STUDIES ON THE ORIGIN OF STREPTOMYCIN RESISTANT MUTANTS IN CHLAMYDOMONAS REINHARDI[1]

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THE inheritance of mutations to streptomycin resistance in *Chlamydomonas reinhardi* was first described by SAGER (1954) who showed that two types of streptomycin resistant mutations occur in this organism and that each type possesses a different pattern of inheritance. Mutations of the first type, designated *sr-1* or *sr-100*, are resistant to 100 μg per ml of streptomycin. These mutant strains exhibit a Mendelian pattern of inheritance, segregating 1:1 in reciprocal crosses to streptomycin sensitive cells (*ss*). Mutations of the second type are resistant to 500 μg per ml of streptomycin and possess a non-Mendelian, uniparental mode of inheritance. These mutant strains are designated *sr-2* or *sr-500*. When a mating is made between *sr-2* cells and *ss* cells, all of the progeny in each tetrad are, with rare exceptions, endowed with the resistance level of the mating type plus (mt^+) parent. Thus, tetrads resulting from the cross *sr-2* $mt^+ \times$ *ss* mt^- are composed entirely of resistant cells, while in the reciprocal cross all of the cells in each tetrad are sensitive to streptomycin.

SAGER (1960) proposed that *sr-1* and *sr-2* mutations arose in different ways. It was suggested that *sr-1* mutations occurred spontaneously in the absence of the selective agent, streptomycin, while *sr-2* mutants were induced by the selective agent. The scheme for the origin of the *sr-2* mutations represents a striking and isolated exception to the rule that selection and mutation are independent processes. We are confronted, therefore, with the possibility that a selective agent induces a characteristic change in the genotype of a cell which allows the cell and its progeny to become "adapted" to existence in the presence of the agent.

The investigations reported in this paper confirm and extend SAGER's observations on the inheritance of streptomycin resistance in *C. reinhardi*, but their main purpose is to present data which pertain to SAGER's hypotheses for the origin of *sr-1* and *sr-2* mutations. The fluctuation test was the experimental method chosen to study the origin of *sr-1* and *sr-2* mutations. This test was designed by LURIA and DELBRUCK (1943) to determine whether bacterial variants resistant to bacteriophage arose spontaneously in the absence of the

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selective agent or "acquired" a hereditary resistance only in the presence of bacteriophage. Fortunately, the experimental method was readily applied to *C. reinhardi*, and it seemed an ideal means for establishing whether *sr-1* and *sr-2* mutations arose in the presence or absence of streptomycin.

MATERIALS AND METHODS

Strains: The wild-type strains (137c mt^+ and 137c mt^-) of *C. reinhardi* used in these experiments were originally obtained by the late PROFESSOR G. M. SMITH in a collection from Amherst, Massachusetts. All of the mutant strains discussed in this paper were derived from this strain including the *sr-1* mutant obtained from SAGER.

Media: The media used in these experiments and their abbreviations are as follows: M = basal minimal medium after LEVINE and EBERSOLD (1958) plus 15 g Bacto Agar (Difco) per liter; MA = M plus 2 g per liter of sodium acetate; Y = MA medium plus 4 g per liter yeast extract (Difco); MS20, MS50, and MS500 media are made from MA medium supplemented with 20 mg, 50 mg, and 500 mg of streptomycin sulfate (Lilly) per liter respectively (streptomycin was added to these media following autoclaving); MAP = A liquid medium in which the concentration of inorganic salts is increased fivefold over that present in M medium and containing 2 g of sodium acetate and 20,000 units of penicillin per liter. Penicillin is not toxic to *C. reinhardi* and was added to reduce the possibility of bacterial contamination during the prolonged growth period of the algal cells in the fluctuation tests.

Genetic methods: Genetic analysis of the streptomycin resistant mutants was carried out using the methods described by EBERSOLD and LEVINE (1959). Resistant and sensitive colonies were differentiated on MS20 medium.

Methods used in fluctuation tests: Fluctuation tests were initiated by removing mt^- cells from Y medium stock plates and suspending them in MAP medium. The cell density of the suspension was determined by hemacytometer count and 0.2 ml aliquots were plated on several plates of MS50 medium to establish the frequency of streptomycin resistant mutants in the initial population of cells. The cell suspension was then diluted into fresh MAP medium to a density of 100 to 200 cells per ml, and 0.4 ml aliquots of the diluted suspension were distributed to several hundred 13 mm tubes with a Brewer Automatic Pipetting Machine. The suspension was agitated with a magnetic stirrer during the time that the cells were being distributed. The tubes were then illuminated for a period of four or five days. At the end of this time, the cells in all of the tubes had grown to stationary phase and the entire contents of each tube were plated onto MS50 medium. The liquid dried into the surface of the agar medium almost immediately. All of the MS50 plates were then incubated in the light for a period of a week to ten days after which they were scored for colonies of streptomycin resistant cells. Viable cell counts, performed on the cultures at the beginning and end of the period of growth, served to estimate the amount of growth which had taken place before the cells reached stationary phase. These counts were

made on MA agar medium. In each of the fluctuation tests to be described, *sr-1* and *sr-2* mutations were distinguished by their behavior in crosses to *ss* cells.

RESULTS

The inheritance of streptomycin resistance: A large number of independently arising mutations to streptomycin resistance were isolated in fluctuation tests. Genetic studies have confirmed the existence of the Mendelian and non-Mendelian mechanisms of inheritance of streptomycin resistance described by SAGER (1954). When reciprocal crosses are made between an *sr-1* mutant and *ss* cells, the resulting tetrads always segregate 1:1 for resistance and sensitivity (Table 1). Twenty-nine of these mutants have been crossed to *ss* cells. In every case 1:1 segregations have been obtained, and a minimum of 20 tetrads has been examined in each cross. In no instance has a difference in reciprocal crosses been observed.

Twenty-six independently isolated *sr-1* mutants have been crossed to a single tester mutant (*sr–1–5*). In general, 20 to 40 tetrads were scored in each cross, but in one cross 266 tetrads were examined. Recombinant tetrads have not been recorded in any cross. This result shows that the *sr-1* mutants are closely linked or allelic. One of the *sr-1* mutants (*sr-1–5*) has been localized in the left arm of linkage group IX. This localization agrees with the one recently published by SAGER and TSUBO (1961). Finally, a cross of an *sr-1* mutant obtained in our experiments to an *sr-1* mutant isolated by SAGER gave rise to no recombinants in 50 tetrads examined. These data establish the Mendelian inheritance of the *sr-1* mutations, and show that these mutations are closely linked to one another.

Mutations have also been isolated which display a non-Mendelian pattern of inheritance which corresponds to the pattern of inheritance of the *sr-2* mutations described by SAGER (1954). Reciprocal crosses of one of these mutants, *sr-2–60*, to *ss* cells are shown in Table 1. If the mutant is mt^+, then the majority of tetrads examined are composed entirely of resistant cells. The reciprocal cross gives rise to tetrads composed only of sensitive cells. In both of the crosses which are shown, a chromosomal mutant, *pf-15* (paralyzed flagella), segregated 1:1.

Two exceptional tetrads were obtained in the crosses of *sr-2–60* to *ss*. In the cross, *sr-2–60* $mt^- \times$ *ss* mt^+ one tetrad was obtained in which all four products of meiosis gave rise to clones containing resistant cells. This sort of exception has been noted by SAGER (1954) who showed that it did not arise as the result of a

TABLE 1

Segregation of sr-1 *and* sr-2 *mutants in crosses to* ss

Cross	1:1	4 resistant: 0 sensitive	0 resistant: 4 sensitive	Other
sr-2-60 mt^{+*} × *ss* mt^-	0	47	0	1
sr-2-60 mt^{-*} × *ss* mt^+	0	1	35	0
sr-1-5 mt^+ × *ss* mt^-	296	0	0	0
sr-1-5 mt^- × *ss* mt^+	23	0	0	0

* A chromosomal mutant, *pf-15*, segregated 1:1 in all tetrads.

mating type mutation or recombination between a gene controlling the transmission of *sr-2* and the mating type locus. Recently, an analysis of a number of exceptional tetrads of this type (GILLHAM, unpublished) has revealed that most, if not all, of these tetrads are actually mixtures of resistant and sensitive cells. At least one, and in some cases all four, products of meiosis are capable of segregating resistant and sensitive cells.

The second exceptional tetrad was obtained in the cross, *ss* $mt^- \times$ *sr-2–60* mt^+. This tetrad consisted of at least three clones and possibly four which contained a mixture of resistant and sensitive cells. In this case the mt^- parent was able to transmit sensitivity to the zygote.

Finally, it should be pointed out that the *sr-2* mutations isolated in this study are resistant to streptomycin concentrations as high as 1,000 μg per ml, while the *sr-1* mutations are resistant to between 50 and 100 μg per ml. A similar difference in resistance level between the two classes of mutants has been noted by SAGER (1954).

Fluctuation tests: The results of the fluctuation tests can be considered first on the basis of some data presented in Table 2. As mentioned earlier, very small inocula were used in each of the fluctuation tests. This was done to prevent the introduction of resistant cells into any of the culture tubes. The largest number of

TABLE 2

Experimental conditions of the fluctuation tests

Experiment		29	37	38
Cells/tube at start of experiment	N_0	44	52.5	84
Cells/tube at end of experiment	N_t	3.89×10^6	4.08×10^6	3.10×10^6
Cell generations/tube	$g = \frac{\log_{10} N_t - \log_{10} N_0}{0.301}$	16.4	16.2	15.1
Frequency of resistant cells in undiluted culture	F	5.70×10^{-7}	3.67×10^{-7}	3.04×10^{-7}
Tubes containing mutants/ total tubes	M/T	55/505	48/495	28/448
Percentage of tubes containing mutants		10.9	9.7	6.25
Tubes containing resistant cells at start of experiment	$s = TN_0F$	0	0	0
Total number of mutations arising in the experiment, calculated	$b = T\log_e(T/T - M)$	57.0	51.5	30.6
Tubes containing more than one mutation of independent origin	$r = b - M$	2.0	3.5	2.6

cells inoculated per tube in any of the experiments was 84. In order to establish whether resistant cells had been introduced into any of the tubes the frequency of resistant cells in the undiluted suspension from which the inocula were made was measured. This frequency was used to calculate the number of cultures into which resistant cells had been introduced. It was zero in each of the fluctuation tests.

The data set forth in Table 2 also show that the proportion of cultures giving rise to at least one mutant ranged between six and 11 percent. This fact made it seem unlikely that many of the tubes contained more than one mutant of independent origin. However, it is possible to estimate the number of tubes containing more than one mutant of independent origin using the formula developed by GREEN and KRIEG (1961). When this formula was applied to the data given in Table 2, it was found that between 3.6 (2.0/55) and 9.4 (2.6/28) percent of the tubes containing mutants would have been expected to include more than a single mutant of independent origin. The average for all three experiments was 6.2 percent (8.1/131). Therefore, the distribution of *sr-1* and *sr-2* mutations which is seen in these experiments is influenced only to a small degree by cultures having more than a single mutation of independent origin.

The data showing the distribution of *sr-1* and *sr-2* mutations in the three fluctuation tests are presented in Table 3. For genetic analysis, a single resistant colony was removed from each plate bearing such colonies. The testing of only a single colony seemed to be valid since it was previously established that only a small percentage of tubes would be expected to harbor more than a single mutant of independent origin.

An examination of the data in Table 3 shows that *sr-1* and *sr-2* mutations have different distributions in fluctuation tests. Over 80 percent of the 29 *sr-2* mutations isolated in these experiments appeared as single colonies on the MS50 plates. In comparison, only 40 percent of the 94 *sr-1* mutations occurred as single colonies. The distribution of *sr-1* mutations has a variance which is much greater than the mean. This is precisely the result expected if the *sr-1* mutations were to have a spontaneous origin. In two of the experiments (29 and 38) the difference between the mean number of colonies per plate and the variance was well over an order of magnitude. The difference between the mean and variance in the third experiment (Experiment 37) was over three orders of magnitude. The reason for the great difference in the third experiment stems from the fact that two plates had very large numbers of resistant colonies. Fluctuations of this type are rare, but when they occur they have a large effect on the variance.

The *sr-2* mutations, on the other hand, show a much smaller fluctuation than the *sr-1* mutations. The largest number of *sr-2* colonies which appeared on any of the plates was two. The five plates with two colonies comprised only 17 percent of the *sr-2* mutations obtained. In each case, both colonies were tested genetically to confirm the *sr-2* genotype. A comparison of the mean number of colonies per plate with the variance demonstrates that they are very close. As a first approximation, it appears that the distribution of *sr-2* mutations in these experiments does not deviate greatly from the Poisson. However, calculation of

TABLE 3

Colonies of sr-1 *and* sr-2 *mutants obtained from similar cultures of sensitive cells in fluctuation tests*

	sr-1 mutants			*sr-2* mutants		
Experiment	29	37	38	29	37	38
Mutant colonies obtained per culture						
0	469	451	425	477	490	443
1	9	17	11	16	4	4
2	5	6	5	3	1	1
3	3	3	3	.	.	.
4	3	2	1	.	.	.
5	1	2	.	.	.	.
6	.	1	.	.	.	.
7	1	1	.	.	.	.
8	.	1	.	.	.	.
9	.	2	.	.	.	.
10	.	.	1	.	.	.
11	1	1	.	.	.	.
12	1	.	.	.	.	.
13	.	3	.	.	.	.
14	1	.	.	.	.	.
17	.	1	.	.	.	.
20	1	.	.	.	.	.
25	.	1	.	.	.	.
31	.	.	1	.	.	.
45	.	1	.	.	.	.
65	1	.	.	.	.	.
70	.	.	1	.	.	.
2,820	.	1	.	.	.	.
3,000	.	1	.	.	.	.
Total cultures	496	495	448	496	495	448
Mean number of resistant colonies obtained per culture	0.35	12.2	0.32	0.044	0.012	0.013
Variance	10.5	3.42×10^4	13.4	0.055	0.016	0.018
P (exact method of FISHER)				0.013	0.030	0.033

the exact P values by the method of FISHER (1950) shows that in every experiment the distribution of *sr-2* mutations does, in fact, depart significantly from the Poisson. The reason seems to be that in all three experiments plates bearing two *sr-2* colonies appeared when none were expected.

Control experiments: Before the results of the fluctuation tests could be interpreted, control experiments had to be executed to rule out certain trivial explanations of the results. In the case of the *sr-1* mutants it had to be shown that the large fluctuations observed in the fluctuation tests were not an artifact of the

method of plating. In the case of the *sr-2* mutants, on the other hand, it had to be established whether the *lack* of fluctuation was caused by selection against these mutants in the growth tubes prior to plating or by the inability of most cells of the *sr-2* genotype to form colonies on MS50 plates in the presence of large numbers of sensitive cells.

1. *Reliability of plating:* It was necessary to show that the plating of parallel samples from a single large culture of cells, a very small proportion of which were resistant to streptomycin, did not lead to fluctuations in the numbers of resistant colonies per plate which were greater than those which could be ascribed to random sampling. If the resistant colonies occur randomly among the plates, then they should follow a Poisson distribution and the variance in numbers of resistant colonies per plate will be equal to the mean. If, on the other hand, the resistant colonies are not distributed at random, the distribution will not be Poisson and the variance in numbers of resistant colonies per plate will be greater than the mean. The fluctuation test would be inapplicable under these conditions since errors in the method of plating could account for any fluctuations in numbers of resistant colonies which are seen in the fluctuation tests themselves.

In order to determine whether the method of plating was reliable, flasks of MAP medium were inoculated with suspensions of cells which had been grown on plates of Y medium. These inocula always contained some resistant cells which had arisen from mutations on the stock plates. After the cells had reached stationary phase, aliquots of the suspension (0.2 to 0.22 ml) were plated on MS50 medium. These aliquots were approximately the same size as those plated from the tubes used in the fluctation tests. The results of three different experiments are shown in Table 4. The data in this table show agreement between the mean number of resistant colonies per plate and the variance in each experiment. Application of the χ^2 test indicates that there is no significant difference between the variance and the mean in any of the experiments. Therefore, it appears that the plating method is reliable and that resistant colonies are distributed at random among the plates.

2. *Plate suppression:* Over 80 percent of the *sr-2* mutants isolated in the fluctuation tests appeared as single colonies on the MS50 plates. In the remainder of the cases two *sr-2* colonies were found. It seemed possible that this lack of fluctuation among the *sr-2* mutants might have resulted because the growth of the majority of the mutant cells was suppressed in the presence of large numbers of sensitive cells. If this were so, the distribution of *sr-2* mutants in the fluctuation tests would yield no information on their origin, but would merely reflect the fact that most of the mutant cells are unable to form colonies in the presence of a great many sensitive cells.

A reconstruction experiment was performed to establish whether the ability of *sr-2* mutants to form colonies is hindered by concentrations of sensitive cells similar to those found on the MS50 plates in the fluctuation tests. Two concentrations of sensitive cells were dispensed to MS50 plates along with a very small number of mutant cells. As a control, mutant cells were distributed to MS50 plates in the absence of sensitive cells. The numbers of mutant cells plated were

the same in all cases. Finally, several MS50 plates bearing only sensitive cells were set aside to establish the frequency of spontaneous mutants in the stock of sensitive cells used for the reconstruction experiment.

The results of the reconstruction experiment are shown in Table 5. Three

TABLE 4

Variance in numbers of resistant colonies obtained from parallel platings of the same culture. No attempt has been made to distinguish sr-1 *and* sr-2 *mutations*

Sample	Experiment 1	2	3
1	1	5	10
2	2	7	10
3	4	6	8
4	7	5	16
5	3	6	18
6	3	13	10
7	1	7	7
8	2	5	10
9	.	5	16
10	.	10	9
11	.	9	13
12	.	6	21
13	.	5	14
14	.	7	.
15	.	9	.
Mean	2.88	7.0	12.5
Variance	3.84	5.4	18.0
χ^2	10.7	11.6	18.7
P	0.15	0.73	0.11

TABLE 5

Reconstruction experiment designed to test the possibility that the growth of sr-2 *mutants on MS50 plates is suppressed by the presence of large numbers of sensitive cells*

Mutant tested	Concentration of sensitive cells per plate	0	3.08×10^6	6.15×10^6
sr-2-60	Average number of mutant colonies per plate*	27.2	37.2	24.5
	Mutant cells per 10^6 sensitive cells	. . .	12.1	4.0
sr-2-235	Average number of mutant colonies per plate	15.2	21.8	30.2
	Mutant cells per 10^6 sensitive cells	. . .	7.1	4.9
sr-2-241	Average number of mutant colonies per plate	20.2	27.8	28.2
	Mutant cells per 10^6 sensitive cells	. . .	8.97	4.7

* In all cases the average is based on a series of four plates. All averages are corrected for spontaneous mutations already present in the stock of sensitive cells.

independently isolated *sr-2* mutants were tested. In no case is there evidence that the growth of mutant cells is suppressed by the presence of sensitive cells. The number of mutant colonies found on plates bearing sensitive cells is similar to the number found on the control plates for each of the three mutants. The lower concentration of sensitive cells used (3.08×10^6 cells/plate) is slightly lower than the lowest concentration found on the MS50 plates in the fluctuation tests (3.10×10^6 cells/plate). The higher concentration (6.15×10^6 cells/plate) is higher than the greatest concentration recorded in the fluctuation tests (4.08×10^6 cells/plate). In neither case is there any indication that the growth of mutant cells is suppressed with respect to the control. Since no growth suppression was noted for any of the three *sr-2* mutants tested, it seems reasonable to conclude that growth suppression of mutants by sensitive cells cannot account for the distribution of *sr-2* mutants seen in the fluctuation tests.

3. *Growth of* sr-2 *mutants in competition with wild type:* Although suppression of growth on plates cannot account for the distribution of *sr-2* mutants in the fluctuation tests, a second possible explanation of the results must be considered. It is conceivable that *sr-2* mutants grow slowly or not at all when placed in the presence of a large excess of sensitive cells in a nonselective medium. Therefore, if an *sr-2* mutant arose in one of the growth tubes employed in the fluctuation tests it would divide only very rarely. When the contents of such a tube were plated onto MS50 medium, only one or two mutant colonies would be observed. As a result, *sr-2* mutants would appear to arise only in the presence of streptomycin, but in reality their distribution on the MS50 plates would be an artifact of selection.

In order to establish whether mutant cells were strongly selected against in competition with sensitive cells a small number of mutants together with a large excess of sensitive cells were dispensed to 250 ml flasks containing 15 ml of MAP medium. The cells in these flasks were then allowed to undergo a period of growth. At the beginning and end of this growth period viable cell counts were made. Colonies of mutant and wild-type cells were differentiated by scoring the former on MS500 plates and the latter on MA plates.

The results of these selection experiments are shown in Table 6. Tests were made with three independently isolated *sr-2* mutants. The number of mutant cells per 10^6 wild-type cells in the initial inoculum ranged between two and three for the mutants *sr-2–241* and *sr-2–235*. In the case of the third mutant, *sr-2–60*, the number of mutant cells per 10^6 wild-type cells was 8.2 at the start of the experiment. All three of the mutants grew in competition with wild type and the amount of growth, although somewhat less than that observed for wild type, was considerable. These experiments show that three different *sr-2* mutants can grow quite well when placed in the presence of a large excess of sensitive cells in the same nonselective medium (MAP) used for growth in the fluctuation tests. Because of this, it seems highly unlikely that selection can account for the distribution of *sr-2* mutants seen in the fluctuation tests.

TABLE 6

Growth of sr-2 *mutants in competition with wild type.* N_0 *and* N_t *are defined in Table 2*

Mutant tested	Flask number		Mutant cells per 10^6 wild-type cells at start of experiment	Cells per ml N_0	Cells per ml N_t	Generations of growth
sr-2-60	1	*sr-2-60*	8.2	10.9	60.0	2.47
		wild type	. .	1.33×10^6	1.57×10^7	3.30
sr-2-235	1	*sr-2-235*	2.40	3.2	53.7	4.10
		wild type	. .	1.33×10^6	2.52×10^7	4.25
	2	*sr-2-235*	2.40	3.2	42.3	3.74
		wild type	. .	1.33×10^6	3.11×10^7	4.56
sr-2-241	1	*sr-2-241*	2.86	3.8	48.8	3.70
		wild type	. .	1.33×10^6	2.66×10^7	4.35
	2	*sr-2-241*	2.86	3.8	39.4	3.40
		wild type	. .	1.33×10^6	2.18×10^7	4.05

DISCUSSION

The results reported in this paper confirm the existence of two different mechanisms for the inheritance of streptomycin resistance in *C. reinhardi*. The results of the fluctuation tests are in agreement with the hypothesis that *sr-1* mutations arise spontaneously in the absence of streptomycin since the number of resistant colonies obtained on the MS50 plates fluctuates over a wide range. In marked contrast, the *sr-2* mutations showed very little fluctuation. The great preponderance appeared as single colonies on the streptomycin plates and the largest number of *sr-2* colonies observed on any plate was two. Reconstruction experiments have shown that this lack of fluctuation is not the result of selection against *sr-2* mutants when grown in competition with sensitive cells nor is it caused by the inability of *sr-2* mutants to form colonies on MS50 plates in the presence of large numbers of sensitive cells. These observations appear to be compatible with two different hypotheses for the origin of the *sr-2* mutants. They are as follows:

A. *The induction hypothesis:* The hypothesis that streptomycin induces *sr-2* mutations was first proposed by SAGER (1960). According to this hypothesis, most, if not all, *sr-2* mutations would be expected to occur as single colonies on the fluctuation test plates because they are induced by streptomycin. If this hypothesis is expanded to include the corollary that spontaneous *sr-2* mutations can occur at a low frequency, then the results presented here can be explained. The corollary is necessary because statistical analysis has demonstrated that the induction hypothesis by itself cannot account for those plates upon which two mutant colonies occurred.

B. *The hypothesis of intracellular selection:* This hypothesis supposes that a sensitive cell of *C. reinhardi* contains a certain number of discrete particles which are responsible for the sensitive phenotype. Any one of these particles is capable of spontaneous mutation to *sr-2*. However, when such a mutation occurs the mutant particle rarely divides because it is at a great selective disadvantage to the sensitive particles which are present in the cell. Once a cell containing an *sr-2*

particle is plated on MS50 medium, the direction of selection is reversed and *sr-2* particles are selected. A colony of cells results in which the *sr-2* phenotype is fully expressed. This hypothesis makes the prediction that a spontaneous *sr-2* mutation could arise at any time during the growth of sensitive cells in the fluctuation test tubes, but that a population of mutant cells would never accumulate in these tubes. This prediction is in agreement with the results of the fluctuation tests.

At present it is not possible to differentiate satisfactorily between the two hypotheses for the origin of the *sr-2* mutations. The hypothesis of intracellular selection does make the important assumption that *sr-2* and *ss* are alternate states of the same particulate determinant. Although this assumption is supported by evidence discussed by SAGER (1954, 1960), it is not necessary in the case of the induction hypothesis. In the latter case it is merely assumed that streptomycin induces a change in a sensitive cell which makes it resistant to streptomycin. The nature of the change and the point of action of streptomycin in the cell are left unspecified. Whichever hypothesis proves to be correct, it is evident that a period of contact with streptomycin is generally necessary for the establishment of the *sr-2* phenotype. *sr-2* mutations do not behave like classical chromosomal mutations in either the genetic sense or in their mode of origin.

SUMMARY

Mendelian and non-Mendelian mechanisms of inheritance of streptomycin resistance in *Chlamydomonas reinhardi* have been confirmed. It has been shown that in fluctuation tests those mutations exhibiting Mendelian inheritance arise spontaneously, but that those which are non-Mendelian in inheritance arise, for the most part, only in the presence of streptomycin. The results are compatible with the interpretation that nuclear mutations to streptomycin resistance arise spontaneously in the absence of streptomycin and that streptomycin only selects these mutations, while streptomycin is necessary for the appearance of most extranuclear mutations to streptomycin resistance.

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