# DIFFERENTIAL GENE RESPONSE TO MUTAGENS IN DROSOPHILA MELANOGASTER

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THE most outstanding recent development in the field of mutagenesis is the discovery that gene mutation is not random, but selective, depending on the nature of both mutagen and gene locus. The evidence for this selective mutagenicity became abundantly clear with the introduction of microorganisms (bacteria and the lower fungi) in the study of mutagenesis. The ease with which large populations of these organisms can be cultured, treated, and subsequently observed, helped in overcoming the technical difficulty arising from the rarity of the mutation event. DEMEREC (1954, 1955), working on *Escherichia coli*, could demonstrate that the rate of reversion (back mutation) at several nutritional deficiency loci varied markedly under similar treatments with different mutagens. Evidence was also available suggesting that this differential response was a function of the genes themselves, rather than the bacterial cell, since there were no particular strains which were conducive to high, or low, mutational rates for all loci. A grave drawback of the bacterial back mutation test, however, is that it is difficult (in some strains even impossible) to subject the arising mutants to recombination allelism tests. Most workers on bacterial mutagenesis, therefore, accept the scoring of the mutants on the basis of their phenotypic manifestations. This will inevitably lead to an erroneous assessment of the true mutation rate per locus since it will be impossible to eliminate cases due to adaptation or suppressor mutations. How far this error could have affected the phenomenon of differential mutability in bacteria is difficult to assess, but it is certain that it could not be the whole explanation. There are a number of mutagen stable loci, which do not respond at all to a diversity of strong mutagens highly effective on other genes.

The results with Neurospora are closely parallel to those with bacteria. Here again the rate of back mutation at different loci, or even at different alleles of the same locus, depends on the mutagen. This has been shown to occur with different radiations like ultraviolet and X-rays (GILES 1951), as well as with different chemical mutagens (KÖLMARK and WESTERGAARD 1953). Of particular interest are the results of KÖLMARK (1953) who demonstrated that two genes in the same nucleus could show different relative response to various mutagens. Unlike bacteria, the Neurospora technique enables the confirmation of the mutations induced, through adequate crosses and subsequent analysis of the segregation ratios among the ascospores. This makes the evidence for differential mutability based on the Neurospora test even more secure than that based on bacteria.

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The recent work (BRENNER, BENZER and BARNETT 1958) on mutagenesis in bacteriophage, has yielded the first evidence for qualitative specific mutability within a gene locus. The mutational sites within the locus affected by proflavin were different from those affected by 5-bromouracil and the induced mutations did not occur at the sites which most frequently mutated spontaneously.

While the evidence for differential mutability in microorganisms is overwhelming, the fundamental mechanism responsible for the phenomenon is still obscure. Indeed it is difficult to be certain that it is exclusively due to differential action on the genetic material itself. The back mutation screening test selects cells which have reverted from being incapable of undertaking a particular synthetic process (amino acid, vitamin, etc.) to cells capable of doing so. There is good evidence (particularly in Neurospora) that the reversion is primarily genetic. Nevertheless, there is the possibility that the recovery process may also be dependent on nongenetic biochemical processes (in the cytoplasm) independent of the mutation itself. If these 'secondary' processes are enhanced or hindered in the presence of certain compounds (the mutagens themselves, or their metabolic complexes around the genetic material) a situation might arise simulating differential mutability, when in fact it is selective establishment of autotrophic populations derived from the same proportion of mutant cells attaining various degrees of biochemical recovery.

The above complication does not occur with higher organisms like Drosophila. The advanced biological organization in Metazoa, enables the separation of the chromosomes carrying the induced mutations from the treated cytoplasm. These chromosomes are invariably transmitted through several cell generations before the mutant effect is manifested and scored. Under such circumstances, therefore, any nongenetic selective effect (due to changed cytoplasm or nucleoplasm) must be postulated to operate on the treated gamete. This would be based on the assumption that particular mutations are accompanied by biochemical cellular disturbances, which are specifically enhanced in the presence of some mutagens so as to result in gametic lethals. Metazoan gametes, however, are extremely tolerant to very gross genetic disturbances. Sperm carrying even large chromosome deletions are fully viable and functional. It would follow, therefore, that whatever lethal effects a mutagen exerted on the gametes, could only be physiological (manifestations of toxicity, producing a 'killing effect' on mutant and normal gametes alike) and would, therefore, be irrelevant to the analysis of mutagenesis.

In view of the above considerations, it is of the greatest significance to have established differential mutability in Drosophila (FAHMY and FAHMY 1956a and 1957a,b). The most interesting observation in this connection concerns the differential yield of recessive 'visibles'. The alkylating compounds induced a great many sex-linked recessive 'visibles' which differed in phenotypic expression and genetic position from those which were frequently encountered in radiation mutagenesis work. This immediately suggested differential mutagenicity at the gene level. However, in view of the subjective element in the detection of mor-

phological mutants, it was felt desirable to subject the phenomenon to more extensive analysis. This was undertaken by two independent methods. The first consisted of the determination of the relative mutation incidence under the effect of different mutagens at the various X chromosome loci. All morphologically detectable mutants in the $F_2$ of Muller-5 experiments were established as stocks and were subsequently subjected to allelism tests. In order to reduce the subjective elements due to the acuity of the observer, we confined this analysis to experiments scored with the greatest care by one of us only (M.J.F.). The second method utilized in the analysis is the most direct and objective technique known in the study of comparative mutagenesis. It consists of testing the mutation rate at certain specific loci with different mutagens under comparable experimental conditions. The results with both of the above methods will be presented in this communication, and they do, indeed, support the concept of differential gene response to mutagens.

## TECHNIQUE

*The mutations and the mutagens:* The present study is an analysis of the mutation incidence at 106 X chromosome loci yielding "visibles" (morphologically detectable changes) which mutated more than once in our own experiments with radiation and chemical mutagens. Of these, 43 loci have been previously detected in radiation mutagenesis work and have been described in the Drosophila mutants literature (mainly BRIDGES and BREHME 1944). These will be referred to in this communication as the "classical loci" and given the symbol "$\alpha$ loci." The remaining 63 loci to be considered are from among another group which has recently been mutated in our laboratory by a diversity of chemical compounds belonging to the so-called biological alkylating agents. These chemically mutated loci have been previously referred to as "new" (FAHMY and FAHMY 1956a) to distinguish them from the already known "classical loci". This nomenclature, however, was taken to imply that all the "new" mutants never occurred in the earlier radiation mutagenesis work. We will, therefore, refer to the hitherto undescribed loci which have mutated in our laboratory mainly by chemical means, by the noncommittal term "$\beta$ loci", without prejudice that some of these loci may prove mutable under radiation. The phenotypic manifestations and genetic positions of 100 $\beta$ loci have already been given (FAHMY and FAHMY 1958b) and the details of a further 200 are in preparation.

The chemical mutagens to be considered all belong to the alkylating compounds and were synthesized at the Chester Beatty Research Institute. For ease of reference the code numbers, as well as the formulae and the full chemical names for the various compounds used, will be given here.

*Polyethyleneimine:*

CB.1246: $(C_2H_4N)_3.C_3N_3$ ; 2:4:6-tri(ethyleneimino)-1:3:5-triazine, or triethylenemelamine (TEM)

*Phenylethylamine mustard:*

CB.3034: $(ClCH_2CH_2)_2N.C_6H_4.CH_2CH_2(NH_2)$ ; p-NN-di-(2-chloroethyl)-

aminophenylethylamine.

*Phenylcarboxylic mustards:*

$(ClCH_2CH_2)_2N.C_6H_4.(CH_2)_nCOOH$

CB.1331: n = 1 ; p-NN-di-(2-chloroethyl)aminophenylacetic acid.

CB.1332: n = 2 ; p-NN-di-(2-chloroethyl)aminophenylpropionic acid.

CB.1348: n = 3 ; p-NN-di-(2-chloroethyl)aminophenylbutyric acid, or "Chlorambucil".

CB.1356: n = 4 ; p-NN-di-(2-chloroethyl)aminophenylvaleric acid.

*Alkyl methanesulphonates:*

*Monofunctional* $R\text{-}OSO_2CH_3$

CB.1528: $R = C_2H_5$ ; ethyl methanesulphonate.

CB.1540: $R = CH_3$ ; methyl methanesulphonate.

*Difunctional* $CH_3O_2SO\text{-}R\text{-}OSO_2CH_3$

CB.2040: $R = (CH_2)_3$ ; 1:3-dimethanesulphonoxypropane.

CB.2041: $R = (CH_2)_4$ ; 1:4-dimethanesulphonoxybutane, or "Myleran".

CB.2058: $R = CH_2.C \equiv C.CH_2$ ; 1:4-dimethanesulphonoxybut-2-yne.

CB.2094 and CB.2095: $R = CH_2CH{=}CHCH_2$ ; the *cis*, and *trans* forms of 1:4-dimethanesulphonoxybut-2-ene.

CB.2511: $R = CH_2(CHOH)_4CH_2$ ; 1:6-dimethanesulphonyl mannitol.

*Halogeno-alkyl methanesulphonates* $X\ CH_2CH_2OSO_2CH_3$

CB.1506: X = Cl ; 2-chloroethyl methanesulphonate.

CB.1522: X = F ; 2-fluoroethyl methanesulphonate.

*Amino-acid mustards:*

*Monofunctional*

CB.1592: $ClCH_2CH_2SCH_2CH(NH_2)COOH$ ; S-2-chloroethylcysteine

*Difunctional*

CB.3007, CB.3025, CB.3026: $(ClCH_2CH_2)_2N.C_6H_4.CH_2CH(NH_2)COOH$ ; p-NN-di-(2-chloroethyl)aminophenylalanine, the DL mixture (Merphalan), the L-isomer (Melphalan), and the D-isomer (Medphalan).

CB.3051: $(ClCH_2CH_2)_2N.C_6H_4\text{-}O\text{-}C_6H_4.CH_2CH(NH_2)COOH$ ; p-/p′-NN-di-(2-chloroethyl)aminophenoxy/phenylalanine.

CB.1385: $(ClCH_2CH_2)_2N.C_6H_4.CH_2CH_2CH(NH_2)COOH$ ; $\alpha$-amino-$\gamma$-p-(NN-di-2-chloroethyl amino)phenylbutyric acid.

For the mutagenicity tests the above compounds were administered as aqueous solutions by intra-abdominal injection round the testes of young imagines of Oregon-K Drosophila males. The carboxylic and amino acid mustards, which are practically insoluble in water, were converted to the sodium salts, and these were sufficiently soluble for the biological tests. Some of the di-methanesulphonates (e.g., CB.2041, 2058, 2094 and 2095) gave true solutions of a maximum concentration of about $0.8 \times 10^{-2}$ M. Whenever higher concentrations of these compounds were tested, they were administered as partial suspensions.

The radiation mutagenicity tests were carried out on males comparable to those used in the genetic experiments with the chemical mutagens. The males were of the same strain, as well as of nearly equal average weight and size. In all

experiments irradiation was done by the same X-ray machine: a "Picker" therapeutic machine, 250 kv-15 milliamps, with inherent filtration of 0.2 mm Cu and added filtration of 0.2 mm Sn + 0.25 mm Cu + 1 mm Al; the half value layer being 1.5 mm Cu. The flies were irradiated in batches of about 1,000 males in a perforated "perspex" cylindrical chamber (2.5 cms diameter × 1 cm height) placed 20 cms from the source. Under these conditions the dose rate delivered was 250–260r per minute. The exact dose rate for each irradiation was checked by a dosimeter, thus enabling an accurate determination of the total dose in each of the genetical experiments (Table 4).

*The detection of mutants:* The present analysis is confined to "visibles" induced in the X chromosome of the "Oregon-K" stock. The greatest majority of the mutants were detected by the Muller-5 technique, though a small number occurred in attached-X experiments and were also included. In Muller-5 tests, cultures yielding only M-5 males ($sc^{s^1}B(InS)w^a sc^s$/Y) and completely lacking in the potentially wild type male class, were scored as sex-linked recessive lethal cultures. The occurrence in $F_2$ cultures of non-Muller-5 males with anomalous phenotypes was taken as an indication of the induction of a sex-linked visible. The flies from these cultures carrying the treated chromosome were used to establish stocks, which were subsequently subjected to further genetic analysis. It thus became possible to determine whether the mutation was complete or mosaic in the germ line, its exact genetic position, and whether it was a recurrence at a known locus or a mutation of a hitherto unaffected gene.

Several control experiments were undertaken with untreated males to determine the spontaneous mutation rate using the above techniques. In 16 Muller-5 experiments there was a mean sex-linked recessive lethal rate of 0.18 ± 0.05 percent. As regards the visibles, there were two mutants (one rudimentary: *r*, and one unidentified body color mutant: a $\beta$ locus) in 13093 Muller-5 $F_2$ cultures, and also two others (one singed: *sn*, and one mosaic yellow: *y*) among 29663 $F_1$ males from attached-X experiments. The spontaneous $\beta$ locus mutant was not allelic to any of the induced mutations considered in the present analysis. The over-all control sex-linked recessive visible rate, therefore, for the X chromosome gene complement is 0.009 percent.

A study was also undertaken of the absolute mutation rate at certain specific X chromosome loci under the effect of X-rays and the amino acid mustard CB.3025 /p-NN-di-(2-chloroethyl) amino-*L*-phenylalanine/. The loci analysed in this way, together with their genetical positions are detailed in Tables 4 and 5, and their phenotypic description has already been published (FAHMY and FAHMY 1958b). The experimental procedure for both the physical and chemical agents consists of treating the wild type males (Oregon-K stock) and subsequently mating them to females either homozygous for several of the marked loci, or heterozygous for two sets of markers, each on one of the X chromosomes. When the normal allele of the marked locus in the paternal X has been affected, the $F_1$ daughter will reveal the mutation. The total number of the treated X chromosomes is given by the number of $F_1$ daughters observed when the mothers are

homozygous, and by half this number when the mothers are heterozygous for any particular marker.

The $F_1$ daughter could reveal the phenotypic manifestations of the gene under test if the paternal X has been affected by the treatment so as to carry:

1) an induced "visible" allelomorphic to the marker or
2) an induced "visible" as above together with a lethal somewhere else on the chromosome or
3) a deficiency or deletion (in itself a lethal) covering the locus of the marker.

Our technique for the differentiation between the above possibilities will be here given in detail:

1) The $F_1$ abnormal daughters from the treated fathers and "visible" marked mothers are classified for phenotype and then mated individually to M-5 males for the genetical analysis.

2) The $F_2$ cultures are fully scored and counted. The occurrence of a sex ratio of approximately unity and the existence of males carrying the marked X, as well as others with the induced mutant is an indication that a fully viable visible has been induced. The absence of the class of male carrying the treated chromosome is an indication of a lethal.

3) Up to 50 $F_2$ females are mated to M-5 males and bred separately to distinguish between females heterozygous for the marked chromosome and those heterozygous for the treated X. This is assessed on the basis of the male classes in the $F_3$.

4) A line is established from the $F_3$ cultures which proved to be heterozygous for the treated chromosome and further subjected to the following tests:

(a) an induced "visible" is confirmed by allelism.
(b) an induced lethal is placed against a 5-point marked chromosome (*sc*, *ct*, *v*, *f* and *car*). This enables the differentiation between the case where there is a lethal at the point of the marker (whether point mutation or deficiency), from the situation where there is an induced visible at the marker together with a lethal elsewhere on the same chromosome.
(c) where the lethal is at the point of the marker, it is crossed to the relevant visible to confirm allelism by "coverage".

It should be noted that the marker chromosomes used were uninverted, which means that crossing over between the marker and the treated chromosomes could occur in the $F_1$ daughter. This could only affect the analysis in the case of induced visible point mutations. To ensure that the induced allele is analysed, four $F_3$ cultures which show only the single mutant, are subjected to the allelism test. The probability that all four cultures were the result of crossing over with the marked chromosome is negligible.

Mosaic visible mutations were taken into account only when they were complete in the germ line, so that they could be analysed by breeding tests. Where a few wild type males occurred in the $F_2$, indicating a mosaic lethal, the mutation was only considered if it could be established as a full germ-line lethal that could then be subjected to our complete genetic analysis.

## OBSERVATIONS

*Relative mutability of the different gene loci:* This analysis is confined to those loci of the X chromosome which mutated more than once in our own experiments with radiation and chemical mutagens over a period of roughly ten years. The over-all number of these loci was 106, of which 43 were $\alpha$ loci and the remaining 63 were $\beta$ loci. In all, 824 mutants occurred at these loci and it was possible to determine the exact locus yielding most of these mutants by direct allelism tests. In a few instances, however, allelism tests were not possible because of the sterility of some of the mutants, but the placing of these mutants and their phenotypes left no doubt whatever as to the loci involved.

Before the discovery of the $\beta$ loci, the gene pool available for the allelism tests consisted entirely of $\alpha$ loci. Only these loci were, therefore, considered in the analysis of the relative visible yield when mutagens used in our earlier experiments were taken into account. More recently, and by virtue of the higher efficiency of some of the alkylating mutagens in the induction of visibles, it was possible to increase our pool of sex-linked visibles considerably. This widened the scope for allelism tests and enabled the analysis of the relative mutation yield at the $\beta$ loci in our more recent experiments.

In order to make the analysis of relative mutability statistically feasible, alleles induced at the same locus by closely related compounds of the same chemical series were pooled together. The mutants induced by CB.1506 (2-chloroethyl methanesulphonate), however, have not been pooled with those of other sulphonates, since there are biochemical and biological reasons (FAHMY and FAHMY 1956b, 1957c) suggesting that this compound's mode of action is atypical. Likewise, the mutations induced by CB.1592 (S-chloroethyl cysteine) were not included with those of other amino acid mustards, since its pattern of cell stage response was somewhat different. The "visibles" compared have been recovered from experiments undertaken at different mutagenic levels (as regards the lethal rate) and from male germ cells treated at various stages of spermatogenesis. However, there is no reason to suspect that these factors affect the relative mutability of different loci in the same sample of treated chromosomes. As regards the effect of dose, there is no evidence for a threshold effect for particular loci, either with radiation or with chemical mutagens. Also there is no indication, so far, that the cell stage at the time of treatment affects the yield of visibles qualitatively. In the present analysis, different alleles of the same locus were not confined to any particular brood of the fractionated progeny of the treated males. Furthermore, germinal selection against mutants induced in early germ cells is unlikely to have played a major rôle. Recessive visibles, being mostly point mutations, are not appreciably eliminated by this selection.

Tables 1 and 2 give the distribution of alleles at specific loci under the effect of X-rays and various classes of alkylating mutagens. It is required to test whether the relative mutation incidence at a given locus differs with the mutagen. Because of the rarity of the mutation process at any one locus, the number of mutants in the various cells of the table is unavoidably small, too small for the application

TABLE 1

*Distribution of alleles at specific α loci with different mutagenic agents*

| Locus | | Position | X-ray | TEM | Carboxylic mustards | Amino acid mustards | Sulphonates | C.B. 1506 | C.B. 1592 | C.B. 3034 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| yellow | *y* | 0.0 | 1 | 4 | 1 | 11 | 12 | 1 | 2 | 1 | 33 |
| silver | *svr* | 0.0 | 1 | 2 | 1 | 5 | 2 | .. | 2 | 1 | 14 |
| twisted | *tw* | 0.4 | 4 | 2 | 1 | 7 | 10 | 3 | 1 | 2 | 30 |
| broad | *br* | 0.6 | .. | .. | .. | .. | 1 | 1 | .. | .. | 2 |
| prune | *pn* | 0.8 | 1 | .. | .. | 4 | 6 | 1 | .. | .. | 12 |
| giant | *gt* | 0.9 | .. | .. | .. | .. | 2 | .. | .. | .. | 2 |
| white | *w* | 1.5 | 2 | 6 | 1 | 8 | 9 | 5 | 1 | .. | 32 |
| diminutive | *dm* | 4.6 | 3 | .. | .. | .. | .. | .. | .. | .. | 3 |
| echinus | *ec* | 5.5 | .. | .. | .. | 3 | .. | 1 | .. | .. | 4 |
| bifid | *bi* | 6.9 | .. | .. | .. | 1 | .. | .. | .. | 1 | 2 |
| ruby | *rb* | 7.5 | 3 | 1 | 1 | 4 | 2 | 2 | .. | 2 | 15 |
| rugose | *rg* | 11.0 | .. | 2 | .. | 2 | 4 | .. | .. | .. | 8 |
| roughex | *rux* | 15.0 | 1 | 1 | .. | 2 | .. | .. | .. | 1 | 5 |
| vesiculated | *vs* | 16.3 | 4 | 2 | .. | 9 | 6 | 3 | 1 | 1 | 26 |
| deltex | *dx* | 17.0 | .. | 1 | 1 | 2 | 2 | 1 | .. | .. | 7 |
| oval | *ov* | 17.5 | .. | .. | .. | 1 | 1 | .. | .. | .. | 2 |
| shifted | *shf* | 17.9 | 1 | 1 | .. | 1 | 8 | 2 | .. | .. | 13 |
| carmine | *cm* | 18.9 | .. | 2 | .. | 4 | 1 | 2 | .. | .. | 9 |
| scooped | *scp* | 19.3 | .. | .. | .. | .. | 1 | 1 | .. | .. | 2 |
| cut | *ct* | 20.0 | 2 | .. | 1 | .. | .. | .. | .. | .. | 3 |
| singed | *sn* | 21.0 | 2 | 4 | .. | 2 | 5 | 1 | 2 | .. | 16 |
| pentagon | *ptg* | 23.2 | .. | 1 | .. | .. | 2 | .. | .. | .. | 3 |
| goggle | *gg* | 25.0 | 1 | .. | 1 | 1 | 1 | .. | .. | .. | 4 |
| tan | *t* | 27.5 | 6 | .. | 1 | 4 | 3 | 1 | 1 | .. | 16 |
| lozenge | *lz* | 27.7 | 5 | 6 | .. | 6 | 1 | 3 | .. | .. | 21 |
| tarry | *ta* | 27.7 | 1 | .. | .. | 7 | 2 | .. | 1 | .. | 11 |
| divers | *dvr* | 28.1 | 1 | .. | .. | 2 | 2 | 1 | .. | .. | 6 |
| vermilion | *v* | 33.0 | 2 | 3 | .. | 6 | 9 | 2 | 2 | .. | 24 |
| miniature | *m* | 36.1 | 1 | 3 | 1 | 6 | 4 | 1 | 3 | .. | 19 |
| dusky | *dy* | 36.2 | 1 | 5 | .. | 3 | 8 | 1 | 3 | .. | 21 |
| furrowed | *fw* | 38.3 | 2 | 3 | .. | 3 | 4 | 3 | .. | .. | 15 |
| wavy | *wy* | 41.9 | .. | 3 | .. | 3 | 2 | .. | .. | .. | 8 |
| sable | *s* | 43.0 | .. | .. | .. | 1 | 2 | .. | .. | .. | 3 |
| garnet | *g* | 44.4 | 1 | 4 | .. | 5 | 7 | 1 | .. | .. | 18 |
| narrow abdomen | *na* | 45.2 | 3 | 2 | 2 | 3 | 5 | 3 | 2 | .. | 20 |
| pleated | *pl* | 47.9 | .. | 1 | .. | 4 | 1 | .. | .. | .. | 6 |
| scalloped | *sd* | 51.5 | .. | .. | .. | 2 | 1 | .. | .. | .. | 3 |
| uneven | *un* | 54.4 | .. | 1 | .. | 2 | 3 | .. | .. | .. | 6 |
| rudimentary | *r* | 54.5 | 5 | 11 | 2 | 14 | 25 | 5 | 2 | 4 | 68 |
| inflated | *if* | 55.0 | .. | 1 | .. | 1 | .. | .. | .. | .. | 2 |
| forked | *f* | 56.7 | 2 | .. | .. | 3 | 3 | 3 | .. | .. | 11 |
| fused | *fu* | 59.5 | 1 | 3 | .. | 6 | 11 | 1 | .. | .. | 22 |
| carnation | *car* | 62.5 | .. | .. | .. | 2 | .. | .. | .. | .. | 2 |
| Total | | | 57 | 75 | 14 | 150 | 168 | 49 | 23 | 13 | 549 |

of $\chi^2$. We therefore applied the $\Psi$ test (C. A. B. SMITH 1951, 1952) for the assessment of heterogeneity of proportions in a contingency table. This method is far less disturbed by small numbers in the individual cells than $\chi^2$. It is also to be noted that the tables under analysis have a large number of rows, a feature which favors the applicability of the $\Psi$ test, since it helps in smoothing out the irregularities due to the smallness of the individual numbers. The null hypothesis for this test is the absence of heterogeneity, which is to be interpreted as meaning that the distribution of alleles among the various X chromosome loci (under consideration in the contingency table) is independent of the mutagen.

Table 1 shows the distribution of alleles at 43 $\alpha$ loci for X-rays and seven classes of alkylating mutagens. The $\Psi$ test for this distribution corresponds to $P = 0.41$, which is clearly nonsignificant. The over-all relative mutability at the "classical" or $\alpha$ loci, therefore, does not vary with the mutagen. Table 2 gives the distribution of alleles at 63 $\beta$ loci, under the effect of X-rays, and five classes of the alkylating mutagens. In this analysis the mutants of TEM and the carboxylic mustards were omitted, since some of the experiments with these agents were undertaken before the accumulation of the $\beta$ loci. The $\Psi$ test for the data in Table 2 gave a $P = 0.01$, which is highly significant. Unlike the $\alpha$ loci, therefore, the relative response of the $\beta$ loci does differ under the various mutagens. Lastly, an over-all analysis has been undertaken of the relative mutability at 106 X chromosome loci ($43\text{-}\alpha + 63\text{-}\beta$) which have mutated more than once in our more recent experiments (i.e., with the mutagens detailed in Table 2). The $\Psi$ test for this over-all distribution gave $P = 0.02$, which is convincingly significant, indicating a differential response. It would be desirable to analyse the distribution of alleles at the various loci for pairs of mutagenic series, to determine the degree of differential action for the various agents. The samples for the individual series, however, are sufficiently large to warrant such analysis only in the case of the amino acid mustards and the sulphonates. A $\Psi$ test for allele distribution at all the $\alpha$ and $\beta$ loci under these two chemical series gave $P = 0.01$, indicating a real difference. This is of great interest, since it shows that different chemical series within the alkylating agents do produce differential mutagenic effects.

The most important outcome of the above statistical analysis is that there is a group of genes (the $\alpha$ loci) which seems to respond to roughly the same degree to radiation and the various alkylating mutagens. Another group of genes (the $\beta$ loci) do vary in their response according to the agent. To put this conclusion to further test, we pooled the number of alleles at the $\alpha$, as well as at the $\beta$ loci for each mutagen and compared their ratios in a $2 \times 6$ contingency table (Table 3). The numbers here are sufficiently high to justify the application of $\chi^2$ test. The total $\chi^2$ for the six mutagenic classes gave a value of 18.8 which for five degrees of freedom corresponds to $P = 0.004$. The relative effect of various mutagens on the $\alpha$ and $\beta$ loci is, therefore, most decisively different.

*Comparative mutation rates at specific loci:* To substantiate the discovery of the preferential response of certain genes to particular mutagens, tests have been undertaken to determine the exact mutation rates at specific $\alpha$ and $\beta$ loci under

TABLE 2

*Distribution of alleles at specific β loci with different mutagenic agents*

| Locus | | Stock | Position | X-ray | Amino acid mustards | Sulphonates | C.B. 1506 | C.B. 1592 | C.B. 3034 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| scute$^{fahmy}$ | *sc$^{fah}$* | 2016 | 0.0 | .. | 2 | 1 | .. | .. | .. | 3 |
| tiddler | *tdd* | 2369 | 0.0 | .. | 1 | 1 | .. | .. | .. | 2 |
| unequal-wings | *uq* | 2197 | 0.5 | 1 | 2 | .. | .. | .. | .. | 3 |
| rough-eye-like | *re$^{2}$* | 1665 | 0.6 | .. | 2 | .. | .. | .. | .. | 2 |
| reduced-size | *rsi* | 1837 | 0.6 | .. | 2 | .. | 1 | .. | .. | 3 |
| murky | *mk* | 2064 | 0.8 | 1 | 4 | 3 | 2 | .. | 1 | 11 |
| faulty-chaete | *fc* | 2021 | 0.9 | .. | 1 | .. | .. | 1 | .. | 2 |
| waisted | *ws* | 2170 | 1.0 | .. | 1 | 1 | 1 | .. | .. | 3 |
| bent-scutellars | *bsc* | 2056 | 1.1 | .. | 3 | .. | .. | .. | .. | 3 |
| misproportioned | *mis* | 589 | 1.3 | .. | .. | 2 | .. | .. | 1 | 3 |
| vestigium | *ves* | 2134 | 1.4 | .. | 2 | .. | .. | .. | .. | 2 |
| cramped-like | *crm$^{2}$* | 2152 | 1.4 | .. | 4 | 3 | 2 | 1 | 1 | 11 |
| stiff-chaete | *sfc* | 2195 | 3.2 | .. | 1 | 1 | .. | 1 | .. | 3 |
| ruddle | *rud* | 1276 | 3.3 | 1 | 2 | 6 | .. | .. | .. | 9 |
| Ocellarless | *Oce* | 1243 | 5.7 | .. | 1 | 2 | .. | 1 | .. | 4 |
| microoculus | *mo* | 1998 | 6.7 | .. | 6 | 5 | 1 | 1 | .. | 13 |
| amber | *amb* | 2105 | 6.8 | .. | 2 | .. | 1 | .. | 2 | 5 |
| lacquered | *lac* | 1607 | 7.3 | 2 | 2 | 9 | 1 | .. | 2 | 16 |
| ommatoreductum | *omm* | 1255 | 12.8 | .. | 2 | 2 | .. | 1 | .. | 5 |
| deflected-wing | *dfw* | 2193 | 21.6 | 1 | 1 | .. | .. | .. | .. | 2 |
| slim-bristle | *smb* | 2368 | 23.1 | .. | .. | 2 | .. | .. | .. | 2 |
| concave-wing | *ccw* | 1287 | 23.4 | .. | 3 | .. | .. | .. | .. | 3 |
| bright-eye | *bre* | 1415 | 24.6 | .. | 1 | 1 | .. | .. | .. | 2 |
| rearranged-tergites | *rea* | 1952 | 25.4 | .. | 2 | 1 | .. | 1 | .. | 4 |
| deltoid-veins | *dlv* | 2665 | 25.9 | .. | .. | .. | .. | 2 | .. | 2 |
| smaller | *sma* | 1726 | 29.9 | .. | 4 | 6 | .. | 1 | 1 | 12 |
| clumpy-marginals | *clm* | 1289 | 32.6 | .. | 2 | .. | 1 | .. | .. | 3 |
| wider-wing | *ww* | 1658 | 32.9 | .. | 2 | .. | .. | .. | .. | 2 |
| outshifted | *osh* | 2262 | 33.0 | 1 | .. | .. | 2 | .. | .. | 3 |
| costakink | *csk* | 1253 | 33.4 | 1 | .. | 1 | .. | .. | .. | 2 |
| slim | *slm* | 2183 | 33.7 | .. | 1 | .. | 3 | .. | .. | 4 |
| thread-bristle | *trb* | 2198 | 37.0 | .. | 2 | .. | .. | .. | .. | 2 |
| water-wings | *wtw* | 2013 | 38.9 | .. | 1 | 1 | .. | .. | .. | 2 |
| crumpled-tips | *crt* | 1227 | 40.3 | .. | 4 | .. | .. | 3 | 2 | 9 |
| upheld | *up* | 1892 | 41.0 | .. | 1 | 2 | .. | .. | .. | 3 |
| puny | *pun* | 1386 | 41.1 | .. | 1 | 1 | .. | .. | .. | 2 |
| tawny | *taw* | 2199 | 41.1 | .. | 2 | 1 | .. | .. | .. | 3 |
| cleft-end | *clf* | 1143 | 41.3 | .. | 2 | .. | .. | .. | .. | 2 |
| copper | *cop* | 1523 | 43.3 | .. | 2 | .. | .. | 1 | .. | 3 |
| rimy | *rim* | 2297 | 48.1 | .. | .. | 1 | 1 | 1 | .. | 3 |
| midget | *mgt* | 1879 | 49.6 | 2 | 4 | .. | 2 | .. | .. | 8 |
| thick-veins | *thv* | 2337 | 49.7 | .. | .. | .. | 1 | .. | 1 | 2 |
| slimma | *sla* | 1953 | 50.0 | .. | 6 | .. | .. | .. | .. | 6 |
| tiny-chaete | *tc* | 2161 | 51.6 | .. | 2 | .. | .. | .. | .. | 2 |
| minute-chaete | *mch* | 2416 | 52.0 | .. | 1 | 1 | .. | .. | .. | 2 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| droopy-wing | *drw* | 2889 | 52.3 | .. | 1 | .. | .. | 1 | .. | 2 |
| under-sized | *us* | X208 | 52.5 | 3 | .. | 1 | 1 | .. | .. | 5 |
| melanoscutellum | *ms* | 1916 | 52.6 | .. | 4 | 1 | 1 | .. | .. | 6 |
| Shaker-downheld | *Shw* | 2119 | 53.3 | 1 | 1 | 2 | .. | 2 | .. | 6 |
| small-wing | *sl* | 2100 | 53.5 | .. | 4 | 5 | .. | 1 | .. | 10 |
| acclinal-wing | *acc* | 2223 | 54.5 | .. | 2 | .. | .. | .. | .. | 2 |
| fine-lash | *fil* | 1464 | 56.8 | .. | 1 | 2 | .. | .. | .. | 3 |
| heldup | *hdp* | 2077 | 59.6 | 1 | 1 | .. | .. | .. | .. | 2 |
| tonochaete | *ton* | 1928 | 60.1 | .. | 1 | 1 | 1 | .. | .. | 3 |
| crooked-setae | *crk* | 1578 | 60.1 | .. | 2 | .. | .. | .. | .. | 2 |
| smalloid | *smd* | 2073 | 61.1 | 2 | 4 | 4 | 2 | 1 | 1 | 14 |
| megaoculus | *meg* | 2078 | 61.9 | .. | 2 | .. | .. | .. | .. | 2 |
| knobby-head | *kno* | 1223 | 63.9 | .. | .. | 2 | .. | .. | .. | 2 |
| melanised | *mel* | 1663 | 64.1 | .. | 2 | .. | .. | .. | .. | 2 |
| warty-like | *wa*$^2$ | 1392 | 64.4 | 1 | 2 | 2 | .. | .. | .. | 5 |
| bronzy | *bz* | 1920 | 64.9 | 1 | 3 | .. | .. | 1 | .. | 5 |
| refringent | *ref* | 2211 | 67.9 | .. | 2 | .. | .. | .. | 1 | 3 |
| little-fly | *lf* | 1981 | 68.3 | .. | 3 | 3 | 1 | 1 | .. | 8 |
| TOTAL | | | | 19 | 119 | 77 | 25 | 22 | 13 | 275 |

TABLE 3

*The ratio of alleles at the α and β loci, with different mutagenic agents*

| Mutagen | α loci mutants | β loci mutants | Total mutants | Ratio β/α loci mutants | $\chi^2$ |
|---|---|---|---|---|---|
| X-rays | 57 | 19 | 76 | 0.33 | 5.001 |
| Amino acid mustards | 150 | 119 | 269 | 0.79 | 5.350 |
| Sulphonates | 168 | 77 | 245 | 0.46 | 3.749 |
| CB. 1506 | 49 | 25 | 74 | 0.51 | 0.416 |
| CB. 1592 | 23 | 22 | 45 | 0.96 | 2.530 |
| CB. 3034 | 13 | 13 | 26 | 1.00 | 1.758 |
| TOTAL | 460 | 275 | 735 | 0.60 | 18.804 |

the effect of two contrasting mutagens. The two mutagens chosen were X-rays as a respresentative of the physical agents and the mustard derivative of phenylalanine [CB.3025, p-NN-di-(2-chloroethyl) aminophenylalanine] as a representative of the alkylating agents. This compound has been chosen for the test, since its mutagenic properties are appreciably different from those of radiation. It is a much weaker chromosome breaker, while being substantially more effective in the induction of point mutations resulting in recessive visibles (FAHMY and FAHMY 1956a).

An effort was made to standardize the experimental procedure in the genetic tests with the radiation and the chemical agents. The mutations were detected in the $F_1$ daughters from treated fathers mated to mothers carrying the marker loci. All the mutants were further analysed by the genetical tests detailed in the section on Technique. The range of mutagenic doses (as assessed by the lethal rate)

utilized with both mutagens was roughly the same. In all experiments only sperm delivered during the first nine days after treatment was tested, so as to ensure the homogeneity of the germ cell stages from which the mutants were recovered. Within this period, under our experimental conditions, only sperm and spermatids at the time of treatment are utilized. Another advantage of using the later sperm stages, is the avoidance of complications due to mutant "clusters" and possible germinal selection that may arise from the use of premeiotic stages.

Table 4, summarizes the data with 23 loci tested for mutability under the effect of X-radiation. Seven of these (insets in the table) are $\alpha$ loci, chosen from among the most common laboratory stocks, the remaining 16 are $\beta$ loci which were initially mutated by various alkylating mutagens. Two of the 16 loci here considered as $\beta$ (*viz.* small-wing: *sl*, and Turned-up-wing: *Tu*) have actually been recorded in the literature as occurring spontaneously or after irradiation. The marker alleles used in the present experiments, however, were chemically induced, involving the possibility that their substructure might be different. This could affect the detection of mutations to the specific alleles used. These loci, therefore, were included among the chemically responsive $\beta$ class. The chosen $\beta$ loci were distributed along the whole length of the X chromosome and were

TABLE 4

*Mutability at specific loci under X-radiation*

| Locus | | Stock | Position | Chromosomes chr. | Dose r | chr. × r ×10⁻⁸ | Deficiencies No. | Deficiencies Rates ×10⁻⁸ | Visibles No. | Visibles Rates ×10⁻⁸ |
|---|---|---|---|---|---|---|---|---|---|---|
| brachymacrochaete | *brc* | 1230 | 0.0 | 28626 | 3834 | 1.10 | .. | ... | .. | ... |
| scute | *sc* | .... | 0.0 | 38352 | 2990 | 1.15 | 2 | 1.74 | 4 | 3.49 |
| notchoid | *nd* | 638 | 2.7 | 34664 | 4266 | 1.48 | .. | ... | .. | ... |
| ruddle | *rud* | 1276 | 3.3 | 47272 | 3418 | 1.62 | 2 | 1.24 | .. | ... |
| Ocellarless | *Oce* | 1243 | 5.7 | 28626 | 3834 | 1.10 | 2 | 1.82 | .. | ... |
| microoculus | *mo* | 1998 | 6.7 | 58833 | 2678 | 1.58 | 2 | 1.27 | .. | ... |
| ommatoreductum | *omm* | 1255 | 12.8 | 28626 | 3834 | 1.10 | 2 | 1.82 | .. | ... |
| cut | *ct* | .... | 20.0 | 38352 | 2990 | 1.15 | 6 | 5.23 | 1 | 0.87 |
| concave-wing | *ccw* | 1287 | 23.4 | 44071 | 2990 | 1.32 | 2 | 1.52 | .. | ... |
| smaller | *sma* | 1726 | 29.9 | 47272 | 3418 | 1.62 | .. | ... | .. | ... |
| vermilion | *v* | .... | 33.0 | 89324 | 3207 | 2.86 | 2 | 0.70 | 4 | 1.44 |
| upheld | *up* | 1892 | 41.0 | 61422 | 2777 | 1.71 | 2 | 1.17 | .. | ... |
| wavy | *wy* | .... | 41.9 | 38352 | 2990 | 1.15 | 7 | 6.10 | 4 | 3.49 |
| copper | *cop* | 1523 | 43.3 | 28626 | 3834 | 1.10 | .. | ... | .. | ... |
| garnet | *g* | .... | 44.4 | 38352 | 2990 | 1.15 | 6 | 5.23 | 5 | 4.36 |
| tiny-chaete | *tc* | 2161 | 51.6 | 44071 | 2990 | 1.32 | .. | ... | .. | ... |
| small-wing | *sl* | 2100 | 53.5 | 44071 | 2990 | 1.32 | .. | ... | .. | ... |
| forked | *f* | .... | 56.7 | 38352 | 2990 | 1.15 | 1 | 0.87 | 3 | 2.62 |
| Turned-up-wing | *Tu* | 2212 | 59.1 | 47272 | 3418 | 1.62 | .. | ... | .. | ... |
| smalloid | *smd* | 2073 | 61.1 | 22278 | 4266 | 0.95 | 1 | 1.05 | 8 | 8.42 |
| carnation | *car* | .... | 62.5 | 66978 | 3418 | 2.29 | 7 | 3.06 | .. | ... |
| melanised | *mel* | 1663 | 64.1 | 32867 | 3316 | 1.09 | .. | ... | .. | ... |
| bronzy | *bz* | 1920 | 64.9 | 44071 | 2990 | 1.32 | 8 | 6.07 | .. | ... |

fully penetrant as well as fully viable and fertile in the homozygous condition. The phenotypic expression of all mutants at these loci is sufficiently clear to enable ease of detection and classification.

As far as the intragenic changes (point mutations: fully viable visibles) are concerned, it is abundantly clear that the $\beta$ loci are comparatively more resistant to radiation than the $\alpha$ loci. Only one of the 16 $\beta$ loci, *viz.* smalloid, was mutated by X-rays, and at an exceptionally high rate ($8.42 \times 10^{-8}$ per r). This is obviously a radiation responsive locus which has been missed by earlier workers. A consideration of the intergenic mutations (deficiencies and deletions covering the markers) conveys a certain degree of differential effect, though to a lesser extent than for the intragenic changes. On the whole the frequency of deficiencies at the $\alpha$ loci is higher than at the $\beta$ loci; eight of the 23 loci were refractory to radiation breakage (i.e., not involved in deficiencies) and all of these were among the $\beta$ loci. This is so, in spite of the fact that the sample-dose product for most of the noneliminated loci is higher than that for the responsive ones, and with a larger dose contribution. Their lack of response, therefore, cannot be entirely due to inadequate sample size, but is an indication that chromosome breakage, even under radiation, is not as random as is generally assumed.

Of the 23 loci tested under radiation, 11 were selected for testing under the effect of the mustard derivative of phenylalanine: ten of the $\beta$ loci (nine X-ray refractory and smalloid) and vermilion of the $\alpha$ loci. The results are detailed in Table 5. The chromosome sample tested per locus is reasonably within the range of that used in the radiation tests. The injected dose of the mustard has been carefully gauged to induce 6-8 percent sex-linked recessive lethals, so as to make the mutagenic doses with the chemical agent approximately equivalent to those in the radiation experiments. The actual mutagenic dose in each experiment was accurately determined by a sex-linked recessive lethal test on an aliquot of the

TABLE 5

*Mutability at specific loci under the "mustard" derivative of phenylalanine (L-p-NN-di(2-chloroethyl) aminophenylalanine)*

| Locus | | Stock | Position | Chromosomes chr. | Dose r equiv. | chr. × r equiv. × $10^{-8}$ | Deficiencies No. | Deficiencies Rates × $10^{-8}$ | Visibles No. | Visibles Rates × $10^{-8}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| brachymacrochaete | *brc* | 1230 | 0.0 | 67445 | 3152 | 2.13 | .. | ... | 1 | 0.47 |
| notchoid | *nd* | 638 | 2.7 | 67445 | 3152 | 2.13 | 12 | 5.63 | 1 | 0.47 |
| ruddle | *rud* | 1276 | 3.3 | 44051 | 3465 | 1.53 | .. | ... | 4 | 2.61 |
| Ocellarless | *Oce* | 1243 | 5.7 | 67445 | 3152 | 2.13 | 1 | 0.47 | 1 | 0.47 |
| concave-wing | *ccw* | 1287 | 23.4 | 87547 | 2563 | 2.24 | .. | ... | 1 | 0.45 |
| vermilion | *v* | .... | 33.0 | 129831 | 2563 | 3.33 | 2 | 0.60 | 3 | 0.90 |
| copper | *cop* | 1523 | 43.3 | 67445 | 3152 | 2.13 | 2 | 0.94 | 1 | 0.47 |
| tiny-chaete | *tc* | 2161 | 51.6 | 48977 | 2563 | 1.26 | .. | ... | 1 | 0.79 |
| small-wing | *sl* | 2100 | 53.5 | 74253 | 2563 | 1.90 | .. | ... | 6 | 3.16 |
| smalloid | *smd* | 2073 | 61.1 | 67445 | 3152 | 2.13 | 1 | 0.47 | 8 | 3.76 |
| bronzy | *bz* | 1920 | 64.9 | 47036 | 2563 | 1.21 | 1 | 0.83 | 2 | 1.65 |

males used in the specific locus analysis, both with radiation and the mustard. The exact r-equivalence for the mustard as detailed in Table 5 has been calculated on the basis of this relative lethal rate.

It is clear from Table 5 that all the loci tested did mutate intragenically at various rates and six were eliminated in deficiencies. Of great interest is the fact that the nine X-ray refractory loci responded intragenically to the mustard. Furthermore smalloid, like vermilion, was responsive to both mutagens.

*Statistical analysis of mutability at specific loci:* The data presented in the previous section lead to the compelling conclusion that a certain amount of differential gene response to mutagens must occur. The question then arises as to how far this conclusion can be substantiated statistically. Two aspects were investigated: a) The conformity to randomness of the mutation incidence at different loci under the same mutagen. b) The relative mutation rates at the same locus under different mutagens.

Random rare events are expected to follow the Poisson distribution. It was, therefore, thought appropriate to test how far the mutation incidence for various groups of loci does follow this distribution. This analysis which is based on Tables 4 and 5, is summarized in Table 6. For each group of loci considered, the incidence 0, 1, 2, 3 and c per $10^8$ chromosome-r (to the nearest whole number) was determined and the mean ($\bar{x}$) was computed and compared with the mutability variance ($V_x$) as estimated from the data. The values of $\bar{x}$ and $V_x$ would not differ significantly for groups of loci that mutate according to the Poisson distribution (i.e., randomly) but would be different if some of the loci in the analysed group mutate at frequencies different from the Poisson expectations.

Table 6 shows that mutability at the seven $\alpha$ loci tested under X-rays follows the Poisson distribution admirably, both for the intra- and intergenic mutations. When the sample of loci contained a high proportion of the $\beta$ class, however, mutability deviated significantly from the Poisson distribution both for the deficiencies and the point mutations, though the deviation was more pronounced for the latter. An attempt was made to determine how far the deviation from the

TABLE 6

*Mutation incidence at various groups of specific loci (in relation to the Poisson distribution)*

| Mutagen | Mutation | Loci tested | Mean mutation rate per $10^8$ chr. × r $\bar{x} \pm s_{\bar{x}}$ | Variance $V_x$ | P |
|---|---|---|---|---|---|
| X-rays | Deficiencies | 7 $\alpha$ | 3.29 ± 0.78 | 4.24 | 0.3 |
| | Visibles | 7 $\alpha$ | 2.14 ± 0.55 | 2.14 | 1.0 |
| | Deficiencies | 23(7$\alpha$+16$\beta$) | 1.70 ± 0.41 | 3.95 | <0.001 |
| | Visibles | 23(7$\alpha$+16$\beta$) | 1.09 ± 0.44 | 4.36 | ≪0.001 |
| | Deficiencies | 11(1$\alpha$+10$\beta$) | 1.18 ± 0.54 | 3.16 | <0.01 |
| | Visibles | 11(1$\alpha$+10$\beta$) | 0.82 ± 0.72 | 5.76 | <0.001 |
| Mustard | Deficiencies | 11(1$\alpha$+10$\beta$) | 1.00 ± 0.52 | 3.00 | <0.01 |
| | Visibles | 11(1$\alpha$+10$\beta$) | 1.73 ± 0.33 | 1.22 | 0.2 |

above distribution was due to the frequency of refractory loci (i.e., the o class of the distribution). The expected frequency of these loci ($f$) is given by the Poisson term

$$f = n\, e^{-\mu}$$

where $n$ is the number of loci in the tested group and $\mu$ is the mean mutation rate per locus for the group. This calculation gave an expected frequency of loci refractory to breakage (deficiencies) of 4.22 as compared to the observed eight ($\chi^2$ with YATE's correction = 2.55 corresponding to P = 0.1) which is not a significant difference. For the point mutations on the other hand, the expected frequency of the o class was found to be 7.76 as compared to the observed 16 ($\chi^2$ with YATE's correction = 7.73 corresponding to a P = 0.01) which is decisively significant. The frequency of the refractory loci, therefore, is not the main reason for the deviation from the Poisson distribution for the deficiency mutations, but it is a major factor for this deviation for the point mutations. This result is also of some relevance to the assessment of the adequacy of the scale at which the radiation experiments were undertaken. The nonsignificance of the shortage of the loci refractory to breakage (giving deficiencies) argues against the possibility that the gross discrepancy of intragenic mutations was due to the small scale of the work.

Most informative is the analysis of the mutability distribution for the same 11 loci (ten $\beta$ loci and one $\alpha$ locus: vermilion) under X-rays on the one hand, and the phenylalanine-mustard on the other. The response of these loci under X-rays is significantly lower than expectation on the basis of the Poisson distribution for both the deficiencies and the viable visibles, though the deviation is more marked for the intragenic mutations. The response of the same loci under the mustard, however, deviates from randomness for the deficiencies, but does follow the Poisson expectations for the point mutations. There can hardly be any doubt, therefore, that some loci among the $\beta$ class do respond differently to X-rays and the chemical agent.

A comparison has been undertaken of the absolute intragenic mutation rate per locus, for those loci which have been tested under the effect of X-rays and the phenylalanine-mustard. For this purpose we used PEARSON's (1948) expression for the comparison of the frequency of rare events.

$$u = \frac{a_1 - r\,\lambda}{\sqrt{[r\,\lambda\,(1\text{-}\lambda)]}}$$

Where $a$ = number of mutants observed ($a_1$ and $a_2$ for the two mutagens)

$$r = a_1 + a_2$$

and $\lambda = \dfrac{n_1\,\kappa}{n_1\,\kappa + n_2}$, $n_1$ and $n_2$ being the samples used,

which in our case is measured by the chromosome-r product, $\kappa$ is a constant given by $x_1/x_2$ which is the ratio of the true mutation rates $x_1$ and $x_2$. $\kappa$ is 1 for expected equality.

$u$, is a random variable which, according to the hypothesis under test, has a zero mean and unit variance. The significance of $u$, therefore, can be tested by referring its value to a table of the normal probability integral.

The intragenic mutation rates are compared in Table 7. The deviation from equal mutability reached decisive statistical significance ($P = 0.02$) for two loci —ruddle and small-wing, both of which were more responsive to the chemical agent. For two more loci, smalloid and bronzy, the deviation from equality reached the verge of significance; smalloid being more responsive to X-rays, and bronzy more so to the mustard. The over-all mutation incidence for the 11 loci does indicate a difference in the average mutation rate, but only on the border line of significance. This, however, is fortuitous, and is entirely due to the exceptionally high response of the smalloid locus to X-rays. When this locus is excluded, the difference in the mutability at the remaining ten loci proves to be highly significant ($P = 0.004$). A comparison was also undertaken for the mutability at the nine $\beta$ loci which proved refractory to X-radiation, taking into consideration the stronger treatment with the chemical agent (as measured by the chromosome-r product). This comparison revealed a very gross deviation from equal mutability under the two mutagens ($P = 0.0002$).

It would be interesting to determine whether differential gene response to mutagens is quantitative: that is a matter of degree, or qualitative: that particular loci are responsive to certain mutagens and completely refractory to others. The

TABLE 7

*Comparison of the mutation rates of the same gene locus under X-radiation and the "mustard" derivative of phenylalanine*

| Locus | | Mustard | | X-rays | | Pearson's ratio | P |
|---|---|---|---|---|---|---|---|
| | | No. $a_1$ | Sample chr. $\times$ r ($\times 10^{-8}$) $n_1$ | No. $a_2$ | Sample chr. $\times$ r ($\times 10^{-8}$) $n_2$ | $u$ | |
| *brc* | 1230 | 1 | 2.13 | 0 | 1.10 | 0.72 | 0.24 |
| *nd* | 638 | 1 | 2.13 | 0 | 1.48 | 0.83 | 0.20 |
| *rud* | 1276 | 4 | 1.53 | 0 | 1.62 | 2.06 | 0.02 |
| *Oce* | 1243 | 1 | 2.12 | 0 | 1.10 | 0.72 | 0.24 |
| *ccw* | 1287 | 1 | 2.24 | 0 | 1.32 | 0.77 | 0.22 |
| *v* | .... | 3 | 3.33 | 4 | 2.86 | 0.58 | 0.28 |
| *cop* | 1523 | 1 | 2.13 | 0 | 1.10 | 0.72 | 0.24 |
| *tc* | 2161 | 1 | 1.26 | 0 | 1.32 | 1.02 | 0.15 |
| *sl* | 2100 | 6 | 1.90 | 0 | 1.32 | 2.04 | 0.02 |
| *smd* | 2073 | 8 | 2.13 | 8 | 0.95 | 1.66 | 0.05 |
| *bz* | 1920 | 2 | 1.21 | 0 | 1.32 | 1.48 | 0.07 |
| Total | 11 loci | 29 | 22.12 | 12 | 15.49 | 1.55 | 0.06 |
| *smd* excluded | 10 loci | 21 | 19.99 | 4 | 14.54 | 2.65 | 0.004 |
| X-ray refractory | 9 loci | 18 | 16.66 | 0 | 11.68 | 3.55 | 0.0002 |

Pearson's ratio.

$$u = \frac{a_1 - r\lambda}{\sqrt{[r\lambda(1-\lambda)]}} \qquad \text{where } r = a_1 + a_2 \qquad \lambda = \frac{n_1 k}{n_1 k + n_2}; \qquad k = 1 \text{ for true equality.}$$

magnitude of the present data, does not permit a decisive answer to this problem. It is clear, however, that at least for ruddle and bronzy, the differential response to the amino acid mustard as compared to X-rays is only quantitative. Although these loci gave significantly lower mutation rates under X-rays (Table 7), they obviously can be mutated by it, since they were detected in our Muller-5 radiation tests (Table 2).

## DISCUSSION

The genome of Drosophila was shown to consist of two classes of genes, which differ in their stability and manner of response to mutagens. On the X chromosome, for example, there are a number of loci, here designated as the $\alpha$ class, which seem to respond to radiation, as well as to a diversity of chemical mutagens belonging to the so-called biological alkylating agents (chloroethylamines, ethyleneimines, and methanesulphonic esters). Morphologically detectable mutants, or 'visibles', at these loci have frequently been identified after the discovery of the mutagenic action of radiation, and a large sample of them has already been analysed and described in the Drosophila mutants literature (mainly BRIDGES and BREHME 1944). These 'visibles' came to be maintained in the various Drosophila laboratories and are looked upon as the 'classical' examples of morphological mutants. More recently, it was noticed (FAHMY and FAHMY 1956a, 1957a) that in our experiments with the alkylating mutagens, a great many mutants occurred in addition to the classical ones. As is demonstrated in this communication, the additional mutants are mainly the result of the response of another group of loci which have been designated the $\beta$ loci to distinguish them from the 'classical', or $\alpha$ loci.

Major differences have already been recognized between the $\alpha$ and $\beta$ classes of loci, as regards

(a) phenotypic manifestations
(b) distribution along the chromosome
(c) response to different mutagens.

Visible mutants at the $\beta$ loci are on the whole markedly more pleiotropic in their phenotypic manifestations, affecting several parts of the fly's body simultaneously. Many of these mutants are slow in development and, therefore, late in eclosion and also have reduced viability and/or fertility. The distribution of the $\beta$ loci recessive 'visibles' along the genetical map of the X chromosome has been compared with the corresponding $\alpha$ loci mutants (FAHMY and FAHMY 1957a, 1958a) and there was clear evidence for a higher relative frequency of the $\beta$ loci visibles at the proximal half of the chromosome, near the centromere. Whereas most of the $\alpha$ loci seem to respond to roughly the same degree under radiation and a diversity of chemical mutagens, the great majority of the $\beta$ loci do respond differentially. Another interesting difference is that the $\beta$ loci are on the whole less responsive to mutagens than the $\alpha$ class. This is clearly manifested in the frequency of allele recurrence under the same mutagens. The mean number of alleles per locus for the $\alpha$ class is $1.78 \pm 0.18$ (based on Table 1, excluding

TEM and the carboxylic mustards) while the comparable value for the $\beta$ loci for the same sample of treated chromosomes and under the same agents is $0.73 \pm 0.06$ (based on Table 2), which is clearly very significantly lower ($P = 10^{-8}$).

It cannot be overemphasized, however, that the classification of the gene loci into $\alpha$ and $\beta$ categories as given in the present communication, is by necessity neither rigid nor final. Any permanent grouping in this respect, is only possible after the accumulation of considerable data on the mutability of each locus to various mutagens. Whether this seemingly prohibitive task can ever be achieved, still remains to be seen. The present data, however, have clearly shown the reality of such a classification, even though it is virtually certain that the details of the grouping will change for some loci.

The differentiation of the gene complement into loci of different stability to mutagens is of great evolutionary significance. Mutation by permitting inherited variability, is perhaps the most important positive factor in the evolutionary process. On the other hand, it is easy to see that equal mutation at both the "advantageous" and "disadvantageous" loci would certainly have a hindering effect on evolution. There will be no chance for natural selection to build up the "desirable" genic combinations. In view of the fact that the majority of the $\beta$ loci "visibles" also have adverse effects on viability and fertility, it is perhaps not surprising that they were "stabilized" in the course of evolution. Of great interest in this connection, is the fact that practically none of the $\beta$ loci "visibles" were reported as having occurred spontaneously. While it is certain that this does not mean that none of them could have arisen in nature, it does, nevertheless, indicate that their spontaneous incidence is extremely rare, much rarer than it is for the $\alpha$ loci.

The mechanism of gene stabilization to mutagens can, at present, be only a subject for speculation. It is generally agreed, however, that gene stabilization could have occurred in evolution through the process of duplication, and it is possible that the same process may have played a rôle in the "protection" of some $\beta$ loci against mutagens. That duplications did occur in evolution, is indicated by the presence of *repeats* in the salivary chromosomes of various Diptera, including Drosophila. These are segments with similar or identical banding patterns, and which occasionally pair with one another in the same salivary nucleus. The so-called doublets (two identical bands) are generally believed to represent a single locus and they are probably the physical manifestation of duplication at the gene level. In a newly arisen gene duplication, the locus involved will be represented four times in a diploid organism. Physiologically, two of these are in effect superfluous and could probably be mutated or even eliminated without adverse effect on the organism. The evolutionary advantage of this in relation to grossly harmful mutations is obvious. Mutations which would be lethal, or which would at least lower the viability or fertility of the organism if they occurred in a nonduplicated locus, would probably have less drastic effects if occurring in a quadruplicated site.

The same situation, of course, holds in the case of a recessive "visible." Both halves of the duplicated locus will have to be mutated simultaneously, or consecutively, before the mutation is expressed phenotypically, even in the hemizygous condition. This will naturally result in a much lower mutation incidence at the duplicated as compared to the single loci. In gene duplicates of greater antiquity, it is highly probable that the two halves underwent some variation by independent mutation. This could be visualized to lead either to two independent loci, or alternatively to one integrated locus, with the various physical entities differentiated to undertake the various biochemical processes necessary for the physiological expression of the gene. From the mutagenesis point of view, loci which are duplications by origin, will be expected to be less mutable, since more than one physical entity of the locus may need to be damaged before the change is manifested. They will also be more likely to respond to agents with less localized "hits." At present, there seems to be no reliable means to estimate the area of action of a single "hit" for the various mutagens. It was shown, however, that recessive lethals induced by some of the alkylating mutagens are more frequently associated with cytologically detectable small deficiencies than the comparable X-ray mutations (BIRD and FAHMY 1953; FAHMY and BIRD 1953; FAHMY and FAHMY 1956a). This may be an indication of more "diffuse hits" by the chemical agents, which would favor simultaneous action on several genic subunits. On this basis, the relatively higher response of $\beta$ loci to the phenylalanine mustard as compared to X-rays, may well be an indication that these loci are physically more complex, probably through being gene duplications by origin.

The differential response to mutagens of the various loci especially within the $\beta$ class, is the most interesting recent development in mutagenesis studies. While its "exact" mechanism is still obscure, some recent advances in genetics and biochemistry may help in its elucidation. Genetical analysis of the highest resolution (BRENNER *et al.* 1958), for example, has revealed the high complexity of gene structure and how its subunits could be affected differently by various mutagens. The *r*II region of bacteriophage $T_4$ was shown to consist of two "cistrons" each of which is composed of a large number of mutable elements of several kinds. The mutational sites in each cistron which responded to 5-bromouracil, were different from those that reacted to proflavin. It is, therefore, clear that an individual locus could be of sufficient organizational complexity to allow for differential response to mutagens. To this there is to be added the variation in organization of different genes along the chromosomes of Metazoa which must have occurred during speciation and evolution. This complexity of organization within genes, as well as between them, implies a multiplicity of "receptors and reception mechanisms" to mutagens which may well result in some degree of differential locus response.

It would be indeed fascinating to identify the various mutagen "receptors" in the genetic material and determine how they interact with the various "active" groups of mutagens. This has not yet been achieved, but there is already some chemical evidence suggesting differences in the mode of action of the alkylating

compounds. There are reasons to believe that these compounds produce their biological damage through the production of "electrophilic" carbonium ions which can combine with the many "nucleophilic" centres of the cell (Ross 1953). These centres in the hereditary nucleoproteins are the sulphydryl (-SH), amino ($-NH_2$) and carboxyl (-COOH) groups in proteins, as well as the phosphate, amino and imino groups and also the ring nitrogen of the purines and pyrimidines of nucleic acids. Evidence is available that reactions with the protein groups may not be relevant to the direct chain of events leading to point mutations. Thus the so-called -SH poisons, like lewisite and chloropicrin are nonmutagenic. More significant in this connection is that ethyl iodide also proved to be nonmutagenic in adult male Drosophila (FAHMY and FAHMY, unpublished), in spite of the fact that it was shown to react *in vivo* with the -SH groups of cysteine or cysteine derivatives (ROBERTS and WARWICK, personal communication). Amine reactors, like triacrylformal (HENDRY, ROSE and WALPOLE 1951), while being fairly toxic to the flies (presumably through attacking the amino groups of proteins) also proved to be completely nonmutagenic (FAHMY and FAHMY 1957d). This leaves reactions with the various groups of deoxyribonucleic acid (DNA) as the possible primary cause for mutagenicity. From purely physico-chemical considerations (Ross 1953), it was argued that with the alkylating mutagens, the phosphate groups of nucleic acids are the most likely recipients of alkylation under physiological conditions.

Reactions *in vitro* between nucleic acid and various alkylating agents (ALEXANDER, COUSENS and STACEY 1957) seemed to support the above view. There was evidence, however, that the esterification of the phosphate groups could vary to a considerable degree, even among closely related mutagens, through electrostatic attraction or repulsion between the reactants. Thus the mustards with a zwitterion (carrying a positive as well as a negative charge, like the phenylalanine derivative) reacted readily, while those with a negative charge (the carboxylic mustards, through giving ionized carboxyl groups) failed to react either partially or completely. Evidence is also forthcoming that nucleic acid groups other than the phosphate, may well be attacked by some alkylating compounds. The nitrogen mustard HN2[di-(2-chloroethyl) methylamine] as well as dimethyl sulphate were shown (LAWLEY and WALLICK 1957; LAWLEY 1957) to alkylate the purine or pyrimidine rings of nucleotides and nucleic acids. The guanine moiety of the purine was the most susceptible, since there were indications that 7:methyl-guanine was formed under the action of dimethyl sulphate.

The "blockage" of some of the DNA groups through alkylation is bound to result in some change in its physico-chemical properties which could, directly or indirectly, affect the attacked gene locus and thus start the chain of events culminating in the biological expression of a mutation. On this basis, it would not be difficult to visualize that mutagens whose active groups show different affinities to the various receptive groups of DNA could produce differential mutability. Furthermore, there is enough chemical evidence to indicate that even the same

primary reaction between the DNA molecule and different alkylating mutagens, could result in different effects as a consequence of the breakdown of the alkylated macromolecule. ALEXANDER *et al.* (1957) have shown that the triesters produced by the alkylation of the phosphate groups of DNA are unstable and are readily hydrolyzed on standing, or at high temperature. The breakdown predominantly occurs at the alkylation linkage, though very infrequently it occurs at the phosphate-sugar linkage. In the latter case, a break in the main chain of the nucleotide is produced, resulting in an appreciable fall of the molecular weight of the DNA recovered by hydrolysis after alkylation. The degree of disruption in the nucleotide chains seems to vary with the alkylating agent. A major difference in this respect has been demonstrated between the mono-, as compared to the difunctional alkylating mutagens. The monofunctional compounds seem to produce "hidden breaks" in only one of the twin chains of the DNA molecule. This does not lead to a disruption of the molecule since it would remain supported by the normal twin. The difunctional agents, however, were shown to act on the two chains of the DNA molecule, thus resulting in its disruption. While it would be merely speculative to interpret the above chemical differences in terms of specific types of mutation, it seems legitimate to expect them to result in some differential mutagenic manifestations.

The concept of random mutability has emanated to a large extent from the simplified notion that the genetic material consists of a succession of identical masses of nucleoproteins, which could be assumed to behave similarly to the same, as well as to different, mutagens. The advocates of the above theory argued that the occurrence of any particular mutation, spontaneously or under the effect of a mutagen, is purely a matter of chance, as a result of an encounter (or "hit") between the mutagen, or part thereof, and the gene locus. It was further suggested that an "encounter" which is sufficient to produce a mutation at one locus, could also produce a mutation at any other. This simplified "scheme" of genic structure and mutagenesis is no longer compatible with recent developments in genetics and biochemistry. Gene structure proved to be far more complex than has hitherto been supposed, thus making differences between loci easier to visualize. These differences may well be due to the genic subunits varying in relative frequency and orientation, rather than in detailed chemical constitution. As regards the mode of action of the mutagens, there is sufficient chemical evidence to show that even closely related compounds like the alkylating agents, could act differently on biological macromolecules (particularly DNA), due to: a) different affinities of the receptive groups, b) electrostatic phenomena between the reactants and c) differences in the fate of the alkylated material. All these differences in genes, as well as in the biochemical mechanism of action of mutagens, do indicate that the observed differential mutability is not only theoretically feasible, but could indeed be a genuine manifestation of gene/mutagen interaction, and not merely the outcome of secondary nongenic phenomena independent of the mutation process itself.

SUMMARY

The gene loci on the X chromosome yielding recessive "visibles" could be classified into two classes on the basis of their response to mutagens. The $\alpha$ class respond to roughly the same degree to radiation and various alkylating mutagens. The $\beta$ class are on the average less responsive to the same mutagens than the $\alpha$ class. The $\beta$ loci also show a marked differential response to various mutagens which is manifested in: a) the proportion of alleles at the various loci recovered from the same sample of treated chromosomes, b) the ratio of mutants at the $\alpha$ to $\beta$ loci, and c) the absolute mutation rate at specific $\beta$ loci under comparable treatments with different mutagens (X-rays and the mustard derivative of phenylalanine). The possible reasons for the observed differential mutability and the evolutionary implications of the phenomenon are discussed.

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