Number of lines with at least one clone tested	Killer tests: Number of lines producing at least one sensitive clone	Killer tests: Total clones tested	Killer tests: Percentage frequency of clones: Killer	Sensitive	Mixed or equivocal
0	Mixed	82	0.00 ± 0.06	40	35	396	51.3	46.5	2.3
4.6	1	8	1.55 ± 0.06	30	12	73	41.1	37.0	21.9
4.6	2	10	1.52 ± 0.07	27	10	96	52.1	30.2	17.7
4.6	3	62	0.38 ± 0.10	39	33	335	49.9	47.2	3.0
4.6	4	78	0.09 ± 0.06	40	38	393	34.9	64.6	0.5

controls showed a lower frequency of normal autogamous clones than in the usual inbred stock, a common experience with hybrids between stocks in *P. aurelia*. Consequently the frequency of normal clones in the one-hour and two-hour groups is very low, and in some cases only one clone out of 25 was testable. Not too surprisingly, there is an excess of killer clones in one of these groups suggesting some macronuclear regeneration. The frequency of killers and sensitives in the control, one-hour, and three-hour groups did not depart very much from equality, suggesting only a small amount of macronuclear regeneration at most, though the data for the one-hour group are uncertain because of the high frequency of equivocal tests. The four-hour group produced an unexplained high frequency of sensitive clones. In any case, a high frequency of macronuclear regeneration certainly did not occur in either the three-hour or four-hour group.

Time available for recovery: We (KIMBALL *et al.* 1957, 1959b; KIMBALL, GAITHER and PERDUE 1961) have developed the hypothesis that premutational damage produced in G1 is lost continuously by repair up to the time of chromosome duplication when the remaining damage is converted to mutation. Since G2 is farther from the next chromosome duplication than G1, more repair might occur; but assuming that the probability of repair per unit time is constant for all stages, it can be calculated that the interval would be much too short to explain the G1–G2 difference unless the interval were increased considerably by radiation.

The increase in generation time produced by 4.6kr of X rays given at various times after division is shown in Figure 2. The later the irradiation the greater the generation time; but no discontinuities are seen between G1 and G2 or between early prophase and later stages to correspond with the discontinuities in the amount of mutation (KIMBALL 1961). A direct check was made by Feulgen microspectrophotometry for the time of the next S period with and without irradiating with 5kr in G2 (Groups 1 and 2 in Table 1). There was no evidence for any change in the interval between the postirradiation division and the following S period, and any change could not have been greater than 15 minutes.

DISCUSSION

The data in this paper show that the difference between G1 and G2 irradiations in producing detectable mutation cannot be attributed to selective loss of mutations before expression at autogamy. It is true that some evidence of what might be selective elimination was found, i.e., loss of lines of descent, loss of micronuclei, and occurrence of macronuclear regeneration. These phenomena, however, have not been shown to be selective in respect to micronuclear mutations and, in every instance, such events were more frequent in the G1 than in the G2 group, the wrong direction to account for the mutation data. The only item, the correlation between mutation and micronuclear number, that directly tests for selection against mutations suggests that selection is not greater and may even be slightly less in the G2 group. Thus, there seems no doubt that irradiation in G2 is much less efficient than irradiation in G1 in inducing final mutation.

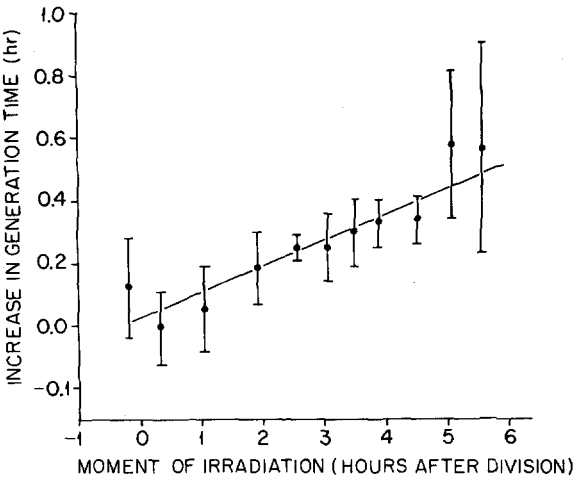

FIGURE 2.—Increase in generation time as a function of the time after completion of cytokinesis when 4.6kr of X rays were given. The 95 percent confidence limits and least squares straight line are plotted. All increases are for the generation during which the radiation was given except for the leftmost point. All specimens in this group were undergoing cytokinesis when irradiated and the increase is for the generation immediately following. The two rightmost points are for groups that had partially completed cytokinesis when they were irradiated. The increase in generation time is plotted only for those specimens that divided after irradiation. Except for these two groups, there were ten control and ten irradiated specimens per point. The mean generation time of the controls was 5.4 hours.

A low rate of induction could mean either that the genetic material is protected against the physical and chemical processes that produce the initial molecular alterations or that for some reason these alterations do not lead to final mutation. Arguments have been given previously for rejecting general protective agents and destructive removal of altered precursors of DNA as explanations for the insensitive period (KIMBALL 1961). We are also inclined to reject destructive removal of diffusible and reasonably stable mutagens because it seems unlikely that such substances act in X-ray mutagenesis in Paramecium (KIMBALL, HEARON and GAITHER 1955). It is possible, however, that the molecular structure of the chromosomes changes at duplication in such a way that critical bonds are protected from radiation attack. No way to bring about this protection is obvious to us; however, the idea must be considered as implausible but not clearly rejectable. The most likely explanations involve some form of repair of premutational lesions in the chromosomes (KIMBALL 1961).

Restitutional reunion of chromosome breaks is the best established form of repair, and several explanations for stage differences in sensitivity have involved this process. For example, WHITING (1945, 1961) suggested that the greater sensitivity of metaphase I than prophase I eggs of Habrobracon is a result of greater tension on the chromosomes in metaphase with a consequent reduced chance of restitution. WOLFF (1961) suggested that more aberrations are detected in Vicia and Tradescantia when the chromosomes are double at irradiation in

part because a whole class, the isochromatid aberrations, is produced by union of breaks in sister chromatids, breaks that could only restitute if produced in single chromosomes. Neither explanation is applicable to the refractory period in Paramecium. The micronuclear chromatin is highly condensed throughout interphase and does not undergo any cytologically detectable change in physical state during the G1–G2 transition. Thus, changes in tension are improbable. The change in sensitivity is the reverse of that in Vicia and Tradescantia. Indeed the small amount of mutation inducible in G2 and early prophase makes it doubtful whether isochromatid aberrations occur at all.

One hypothesis involving breakage and reunion is more plausible than the others. This may be called the "splint" hypothesis, which was suggested by THOMPSON (1962) to account for the observation that more recessive lethals were produced in structural heterozygotes than in structural homozygotes when meiotic stages in Drosophila males were irradiated. THOMPSON suggested as one explanation for his results that a closely paired homologue in a structural homozygote may be able to hold a broken chromosome in place, thus facilitating restitutional reunion. The closely paired sister chromatids of G2 and early prophase could serve the same function. To account for the whole G1–G2 difference, it would be necessary to assume that the great majority of the postautogamous lethality and slow growth in Paramecium resulted from breakage phenomena. This seems unlikely (KIMBALL, in preparation), but the splint hypothesis could give an explanation for part though not all the difference.

Another group of ideas has centered around the metabolic aspects of repair of chromosomal lesions. The hypothesis has been developed for Paramecium (KIMBALL *et al.* 1959, 1961; KIMBALL 1961) that premutational lesions are subject to metabolic repair up to the time of the first postirradiation chromosome duplication when any remaining damage is converted to final mutation. On this basis, premutational lesions induced in G2 would be subject to repair until the next S period, provided such lesions could persist through mitosis. The interval between G2 and the following S period is not long enough, however, to account completely for the low number of mutations if repair of a single class of damage occurs at the G1 rate. Thus in Table 1 the difference between the amount of mutation induced in G2 and G1 four hours later is about 1.5 *M*, after correction of both G1 and G2 mutation to a dose of 4.5kr. If equal amounts of premutational damage were induced in G2 and G1, then 1.5 *M* must have disappeared in the intervening four hours, a rate of repair of 0.4 *M* per hour. This is a minimum estimate of the rate since the generation time was longer than usual in this experiment. The rate of repair in G1 is no greater than 0.2 *M* per hour with a dose of 4.5kr (KIMBALL *et al.* 1961). A second indication that repair at the G1 rate cannot account completely for the low mutation in G2 is that there is no sharp change in generation time following irradiation in midprophase although there is a sharp rise in mutation induction (KIMBALL 1961). Thus the time available between G2 and the next S is insufficient to account by itself for the low amount of mutation in G2 if the rate of repair of premutational damage remains constant and there is only one class of reparable damage. The time might be sufficient to remove a class of

damage that was reparable in both G1 and G2 if there were another class that was reparable only in G2.

The studies with G2 paramecia have suggested that no more than one tenth, quite possibly less, of all initial damage is irreparable. Studies with stationary phase paramecia, in which the chromosomes remain unduplicated (KIMBALL and PERDUE, unpublished), have shown, however, that about one third of the mutation remains no matter how long the time for repair (KIMBALL *et al.* 1959b). The disparity between these two estimates of irreparable damage could be explained in one of three ways: (1) There is an irreparable form of initial damage produced in stationary phase that is not produced in G2; (2) the same kinds of initial damage are produced at all stages but one kind is reparable mainly in doubled chromosomes; (3) there is a conversion of premutational damage to final mutation in the absence of chromosome duplication, and it is this mutation that remains after a prolonged stationary phase. It seems improbable that the initial attack of radiation on the chromosomes is sufficiently different at the different stages to allow (1) to be true. The "splint" hypothesis would provide a mechanism for (2) and recent evidence (KIMBALL, in preparation) for some postautogamous lethality caused by rapidly rejoining chromosome breaks might provide a mechanism for (3). In any case, if either (2) or (3) is correct, the residual mutation after a prolonged stationary phase is not mutation that is irrevocably formed at the time of irradiation, and probably very little if any mutation of this sort is formed in Paramecium.

It seems possible, then, that much, though probably not all, of the difference between the amount of mutation induced in G1 and G2 can be attributed to a combination of two factors, the longer time for repair and especially effective repair of chromosomal breaks in doubled chromosomes.

A stage during the cell cycle that is very insensitive to the induction of recessive lethals is not unique for Paramecium. In Saccharomyces an early budding stage, thought to be prophase, is about one-tenth as sensitive as other stages (BEAM 1959). Prophase is also very insensitive compared to metaphase for recessive lethal induction in the oocytes of Habrobracon (ATWOOD, VON BORSTEL and WHITING 1956). Possibly the same situation exists in Drosophila oocytes (PARKER 1960) though in this case the nuclear condition has not been clearly described. Great changes in sensitivity also occur during spermatogenesis of Drosophila (see SÄVHAGEN 1961 for review) and in the silkworm (TAZIMA 1961) but the relations to the ordinary cell cycle are not clear. The relation to the time of chromosome duplication has not been investigated in any of these cases; consequently, it is possible that G2 as well as part of prophase is insensitive as it is in Paramecium.

A highly insensitive period for inactivation by radiation and P^{32} decay exists in bacteriophage, but apparently it begins before DNA synthesis and may involve gene expression rather than duplication (PRATT, STENT and HARRIMAN 1961; STENT 1961). It is likely, therefore, that this phenomenon has nothing in common with that in higher organisms.

In any case, large fractions of the initial radiation damage to the chromosomes can be removed or repaired under appropriate circumstances. Indeed it is prob-

able that little if any damage is irreversibly formed at the moment of absorption of radiation energy.

SUMMARY

It has been shown previously that very few recessive lethal and slow growth mutations can be detected in paramecia irradiated during G2 and early prophase. This paper rules out various artifacts that might influence detection of mutations and shows that this is indeed a period during which very few mutations can be induced. Various hypotheses to account for the observations are discussed in relation to work with other organisms. It is concluded that some form of post-irradiation repair, possibly of more than one kind, is probably involved. Little if any final mutation appears to be formed at the time of irradiation; almost all passes through an intermediate, reparable stage.

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