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INHERITANCE OF MUTATIONAL CHANGES INDUCED BY RADIATION IN PARAMECIUM AURELIA

R. F. KIMBALL

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R. F. Kimball

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INHERITANCE OF MUTATIONAL CHANGES INDUCED BY RADIATION IN PARAMECIUM AURELIA

R. F. Kimball[1]

In a previous paper Kimball (in press) has described the induction by radiation of changes in vigor in Paramecium aurelia which first expressed themselves in the exautogamous progeny of rayed animals. In that paper, it was assumed that the changes were due to mutations in the micronucleus. This paper will present the genetic evidence which bears on this assumption.

The radiation studies suggested that most of the reduction in vigor after autogamy was due to the combined action of a number of mutant genes with individual effects too small to be detected rather than to genes with large effects. Thus, the phenomena of inheritance observed should be those characteristics of multiple gene inheritance.

METHODS

For this work stock 90 of variety 1 was used. Four experiments were carried out. In the first two, beta radiation was given by means of the phosphorus plaque technique previously described. In experiment 1 approximately 13,000 rep were given; in experiment 2, approximately 6500. The dosage rate was about 200 rep per minute. The irradiation in experiments 3 and 4 was by x-rays from a Maximar model machine operated at 250 kv and 15 ma. There was an inherent filtration of 3 mm of aluminum but no added filtration. Measurements were made immediately before exposure with a Victoreen chamber placed in the same position as the exposure chamber. The

1 Valuable assistance was given by Nenita T. Gaither and Margaret E. Griess.

animals in 0.5 cc of culture fluid were put in a cavity 1.8 cm in diameter drilled in a small lucite block, and the cavity was covered with cellophane tape. The block was placed under the tube so that the animals were about 46 cm from the target. In experiment 3, a dose of 6600 r was given; in experiment 4, 7000 r. The dosage rate was about 245 r per minute.

After irradiation, single rayed animals were isolated and allowed to divide. Every day a single descendent of each rayed animal was isolated to carry on the line of descent. A few days after irradiation the descendants of animals left over after the first or second isolation were mixed with unirradiated animals of the opposite mating types and conjugant pairs isolated from the mixture. When the conjugants had separated, the two members of each pair were put in separate containers. One product of the first or second division of each exconjugant was saved and the rest discarded to prevent mixture of different caryonides and the reconjugation which such mixture would allow. The animals which were saved were left in a limited quantity of culture fluid for four days and then observed for total growth. All those which failed to produce a maximum and starved population in this period were classified as of reduced vigor. This is the same test for vigor previously described in some detail by Kimball (in press). The group of exconjugants obtained in this way will be called the BC1 generation.

Meanwhile the line of descent from the rayed animals was continued by daily isolation and about a week after irradiation the left-over animals from each line were found to be in autogamy. Twenty-five autogamous animals were isolated from

each rayed line and likewise subjected to the four-day total growth test. This generation will be called the A1.

The exconjugants of the BC1 generation likewise were continued by the daily isolation technique. About a week after conjugation in experiments 1 and 2, some of the progeny from several of the clones were caused to conjugate again with unirradiated animals to yield a BC2 generation. In all four experiments, two to three weeks after conjugation, autogamy was found to be occurring in the left-overs from daily transfer, and 25 autogamous animals were isolated from each BC1 clone. This will be called the A2 generation. In experiment 1, BC3 and A3 generations were obtained in the same manner from the BC2 clones. In all four experiments, unirradiated animals from the same clone as the original irradiated ones were carried through the same breeding procedure to serve as controls.

GENETIC EXPECTATIONS

According to the conclusions of Kimball (in press), animals given the doses of radiation employed here are probably heterozygous for a number of mutant genes affecting vigor. When such heterozygotes are crossed to unrayed animals the exconjugants will be, on the average, heterozygous for a number of mutant genes but will rarely, if ever, be homozygous for any of them. Different exconjugant pairs from the same rayed animals will be heterozygous for different combinations of mutants as a result of segregation during gamete nucleus formation in the rayed member of the pair. However, the two members of a pair should be of identical genotype after conjugation. The average number of mutant genes per micronucleus

in the BC1 generation should be half that in the original rayed animals. Exautogamous animals should be completely homozygous and, if derived from heterozygous animals, should frequently be homozygous for some of the mutant genes. Different exautogamous animals from the same heterozygote should be homozygous for different combinations of mutants as a result of segregation during gamete nucleus formation. For the details of the genetic and cytological information upon which those expectations are based, Sonneborn (1947) may be consulted.

In testing the backcross generations for agreement with these expectations, the phenotype of the backcross clones themselves, as expressed in four-day total growth, might be used. However, this method would only detect mutants with some dominant effect. A more satisfactory method is to obtain exautogamous progeny from each backcross clone. If a large number of autogamous progeny were obtained, all possible homozygous combinations of the mutant genes in the backcross clone should appear and the fraction of exautogamous clones of reduced vigor should be characteristic for the genotype of the backcross clone. In general, the more mutant genes, the larger this fraction would be. In actual practice only 25 autogamous progeny were obtained from each clone so that this measure is expected to be subject to considerable sampling error.

PHENOTYPE OF THE BACKCROSS CLONES

The data on the phenotype of the backcross clones as expressed in four-day total growth are given in table 1. Each clone was classified as of normal vigor (+) or of reduced

TABLE 1

Generation	Experiment	Number of Original Rayed or Control Animals	Number of Clones from Previous Generation From Which Derived	Number of Pairs			Total
				+ +	+ −	− −	
BC1	1	5	–	112	4	0	116
	2	9	–	206	16	6	228
	3	30	–	107	8	4	119
	4	13	–	98	23	9	130
	Total	57	–	523	51	19	593
BC2	1	1	6	45	17	28	90
	2	3	60	541	37	6	584
BC3	1	1	34	407	20	60	487
BC1 Control	1	1	–	30	0	0	30
	2	6	–	140	15	5	160
	3	10	–	37	3	0	40
	4	5	–	47	3	0	50
BC2 Control	1	1	2	20	0	0	20
	2	1	20	162	6	0	168
BC3 Control	1	1	10	147	3	0	150
Total Control	–	22	–	583	30	5	618

vigor (-). Thus, there were three classes of pairs depending upon the phenotype of the two members.

The BC1 of experiments 1, 2, and 3 and the BC2 of experiment 2 do not differ significantly from each other, as shown by a chi-square for heterogeneity of 9.3 for six degrees of freedom, nor does their total differ significantly from the total for all the controls as shown by a chi-square for heterogeneity of 4.3 for two degrees of freedom. The BC1 of experiment 4 differs significantly from the total controls as shown by a chi-square of 50 for two degrees of freedom. The BC2 and BC3 of experiment 1 also differ significantly from the controls as shown by high values of chi-square. The later generations of experiment 1 show other pecularities, and a detailed discussion will be reserved for another section. Possibly, the divergence of the results of experiment 4 from the others might be due to the chance occurrence of more dominant mutations in it than the others, but the number of discordant pairs makes difficulties for this interpretation.

Except for experiment 4, there seems to be no evidence for dominant effects in BC1. This is so despite the fact that all 57 rayed animals from which this generation was derived gave evidence in their A1 progeny of effects of the radiation. Many of them produced more than 50% clones of reduced vigor, and the smallest percentage was 24% as compared to controls which rarely exceed 16%. It is possible that dominant changes are sufficiently less frequent than recessives with small effects and that the relatively small group of rayed animals so far studied is inadequate to clearly demonstrate them.

The occurrence of discordant pairs is not expected on simple genetic theory. Since such pairs also occur in the controls, part at least have no connection with the effects of radiation. The numerous discordant pairs in experiment 4 cannot be accounted for in this way. It is possible that some may have been the result of cytogamy. This process, which was not distinguished from true conjugation in the present work, is essentially autogamy in the two members of the pair. The member of rayed ancestry of a cytogamous pair would be just as likely to be of reduced vigor as any autogamous animal from the same rayed animal. The member of unrayed ancestry would be just as unlikely to be of reduced vigor as the autogamous descendants of the corresponding unrayed animals. It should be noted that in most cases later generations were obtained from BC1 pairs,.both members of which were normal. Thus, the animals of these generations were descended probably from few, if any, cytogamous pairs. Most pairs from which later generations were obtained were probably true conjugants.

THE EXAUTOGAMOUS PROGENY OF BC1 CLONES

As has already been pointed out, the A2 generation should furnish a measure of the genotype of the BC1 clone from which it was derived. An A2 generation was obtained from both members of 124 BC1 pairs and from one member only of 23 other pairs. From each member about 25 autogamous animals were obtained. An A2 generation was not always obtained from the same number of BC1 pairs for different rayed animals.

For purposes of a statistical analysis to be given below, it was desirable to have a body of data in which an A2 was obtained from the same number of BC1 pairs for each rayed animal. Therefore, for each rayed animal, for which at least two pairs were available, two were chosen at random using Snedecor's (1946) table of random numbers. This random sample of the total data is presented in table 2. It consists of the A2 generation from 74 pairs derived from 37 rayed animals from all four experiments. The body of the table shows the fraction of A2 clones of reduced vigor. The two members of the BC1 pairs are labeled "a" and "b" arbitrarily since it was not known which came from the rayed and which from the unrayed member. Control data has been omitted to save space. Suffice it to say that only occasionally was the fraction of reduced vigor in the controls higher than 0.16; i.e., four clones out of 25, though a few very high fractions were found, especially in experiment 4.

An examination of the table clearly demonstrates that the effect of the radiation is transmitted to both members of the pairs for, in many cases, both members produced much higher fractions than were characteristic of the controls. Sonneborn's (1947) work suggests that ordinarily the pronuclei are exchanged at conjugation with very little, if any, cytoplasm accompanying them. Thus, the transmission to both members is suggestive, though not by itself conclusive, evidence that micronuclear mutations are involved.

The expectation that the two members of a pair should be of identical genotype and so alike in the fraction of

TABLE 2

Fraction of A2 Clones of Reduced Vigor

Experiment	Rayed Animal	BC1 Pair	Member of Pair a	Member of Pair b
1	1	1	0.96	0.96
		2	0.84	1.00
	2	1	0.96	1.00
		2	1.00	1.00
	3	1	0.04	0.08
		2	0.28	0.00
2	1	1	0.76	0.00
		2	0.20	0.00
	2	1	0.04	0.12
		2	0.04	0.16
	3	1	0.16	0.24
		2	0.00	0.00
3	1	1	0.56	0.32
		2	0.00	0.24
	2	1	0.56	0.40
		2	0.76	0.28
	3	1	0.32	0.44
		2	0.96	0.68
	4	1	0.36	0.56
		2	0.64	0.80
	5	1	0.48	0.40
		2	0.56	0.84
	6	1	0.96	0.88
		2	0.16	0.20
	7	1	0.80	0.20
		2	0.88	0.96
	8	1	0.32	0.44
		2	0.56	0.20
	9	1	0.76	0.64
		2	0.52	0.52
	10	1	0.60	0.44
		2	0.16	0.44
	11	1	0.60	0.44
		2	0.60	1.00
	12	1	0.52	0.96
		2	0.32	0.28
	13	1	0.48	0.68
		2	0.12	0.12
3 (Cont.)	14	1	0.44	0.44
		2	0.28	0.48
	15	1	0.48	0.96
		2	0.68	0.80
	16	1	0.00	0.00
		2	0.08	0.00
	17	1	0.48	0.40
		2	0.28	0.52
	18	1	0.16	0.04
		2	1.00	0.52
4	1	1	0.96	1.00
		2	0.52	0.84
	2	1	0.28	0.32
		2	0.68	0.72
	3	1	0.68	0.72
		2	0.88	0.60
	4	1	0.88	0.80
		2	0.80	0.16
	5	1	0.20	0.16
		2	0.00	0.00
	6	1	1.00	0.92
		2	1.00	0.68
	7	1	0.36	0.44
		2	0.28	0.64
	8	1	0.08	0.56
		2	0.00	0.00
	9	1	0.32	0.36
		2	0.92	0.68
	10	1	0.96	1.00
		2	0.88	0.96
	11	1	0.80	1.00
		2	1.00	0.44
	12	1	0.20	0.80
		2	0.80	1.00
	13	1	0.00	0.00
		2	0.12	0.08

exautogamous clones of reduced vigor which they produce is not met with any exactness by the data in table 2. At least two factors might be expected to cause departures from identity. The first is deviation due to sampling error which, with samples of 25, is expected to be high. The second is spontaneous mutation. The exconjugants had to multiply vegetatively for two to three weeks before exautogamous clones could be obtained. During this time, spontaneous mutation could occur and destroy the original identity of genotype. This is all the more possible since the BC1 clones were kept as daily isolation lines so that chance choice for transfer of an animal with a new mutation would immediately establish the mutation in the line of descent.

Even if spontaneous mutation were relatively frequent the two members of a pair should tend to be more alike than different pairs from the same rayed animal since different pairs would often be of different genotype immediately after conjugation and spontaneous mutation could only add to a difference already existing. To see if this were so, an analysis of variance was carried out on the data of table 2. The fractions were first converted to angles using the table of this transformation in Snedcor (1946). The sums of squares attributable to differences between the progeny of different rayed animals, to differences between pairs from the same rayed animal, and to differences between members of the same pair were found and divided by their degrees of freedom to give the mean squares shown in table 3.

TABLE 3

Analysis of Variance of the A2 Data in Table 2

	Mean Square	Degrees of Freedom
(1) Variance attributable to differences between the progeny of different rayed animals	1823	36
(2) Variance attributable to differences between the pairs from the same rayed animal	477	37
(3) Variance attributable to differences between members of the same pair	203	74

Variance ratio of (2)/(3) = 2.35
Probability = less than 0.01

As would be expected, the mean square for differences between rayed animals is considerably greater than the other two. The ratio of the mean square for differences between pairs from the same rayed animal to that for differences between members of the same pair is 2.35. According to the table of the variance ratio given by Snedecor (1946), a value as great or greater than this would have less than one chance in a hundred of occuring if the two mean squares were really the same. It can be concluded that the two members of a pair are more alike than are different pairs from the same rayed animal.

The final expectation that can be tested with the exautogamous generations is that the mean number of mutant genes per micronucleus should be halved by backcrossing to unrayed animals. Kimball (in press) gives an equation by which the mean number of mutant genes can be calculated from the average fraction of their autogamous progeny of reduced vigor. The equation in its most general form is:

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

where F is the average fraction of exautogamous clones of reduced vigor; m is the mean number of mutant genes per micronucleus; and c_0, c_1, c_2, etc. are constants representing the probability that clones with respectively none, one, two, etc. homozygous mutant genes will be of normal vigor. The dosage data led to the conclusion that c_0, c_1, c_3 are unity and c_5, c_6, etc. are zero with a question as to what value to assign c_4. For present purposes, it will be assumed to be zero, a value which gives a good fit to the dosage data. Using this equation, m can be calculated for the rayed animals from the A1 data;

and for the BC1, from the A2 data.

Experiment 1 is entirely unsuited for such calculations since the A1 generation had so high an average fraction of reduced vigor (0.96) that m for the rayed animals cannot be calculated with any confidence. This is true because, as F approaches unity, a slight change in its value makes a great change in the calculated value of m. Experiment 2 is not especially satisfactory because of the low number (three) of rayed animals used. The values of the constants in the equation are average probabilities for many genes. With a small sample of mutant genes such as would be obtained from only three rayed animals, the values might be entirely different. However, calculations for this experiment have been included since the values of F fall within the usable range. Experiments 3 and 4 are both reasonably suitable.

The calculations are set forth in table 4. Since the average fraction of reduced vigor in the controls was somewhat variable, was uniformly greater for the A2 than for the A1, and was unusually high in the A2 of experiment 4, it seemed necessary to introduce a correction for it. There are several ways in which the correction could be made depending upon the cause of reduced vigor in the controls. It seemed best to adopt a uniform hypothesis for the cause of reduced vigor and assume that, in the controls, it was due to spontaneous mutation. This is probably not entirely true; but, in the absence of data as to the nature and relative frequency of other causes, this appears to be the best approach. For this assumption, the proper correction would be the

subtraction of the mean number of mutant genes calculated from a given control group from that for the corresponding rayed group. The remainder would be the mean number of mutant genes derived from those induced by the radiation.

The last column of table 4 shows the ratio between the mean number of mutant genes in the rayed animals and in the BC1. For experiments 3 and 4, the value is quite close to the expected value of two particularly when the number of approximations involved in the calculations is taken into account. The value for experiment 2 is not so close to expectation but it has already been pointed out that this experiment is not particularly satisfactory for such calculations.

ANOMALOUS INHERITANCE IN EXPERIMENT 1

It has already been mentioned that the BC2 and BC3 of experiment 1 show some unexpected features. The BC1 of this experiment did not include significantly more clones of reduced vigor than did the controls, but many such clones appeared in the BC2 and BC3. That the difference is real is shown by the contingency chi-square for agreement with the total controls. For the BC2, it was 182 and for the BC3, 59. Both values for two degrees of freedom have a negligible chance of occurring as a result of chance deviations. It can also be shown that there is a distinct tendency for the two members of a pair to be alike. The chi-squares for agreement with a random distribution of reduced vigor to the two members of a pair are 33 for the BC2 and 338 for the BC3. Both chi-squares indicate a highly significant departure from a random distribution, obviously in the direction of too few

TABLE 4

The Mean Number of Mutations Per Micronucleus in the Rayed and BC1 Animals as Calculated from the A1 and A2 Data

Experiment and Treatment	Number of Animals	Generation	Total Clones	Fraction of Clones of Reduced Vigor	Mean Number of Mutant Genes	Rayed Minus Control	Ratio A1/A2
2 - rayed	3	A1	75	0.753	10.3	10.3	3.7
	3	A2	1469	0.127	4.0	2.8	—
2 - control	6	A1	150	0.000	0.0	—	—
	1	A2	500	0.004	1.2	—	—
3 - rayed	30	A1	749	0.826	11.5	9.6	2.3
	30	A2	2750	0.438	6.8	4.2	—
3 - control	10	A1	250	0.016	1.9	—	—
	10	A2	950	0.043	2.6	—	—
4 - rayed	13	A1	325	0.698	9.5	6.8	1.7
	13	A2	1300	0.582	8.2	4.0	—
4 - control	10	A1	250	0.050	2.7	—	—
	5	A2	401	0.161	4.2	—	—

+ - pairs. Thus, whatever is involved tends to affect the unrayed as well as the rayed member of the pair within a four-day period after conjugation. The first explanation that comes to mind is that dominant mutations are concerned; but, if this is so, it is difficult to understand how they passed through the BC1 generation without expressing themselves.

A more detailed examination of the data reveals further pecularities which are brought out in table 5. It was found that almost all the BC2 and BC3 pairs in which both members were of reduced vigor came from a few BC1 and BC2 clones. Moreover, in all cases in which both members of BC1 or BC2 pairs were followed, if one member produced much reduced vigor at the next conjugation, the other did not. The member which produced many clones of reduced vigor on conjugation with unrayed animals produced at autogamy many clones which failed to survive the four-day period. The member which produced few clones of reduced vigor at the next conjugation produced few clones at autogamy which failed to survive the four-day period though it produced many which were of reduced vigor. Finally, it should be noted that the BC2 clones which produced much reduced vigor at conjugation and death at autogamy were derived from the members of the BC1 pairs which did not show this behavior. The other member of these same pairs did show it. Apparently, the anomalous behavior can be transmitted through clones which do not exhibit it. No adequate explanation for these phenomena can be offered though it is clear that some kind of inheritance is involved. It is to be noted that table 5 includes only those pairs which showed the anomalous behavior.

TABLE 5

Anomalous Inheritance in Experiment 1

Pair	BC1 Origin	Member of Pair a: BC2 or BC3 ++	a: BC2 or BC3 +-	a: BC2 or BC3 --	a: A2 or A3 +	a: A2 or A3 r	a: A2 or A3 d	b: BC2 or BC3 ++	b: BC2 or BC3 +-	b: BC2 or BC3 --	b: A2 or A3 +	b: A2 or A3 r	b: A2 or A3 d
BC1-39	---	10	2	3	0	25	0	3	4	8	0	6	19
BC1-40	---	15	0	0	5	18	2	2	5	8	0	10	15
BC1-41	---	1	5	8	1	8	16	14	1	0	0	18	7
BC2-32	41b	not obtained			not obtained			1	3	1	0	14	11
BC2-38	41b	11	2	0	5	19	1	1	1	13	0	6	19
BC2-45	41b	not obtained			not obtained			2	1	11	0	5	20
BC2-76	40a	0	4	10	0	7	18	13	1	0	not obtained		
BC2-88	40a	14	5	2	0	25	0	1	2	19	2	6	17

+ = normal vigor, - = reduced vigor, d = died within four days,

r = reduced vigor but survived four days or more.

This includes all three BC1 pairs from which later generations were obtained but only a part of the BC2 pairs from which a BC3 and A3 were derived.

HOMOZYGOUS CLONES OF REDUCED VIGOR

A number of viable exautogamous clones from the backcross progeny of rayed animals have been obtained, some of which had distinctly lower fission rates than the controls. Such clones were presumably homozygous for one or more mutants with a relatively mild total effect upon vigor. Crosses with unrayed animals have shown that the low fission rate can be transmitted through both members of conjugant pairs, but the data is still too limited to warrant detailed presentation.

A most noticeable feature of these homozygotes was the great variability within the clone. When a number of animals were isolated from such a clone, the descendants of some had normal fission rate, or nearly so. Those of others had a distinctly lower rate, while still others failed to survive at all. When animals were isolated from the apparently normal variants, this same variability reappeared. This great variability makes detailed investigations of inheritance with this material very difficult.

DISCUSSION

Sonneborn's (1947) review of the genetics of <u>Paramecium aurelia</u> has brought out the diversity of the types of inheritance and has shown that a number of persistent diversities in this organism are not due to genic differences. This emphasizes the need for caution in interpreting persistent diversities as

having a genic basis. Therefore, alternatives to the gene mutation hypothesis presented in this paper will be examined in some detail.

The first alternative to be considered is cytoplasmic inheritance. The fact that the changes in vigor first appear after autogamy does not itself rule out this possibility since sudden changes in cytoplasmically determined characters are known to occur during this process. The transmission to both members of pairs between rayed and unrayed animals is not to be expected with cytoplasmic inheritance if no cytoplasmic exchange occurs between the conjugants. However, evidence that cytoplasmic exchange does not ordinarily occur has so far been obtained for the B group of varieties only and is not available for the A group to which stock 90 belongs. As a matter of fact, evidence for cytoplasmic inheritance at conjugation has not been found so far in the A group. Even if these two characteristics of the data could be made to fit an hypothesis of cytoplasmic inheritance, there is no obvious reason why the two members of a pair should tend to be more alike than different pairs from the same rayed animal. It seems improbable that sufficiently massive exchange of cytoplasm would regularly occur to insure identity of cytoplasm in the two exconjugants from a pair. On the other hand, the rayed members of different pairs from the same rayed animal were derived by fission from a single animal a few days prior to conjugation, and thus should be more or less alike in cytoplasmic constitution. Thus, with cytoplasmic inheritance it would appear more likely that the two

members of a pair would tend to be less alike than different pairs from the same rayed animal. Again, with cytoplasmic inheritance there is no obvious reason why m, as calculated from the equation given in this paper, should be approximately halved by crossing to unrayed animals. Finally, the small amount of reduced vigor in the BC1 progeny of animals exposed to relatively high doses of radiation contrasts with the relatively large amounts in the A1 and A2 progeny of these same animals. Such behavior is not at all characteristic of cytoplasmic inheritance as so far known, for in those cases in which a cytoplasmically determined variation expresses itself suddenly after autogamy it can also do so at conjugation. Thus, cytoplasmic inheritance either fails to explain particular features of the data or is at variance with them.

A type of inheritance that is possibly non-genic and is known to occur in stock 90, is caryonidal inheritance which Sonneborn (1947) discusses in some detail. Caryonidal inheritance does not give an explanation for the primary results of the present work. It is possible that some of the mutant genes are analogous to the gene already known in variety 1 which makes it possible for the two caryonides of a clone to be of different mating type. However, this would not change the major conclusion though it would affect the calculation of the number of mutant genes from the average fraction of reduced vigor.

The question of whether gross changes in the nuclear apparatus may be of primary importance in determining

reduction in vigor needs to be considered. For example, it would be conceivable that radiation destroys or prevents the division of micronuclei thus producing amicronucleate animals. Such amicronucleate animals would probably be viable as long as they multiplied vegetatively, but the condition might lead to reduced vigor and death after autogamy. Overlooking difficulties in fitting the detailed findings to this hypothesis, it can be said that an amicronucleat condition is certainly not common in the progeny of rayed animals. The vegetative descendants of a number of rayed animals have been shown to have micronuclei by microscopic examination. In the course of staining cultures from several hundred rayed animals as part of the routine procedure for obtaining autogamous animals, typical macronuclear anlagen have been seen time and again. There is no known mechanism by which such anlagen could be formed in amicronucleate lines of descent.

Finally, it might be possible that macronuclear regeneration occurs with a high frequency in the autogamous progeny of rayed animals. Since this process leads to changes which would be classified as reductions in vigor, such an occurrence might be used to explain the appearance of reduced vigor after autogamy. How it could be used to explain the various phenomena of inheritance is not clear. In any case, the routine examination for autogamy of cultures from rayed animals has not shown the typical picture of macronuclear regeneration except occasionally; and the impression has been gained, though no detailed records were kept, that it

is not more frequent than in the controls.

In conculsion, it can be said that most of the evidence is consistent with genic inheritance and that the major expectations of this hypothesis which have been tested have been met. Non-genic inheritance, unless quite different from any now known, does not explain the data and is often at variance with it. On the other hand, the anomalous inheritance in experiment 1 emphasizes the possibility that other types of inheritance than the one postulated may play a role, though probably a secondary one.

There is one point concerning cytoplasmic inheritance that is worth emphasizing. The present experiments were not especially designed for its production or detection. The fact that no effects were produced by the doses used as long as the animals multiplied vegetatively suggests that higher doses or more sensitive detection methods or both will be necessary if effects on cytoplasmic components of inheritance are to be detected. This paper gives no evidence against the occurrence of cytoplasmic inheritance in variety 1, but merely shows that it was not a major factor in the particular phenomena considered.

The statement made by Kimball (in press) that nothing in the data in that paper excluded small deletions from consideration applies equally well to this paper. For the sake of brevity, the changes have been called gene mutations with the understanding that a part may be small deletions. For reasons given in the previous paper, it seems unlikely that large deficiencies or two-break aberrations could play a major role.

SUMMARY

1. A study has been carried out in Paramecium aurelia of the inheritance of effects of radiation which first expressed themselves as reductions in vigor of some of the exautogamous progeny of rayed animals.

2. The technique used in investigating the inheritance was to cross the rayed animals to unrayed ones by conjugation. In some cases, the products of the cross were again crossed to unrayed animals. The genotypes of the products of the crosses were tested by sending their descendants through autogamy to bring about complete homozygosis.

3. The clones from both members of the conjugant pairs were in the majority of the cases of normal vigor.

4. The exautogamous progeny from both members of the pairs often included a high proportion of clones of reduced vigor indicating that the effect was transmitted to both members as would be expected if nuclear mutations were involved.

5. There was clear evidence that the two members of a pair produced exautogamous generations which were more alike, on the average, than were those from different pairs from the same rayed animal. This finding is in agreement with the fact that the two members of a conjugant pair should be of identical genotype as a result of conjugation.

6. Using an equation developed by Kimball (in press) on the basis of data from dosage experiments, it was possible to calculate the average number of mutations per micronucleus in the rayed animals and in the animals resulting from the cross of rayed to unrayed. The average number in

the former was approximately twice that in the latter as would be expected if gene mutations were involved.

7. In one experiment, an anomalous type of inheritance was found for which no adequate explanation can be offered.

8. Viable homozygous clones of lowered fission rate have been obtained by autogamy in the descendants of rayed animals. The lowered fission rate can be shown to be transmitted through both members of conjugant pairs between the low fission rate clones and normal animals.

9. A noticeable feature of such viable but low fission rate clones is the great intraclonal variability in respect to fission rate and even viability itself.

10. The conclusion was drawn that non-genic changes were not a major contributing factor to the reduced vigor resulting from radiation.

11. It can be concluded that the results of the crosses have confirmed the assumption made by Kimball (in press) that reduced vigor in the exautogamous progeny of rayed animals is an expression of micronuclear mutations induced by the radiation.

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