CYTOGENETIC ANALYSIS OF THE ACTION OF CARCINOGENS AND TUMOR INHIBITORS IN DROSOPHILA MELANOGASTER.

VIII. SELECTIVE MUTAGENIC ACTIVITY OF S-2-CHLOROETHYLCYSTEINE ON THE SPERMATOGONIAL STAGES

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THE greatest majority of the physical and chemical mutagens so far investigated in *Drosophila* proved to be far more active on the postmeiotic stages of spermatogenesis than on the premeiotic cells (X-radiation: LÜNING 1952; various alkylating compounds: BIRD and FAHMY 1953; FAHMY and FAHMY 1954, 1955, 1957a, 1958a,b, 1960a). The mustard derivatives of naturally occurring amino acids deviated from this predominant pattern in so far as they proved to be mutagenetically active on all stages of the germ line, particularly the gonidia (FAHMY and FAHMY 1960a). This selectivity for the anabolically active cells was attributed to "metabolic transport", whereby the amino acid mutagenic molecule could be conveyed through the cytosome to sites of nucleoprotein synthesis during chromosome replication. As a further test of this hypothesis, the mutagenicity of a monofunctional mustard derivative of a naturally occurring amino acid, *viz.* S-2-chloroethylcysteine, was analyzed. It was thought possible that in a molecule with a single alkylating group (the chloroethyl radical), the relative rôle of the alkylating to the prosthetic moiety in mutagenesis may be weighted in favor of the latter, thus exaggerating selectivity of action on the metabolically active cells.

The mutagenic cell-stage response of S-2-chloroethylcysteine is also of interest in relation to the mode of action of 2-chloroethyl methanesulphonate (FAHMY and FAHMY 1956a). The activity of this sulphonate on spermatogonia, in contrast to several other mesyloxy-esters, suggested the production *in vivo* of a secondary mutagen, probably an amino acid mustard (FAHMY and FAHMY 1957a). Biochemical evidence (ROBERTS and WARWICK 1957, 1958) indicated that the secondary mutagen is likely to be S-2-chloroethylcysteine. The mutagenic properties of this compound were accordingly investigated, with a view to the further elucidation of the suggested *in vivo* conversion of 2-chloroethyl methanesulphonate.

MATERIAL AND TECHNIQUE

The mutagen used in this investigation is S-2-chloroethylcysteine, CB.1592: $\text{ClCH}_2\text{CH}_2\text{SCH}_2\text{CH}(\text{NH}_2)\text{COOH}$. The synthesis of this compound, as well as preliminary reports on its mutagenic activity have already been published (Con-

NORS and ROSS 1958; FAHMY and FAHMY 1958c). For the mutagenicity tests, the compound was administered in solution as the hydrochloride, around and within the testes of adult Oregon-K flies by our microinjection technique (details in FAHMY and FAHMY 1960c).

The sex-linked recessive lethals and visibles were determined by the Muller-5 technique, and the method of scoring has already been detailed (FAHMY and FAHMY 1955). In these experiments the treated males were bred *en masse* with an equal number of females. In some experiments a simultaneous determination was undertaken of the mutation rates induced in the X and II chromosomes, which were recovered from the same population of treated cells. For this purpose the treated males were mated individually to females homozygous for a modified Muller-5 X chromosome */sc⁸¹ In-S w^asc⁸/* and with the second chromosome pair heterozygous for *Cy* (associated with inversion) and *BIL*. The F₁ female progeny was utilized in the assessment of the sex-linked recessive lethal rate by the usual Muller-5 procedure. The F₁ male sibs were mated individually to *Cy/BIL* females, and their heterozygous F₂ *Cy* progeny were then inbred *en masse*. The F₃ cultures were observed, and the complete absence of the wild type class was taken as an indication of the induction of a II chromosome recessive lethal. Separate records for each treated male were kept to enable the exclusion of individuals heterozygous for spontaneous lethals before treatment.

The progeny of the treated males was fractionated into broods according to our standard technique (FAHMY and FAHMY 1954, 1955). The age of the male at the time of treatment was fixed at 30 ± 5 hours after eclosion. The treated males were mated to a succession of virgin females at set periods of three days, and the mutation rates in the various broods were determined separately.

Some of the sex-linked lethals were placed genetically and analyzed cytologically for chromosome aberrations in the salivary chromosomes. The salivary glands of larvae heterozygous for the lethals were used for the cytological study. The chromosomes were prepared by the aceto-orcein squash technique.

OBSERVATIONS

The brood-mutation curve: The mutation rates in the successive broods of the fractionated progeny of the treated males are presented in Tables 1 and 2 and Figure 1. The shape of the brood-mutation curve is characteristic and is fundamentally the same for the X chromosome and the second autosome. The mutation rate remains fairly constant and at a low level for three or four broods, and then rises sharply for the later progeny. This demonstrates that the stages of spermatogenesis can be broadly classified into two categories as regards their response to S-chloroethylcysteine: low sensitivity stages which are used during the first few days after treatment, and highly sensitive stages which are utilized in the later broods.

The point of upward flexion in the brood-mutation curves (Figure 1), due to the transition in the utilization of stages of markedly different sensitivity, varied with the method of breeding. In mass-mating experiments the transition point

TABLE 1

Sex-linked recessive lethals and visibles in successive broods at different doses

Conc. ($\times 10^{-2}$ M)	Brood (three days)	Chr. tested	Mass matings		Visibles		Chr. tested	Pair matings		Visibles	
			Lethals No.	Per- cent	No.	Per- cent		Lethals No.	Per- cent	No.	Per- cent
0.19	I	532	...	...	...	...	483	...	...	...	...
	II	531	8	1.5	2	0.4	503	1	0.2	...	...
	III	521	7	1.3	...	...	514	14	2.7	...	...
	IV	441	51	11.6	5	1.1	509	9	1.8	2	0.4
	V	499	119	23.8	25	5.0	472	51	10.8	23	4.9
	VI	503	24	4.8	26	5.2	495	41	8.3	20	4.0
	VII	61	8	13.1	1	1.6	299	34	11.4	16	5.4
	VIII	16	...	...	...	...	173	8	4.6	3	1.7
Total		3104	217	7.0	59	1.9	3448	158	4.6	64	1.9
0.27	I	690	4	0.6	...	...	488	...	...	...	...
	II	664	7	1.1	...	...	427	4	0.9	1	0.2
	III	699	3	0.4	...	...	443	4	0.9	1	0.2
	IV	123	11	8.9	2	1.6	116	5	4.3	...	...
	V	201	30	14.9	4	2.0	403	29	7.2	15	3.7
	VI	60	9	15.0	...	...	286	57	19.9	10	3.5
	VII	638	75	11.8	35	5.5	...	...	...	...	...
Total		3075	139	4.5	41	1.3	2163	99	4.6	27	1.3
0.41	I	498	3	0.6	1	0.2					
	II	498	8	1.6	2	0.4					
	III	446	3	0.7	...	...					
	IV	299	19	6.4	4	1.3					
	V	299	53	17.7	5	1.7					
	VI	147	44	29.9	16	10.9					
	VII	4	2	...	...	...					
Total		2191	132	6.0	28	1.3					

TABLE 2

Second autosome recessive lethals in successive broods at different doses

Brood (three days)	Chr. tested	Conc. 0.19×10^{-2} M		Chr. tested	Conc. 0.27×10^{-2} M	
		Lethals No.	Percent		Lethals No.	Percent
I	271	1	0.4	143	...	...
II	297	2	0.7	369	3	0.8
III	260	12	4.6	445	14	3.1
IV	274	10	3.6	71	...	...
V	318	92	28.9	230	74	32.2
VI	346	139	40.1	156	75	48.1
VII	201	94	46.8	...	...	...
VIII	109	63	57.8	...	...	...
Total	2076	413	19.9	1414	166	11.7

FIGURE 1.—The brood-mutation curves for the sex-linked (left ordinate) and second autosomal (right ordinate) recessive lethals. X chromosome experiments: continuous lines for mass matings; x—x for pair matings. Second chromosome experiments in dashed lines.

invariably occurred on the ninth day after treatment, indicating that the sensitive stages start to be recovered in the fourth brood. In pair matings, on the other hand, the transition occurred on the 12th day in three experiments, showing that the utilization of the sensitive cells had been delayed till the fifth brood. In the fourth experiment, however, (curve X-P2, Figure 1) the rise in mutation rate actually occurred in the fourth brood for the X chromosome mutations, but not for the concomitant second chromosome test. While the reason for this is unknown, it nevertheless shows that the effect of the breeding procedure (whether in pairs or *en masse*) is perhaps not crucial. The over-all picture from all experiments is that the cells sensitive to the cysteine mustard start to be utilized in the fourth or fifth

broods (10–15 days after treatment), with a higher tendency towards the end of this period (13–15 days).

The statistical analysis of the fluctuations in the mutation rates in successive broods was undertaken by YATES (1955) χ -method for the combination of probabilities from a series of 2×2 tables. The low mutation rates in the first three broods necessitated the pooling of data from this early progeny. The analysis (Table 3) indicates clearly the high significance of the brood-mutation pattern detailed above. One major difference, however, occurs between the X and second chromosome experiments. The mutation rate of the autosomes shows a consistent increase in the later broods (from the fifth onwards), whereas that of the sex-chromosomes reveals marked fluctuations.

Brood cell-stage relationship: The interpretation of the brood-mutation curve in terms of cell-stage sensitivity necessitates an exact determination of the time of sampling of the various stages of the germ line. It is, of course, clear that the least sensitive stages are those utilized in the earlier broods, which must have been sperm or maturing spermatids at the time of treatment. Equally certain is the fact that the most sensitive stages gave the sperm for the latest progeny and must have been mainly spermatogonial stages. There remains to be determined the exact moment in spermatogenesis when the transition occurs from low to high sensitivity. This was approached through an examination of the correlation between the time of the rise in the mutation rate and the start of the utilization of sperm derived from proliferating spermatogonia.

A mutation induced in a spermatogonium will be repeatedly multiplied through the successive mitoses and will ultimately lead to the production of a bunch of sperm carrying the same mutant. It would follow, therefore, that the first appearance of mutant "clusters" in the fractionated progeny of the treated males will

TABLE 3
Distribution of χ for the increase in mutation rate in consecutive broods

Exp.	Conc. ($\times 10^{-2}$ M)	Broods				
		1st III-IV	IV-V	V-VI	VI-VII	VII-VIII
Mass matings						
X-M1	0.19	11.1	4.9	-8.6	2.7	..
M2	0.27	8.4	1.6	0.0	-0.7	..
M3	0.41	6.2	4.3	2.9	0.9	..
Total		25.7	10.8	-5.7	2.9	..
P		$<10^{-9}$	$\approx 10^{-9}$	<0.001	0.05	..
Pair matings						
X-P1	0.19	1.4	5.9	-1.3	1.4	-2.5
P2	0.27	4.1	1.1	5.0	..	..
Total		5.5	7.0	3.7	1.4	-2.5
P		$<10^{-4}$	$<10^{-6}$	0.004	0.08	0.006
II-P1	0.19	1.8	8.1	3.0	1.5	1.9
P2	0.27	..	..	3.1	..	..
Total		1.8	8.1	6.1	1.5	1.9
P		0.04	$<10^{-9}$	$\approx 10^{-9}$	0.07	0.03

be a marker for the utilization of sperm derived from treated spermatogonia still capable of mitoses. Mutant clusters were scored among the sex-linked recessive visibles, and were also tested for among second chromosome lethals from a sample of the treated males (Table 4). No mutant clusters occurred during the first four broods, but they started to appear in the fifth brood and tended to increase in frequency in the later progeny. The size of the individual clusters also became larger in the sixth and later broods. The largest bunch recovered comprised 19 mutants and extended over three broods (sixth–eighth inclusive).

It can safely be concluded, therefore, that sperm derived from treated spermatogonia (before the growth period, while the cells are capable of multiplication) start to be utilized appreciably from the fifth brood, 13–15 days after treatment. This means that in the preceeding four broods the postspermatogonial stages of the germ line are used. It should be noted, however, that the absence of clusters among the small sample of mutants recovered from the fourth brood is not conclusive evidence against the utilization of some proliferating spermatogonia. This absence does not reach statistical significance as compared to cluster recovery in the fifth brood ($P = 0.28$). Furthermore, spermatogonia which mutated towards the end of their mitotic proliferation could only result in the production of a few sperm with the same mutation, which will naturally lower the probability of mutant recovery in clusters.

The above considerations indicate that in the present fractionation experiments the sperm and the various stages of spermatogenesis are mainly utilized during the first three broods, 0–9 days after treatment. In the fourth brood (10–12 days) the situation is not very clear, but it is highly probable that the sperm for this period is derived from a mixture of cells: spermatocytes at various stages of meiosis and old spermatogonia towards the end of their proliferation cycle or immediately afterwards (auxocytes). For the fifth and later broods the situation is very clear and decisive. The sperm used in this late progeny is predominantly, if not exclusively, derived from young spermatogonia still capable of mitotic proliferation after treatment.

In the pair mating experiments, a substantial rise in mutation rate did occur

TABLE 4

*The distribution of the single (s) and "cluster" (c) mutants in the successive broods.
The number of mutants involved in the clusters are bracketed*

Experiment	Mutants tested	Broods								Overlapping clusters V +		
		I-IV		V		VI		VII			VIII	
		s	c	s	c	s	c	s	c		s	c
X-M1	59	7	..	11	4(10)	2	6(21)	1	....	.	....	3(7)
M2	41	2	..	3	....	.	....	1	7(31)	.	....	1(4)
M3	28	7	..	1	....	3	2(9)	.	....	.	....	2(8)
X-P1	64	2	..	13	1(8)	7	4(11)	5	2(5)	1	....	2(12)
P2	27	2	..	8	2(6)	5	1(4)	.	....	.	....	1(2)
II-P1	143	3	..	7	1(3)	9	4(13)	6	1(2)	10	6(24)	8(66)
Total	362	23	..	43	8(27)	26	17(58)	13	10(38)	11	6(24)	17(99)

in the fifth brood, coincident with the first utilization of sperm from treated young spermatogonia. This shows that S-chloroethylcysteine is almost exclusively active on proliferating spermatogonia. On the other hand, in mass mating experiments, the rise in mutation rate occurred in the fourth brood. This could best be explained as due to a speedier differentiation of the germ line (perhaps as a result of a higher mating frequency in mass cultures) leading to an earlier recovery of some of the responsive young spermatogonia.

Relative mutability in the X and second chromosomes: In the X chromosome experiments, the mutation rate tended to remain the same, or even to fall slightly, after the fifth brood. In contrast, the second chromosome mutation rate showed a continuous rise from the fifth brood throughout the later progeny. This immediately suggested germinal selection against the hemizygous mutations induced in the early germ cells. An attempt was then made to analyze the exact time of onset and the degree of germinal selection in the various progeny fractions. For this purpose a study was undertaken of the relative mutation rates in the successive progeny fractions for the X and second chromosomes from the same treated cell population. Chromosome II is double the length of the X and would, therefore, be expected to yield twice its mutation frequency for the same dose. Table 5 gives the χ distribution for the deviation of the observed mutation frequencies for the two chromosomes from the theoretical 2:1 ratio for the successive broods. There was no significant deviation from expectation for the first four broods. In the fifth and later broods, however, a very gross excess of second chromosome mutants were recovered. Germinal selection, therefore, starts to operate against the recovery of some of the hemizygous lethals induced in the germ cells providing the fifth brood, which, as deduced earlier, includes proliferating spermatogonia. The more pronounced shortage of sex-linked lethals in the sixth and later broods indicates that the effect of germinal selection becomes more drastic the younger are the mutated gonidia and the longer is the period needed for their differentiation.

Visible to lethal ratio: Visible mutations were only scored in the X chromosome experiments (Table 1). It is quite obvious that their yield in the successive broods follows roughly the same pattern as that of the lethals. The visible rate is extremely low for the first four broods and then becomes appreciably higher in the later progeny. It is virtually certain, therefore, that the visibles, like the lethals, are

TABLE 5

Distribution of χ for the comparison of the mutation rates in the second:X ($\times 2$) chromosomes for different broods from concomitant experiments

Conc. ($\times 10^{-2}$ M)	Broods					
	I-III	IV	V	VI	VII	VIII
0.19	-0.3	0.1	2.3	7.7	5.7	8.8
0.27	1.2	..	5.3	2.4	..	..
$\Sigma\chi$	0.9	0.1	7.6	10.1	5.7	8.8
P	0.3	0.5	$<10^{-7}$	$<10^{-9}$	$<10^{-8}$	$<10^{-9}$

almost exclusively induced in the spermatogonial stages. This makes it almost impossible to determine the visible to lethal ratio characteristic of the cysteine mustard. The mutants from the insensitive postmeiotic stages are too few to give a reliable ratio, and those from the sensitive spermatogonia are bound to show a distorted value in favor of the visibles, because of germinal selection against the lethals. The analysis of the variation of the visible to lethal ratio in the successive broods, however, could be of some value in the understanding of the relative recovery of the two mutant types induced in spermatogonial stages. To make such an analysis statistically feasible, extensive pooling was necessary. Mutants recovered in the same brood in different experiments were summed, and the first three broods derived from the low sensitivity stages were grouped as a single fraction (Table 6). Such a method of pooling presumes that the ratio of visibles to lethals is independent of dose and postmeiotic cell stage. While it was not possible to confirm this property for the cysteine mustard, because of the complications of germinal selection, there is no reason to doubt its reality. Ample evidence is now available (FAHMY and FAHMY 1955, 1960b) that whenever germinal selection is not pronounced, the same compound induces a constant and characteristic visible/lethal ratio.

The statistical analysis of the fluctuation of the visible to lethal ratio in the various fractions of the germ line is given in Table 6. This ratio shows the first significant change in favor of visibles between the fourth and fifth broods. The proportion of visibles is even greater in the sixth brood, and the high ratio is maintained in later progeny. This is further evidence that germinal selection operates against the recovery of the hemizygous lethals from proliferating spermatogonia.

Effect of dose: The various doses investigated, and their range, do not permit an exact determination of the relationship between the concentration administered and the mutation rate recovered. There are sufficient data, however, to assess whether a dose effect occurs. This was tested statistically by the χ method (Table 7). Evidence for a dose effect occurred in the comparison involving the X chromosome experiments with dose doubling (X-M1, and M3). There was also suggestive evidence for a dose effect in two of the comparisons where the dose

TABLE 6

Variation in the over-all sex-linked recessive visible to lethal ratio in different germ-cell fractions

Brood	Visibles	Lethals	Total	Ratio v/l	χ^2
I-III	7	66	73	0.11	0.3
IV	13	95	108	0.14	
V	72	282	354	0.26	
VI	72	175	247	0.41	6.2
VII	52	119	171	0.44	0.1

ratio was 1.4 (X-P1 and P2; II-P1 and P2), but not in the third (X-M1 and M2). The most interesting fact that emerges from the analysis is that a very decisive and highly significant dose effect invariably occurs in the sixth brood, which is almost exclusively derived from treated proliferating spermatogonia.

The nature of the lethals: A random sample of 102 sex-linked recessive lethals induced by S-chloroethylcysteine was analyzed cytologically for detectable chromosome aberrations in the salivary gland chromosomes. To make the sample representative, the mutants were drawn from the various X chromosome experiments; they were accordingly induced by various doses and were recovered from different broods. Since the cysteine mustard is preferentially active on the proliferating spermatogonia, it was essential to guard against examining "mutant clusters" of common origin. For mutations arising in the fourth and later broods, therefore, only one lethal per treated male was used in the cytological study. This meant the selection of a few lethals from cell samples with grossly different mutation rates. Under such circumstances it would be virtually impossible to give an accurate assessment of the mutagenic dose in the sample analyzed. A rough estimate of the strength of the treatment, however, was calculated as the weighted mean mutation rate in the broods from which the observed mutants were drawn; the weight was taken as the number of mutants selected from the same brood.

Table 8 shows the summary of the data with the monofunctional cysteine mustard, as compared with those obtained with a difunctional mustard derivative of another amino acid, *viz.*: L-phenylalanine. The mutagenic dose for the two

TABLE 7

Distribution of χ for the effect of dose on mutation rate in the various broods of comparable fractionation experiments

Comparison	Broods				$\Sigma\chi$	P
	I-III	IV	V	VI		
X-M1 and M2	-0.9	-0.8	-2.6	3.2	-1.4	0.2
X-M1 and M3	0.1	-2.4	-2.0	8.8	4.5	0.01
X-P1 and P2	-1.2	1.7	-1.8	4.7	3.4	0.04
II-P1 and P2	-0.1	..	0.8	1.7	2.4	0.08
$\Sigma\chi$	-2.1	-1.5	-5.6	18.4	..	..
P	0.1	0.2	0.003	$<10^{-9}$	..	..

TABLE 8

Chromosome aberrations among sex-linked recessive lethals induced by S-chloroethylcysteine (CB.1592) and p-N-di-(2-chloroethyl)aminophenylalanine (CB.3025)

Compound	Stocks examined	Lethal rate percent	Gross rearrangements		Deficiencies	
			No.	Percent	No.	Percent
CB.1592	102	6.0	1	0.9	23	22.5
CB.3025	134	7.0	3	2.2	50	37.3

compounds can be looked upon as roughly the same. It is abundantly clear that both agents are very weak chromosome breakers. The single major rearrangement observed in the cysteine mustard sample was a deletion, where 21 bands were eliminated. It was recovered from the third brood and was probably, therefore, induced in a spermatid. It is perhaps noteworthy that no major rearrangements were induced in the premeiotic stages, in spite of the fact that the lethal rate in some of these cell samples reached a maximum of 20 percent. It would seem highly probable, therefore, that the cysteine mustard is totally ineffective in breaking the chromosomes of spermatogonial stages.

Small deficiencies (involving less than one percent of the length of the salivary X chromosome) were induced by the cysteine mustard at an over-all rate of 22.5 percent which is significantly lower than that under the phenylalanine derivative ($\chi^2 = 5.9$ for one degree of freedom, $P \approx 0.01$). This may indicate that monofunctional mutagenic agents are less effective in the induction of small deficiencies than are the difunctional compounds of the same chemical series.

DISCUSSION

S-2-chloroethylcysteine is the first instance so far discovered of a mutagen which is virtually specific to a particular cell stage of the male germ line. The compound is ineffective on mature sperm, produces a very low activity on the stages of spermateliosis, but is extremely effective on the early germ cells—particularly the earlier spermatogonia; the mutation rate in these early cells sometimes reaches twenty-fold that of the spermatids. Advantage was taken of the high mutagenic selectivity of the cysteine mustard on the spermatogonia, to determine the start of utilization of these cells in our standard brood-fractionation experiments (see technique). For this purpose three independent criteria indicative of the utilization of mutated spermatogonia were investigated: (1) the appearance of "clusters" of identical mutants, (2) the disturbance of the sex-linked visible/lethal ratio, (3) the recovery of the X, relative to the second chromosome lethals. The evidence from the above sources pointed to the same conclusion: the first appreciable utilization of proliferating spermatogonia occurs in the fifth brood (13–15 days after treatment), coincident with the marked rise in the mutation rate. There were indications, however, that some of the spermatogonia may also be used in the fourth brood in admixture with the youngest spermatocytes (auxocytes) in the premeiotic interphase. The pattern of cell-stage utilization in the present brood-fractionation experiments with the cysteine mustard, therefore, is essentially the same as that occurring in comparable experiments with other alkylating agents (FAHMY and FAHMY 1955, 1957a, 1960a). This adds further support to the conclusion (FAHMY and FAHMY 1958c) that, with moderate doses of the alkylating mutagens and under standard experimental conditions, the speed of germ line differentiation is not drastically disturbed, thus resulting in a reasonable consistency in the cell-stage/brood correlation in different fractionation experiments. Spermatogonial stages (capable of mitoses) are predominantly used starting from the fifth brood, whereas prespermatogonial stages are used in the earlier progeny.

The activity of the cysteine mustard on cells recovered in the late progeny might imply that it is a function of time, being due to the prolonged action of a slow reacting compound. This possibility can immediately be excluded on the basis of the known chemical properties of the compound. In buffered aqueous solution at pH 7.5 and 37°C the half life of chloroethylcysteine hydrochloride, with respect to the hydrolysis of the chloroethyl group, is seven minutes (W. DAVIS, in CONNORS and ROSS 1958). This means that in the present experiments the administered drug is probably completely inactivated by hydrolysis in the haemocoel of the flies in under the hour. The earliest sample of sperm tested in the genetic experiments was collected during the first three days after treatment, long after the complete inactivation of the compound. It would accordingly follow, that the observed fluctuations in the mutation rate in the various broods is a genuine manifestation of cell stage sensitivity. It is due to differential damage during the compound's period of activity and is independent of the sampling times in the genetic tests.

A surprising feature of the mode of action of S-chloroethylcysteine is its extremely low activity on the postmeiotic stages, particularly the mature sperm. Furthermore, the mutation rate in these stages did not manifest any dose response. These features could imply the lack of penetrance of the compound into the sperm and spermatids. This possibility is rendered most unlikely, however, by the fact that the compound does penetrate and mutate the spermatogonial stages whose nuclei are far better protected than those of the sperm by the surrounding cytoplasm. A more plausible explanation for the inactivity on the sperm is that the cysteine mustard may well be ineffective on the fully formed, highly condensed, chromosomes. Conversely, the compound would be expected to be most active on the attenuated chromosome. This would be in harmony with the agent's effectiveness on the genes of the proliferating spermatogonia. Maximal chromosome attenuation would naturally occur during the synthesis of the new replicate early in the mitotic cycle, and it is probable that this is the moment of the maximal sensitivity.

The recessive lethals induced by the cysteine mustard conform with those induced by other amino acid mustards (FAHMY and FAHMY 1956b, 1957b) in so far as they are virtually lacking in major chromosome aberrations. The cytological data with this mustard, however, while indicating that the agent is a very weak chromosome breaker, do not permit an accurate assessment of this property. A proportion of the rearrangement lethals, if induced in the premeiotic stages where the mutagenicity is highest, is bound to be eliminated by germinal selection. An interesting deviation of the cysteine-mustard lethals from those of other difunctional amino acids is that they are associated with a significantly lower rate of deficiencies and small deletions (not exceeding ten salivary chromosome bands). Among lethals induced at roughly the same over-all rate, there was nearly double the deficiency rate under the difunctional mustard derivative of phenylalanine as under the monofunctional cysteine derivative. Here, as in the case of major rearrangements, differential germinal selection could have depressed the rate of deficiency lethals recovered from spermatogonia, and the data

were not sufficient to determine its degree. Nevertheless, it is highly probable that the low deficiency rate under the monofunctional mustard is a genuine phenomenon and not merely an artifact due to selection. Recent work with the mono- and difunctional sulphonates which are both active on the postmeiotic stages (where no germinal selection occurs) yielded results parallel to the present data with the mustards. For the same mutagenic dose, the rate of deficiency lethals was far lower for the monofunctional than for the difunctional sulphonic esters (FAHMY and FAHMY, unpublished).

Reference has been given to the biochemical evidence that S-2-chloroethylcysteine could be one of the metabolites produced *in vivo* after the administration of 2-chloroethyl methanesulphonate. The present genetical evidence is in harmony with the biochemical findings. The mutagenic cell-stage response under the two compounds is strikingly comparable, particularly in so far as both are preferentially active on the proliferating spermatogonia. This similarity of pattern also supports the hypothesis that the deviation of the brood-mutation curve for the chloro-ester from the typical sulphonate pattern (with no activity on the gonias) could be due to the conversion of the sulphonate into the amino acid mustard *in vivo* (FAHMY and FAHMY 1957a, 1958c).

SUMMARY

The fractionated progeny of males injected with S-2-chloroethylcysteine showed a marked selective mutagenicity on the early germ cells, which start to be utilized 10–15 days after treatment. The sperm sample used towards the end of this period (13–15 days), (a) contained clusters of identical mutants, (b) showed a disturbed sex-linked visible to lethal ratio in favor of the visibles, and (c) gave a disproportionate excess of second to X chromosome lethals. This shows that the selective mutagenicity of the cysteine mustard is on the proliferating spermatogonia. The recessive lethals induced were virtually free of major rearrangements and were associated with a low rate of small deficiencies. The pattern of cell stage response of the cysteine mustard was similar to that of chloroethyl methanesulphonate.

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