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MUTAGENIC EFFECTS OF THE MONOFUNCTIONAL ETHYLATION AND METHYLATION REACTIONS IN DROSOPHILA*

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The monofunctional alkylating agents, diethyl sulphate, ethyl methanesulphonate, and methyl methanesulphonate, have been studied as mutagens on *Drosophila* spermatozoa when administered by the adult feeding method: all three compounds show pronounced mutagenic effects. A comparison has been made of the relative mutagenic effectiveness of the ethylation and methylation reactions on *Drosophila*.

Introduction

The alkylating agents are one of the most important groups of chemical mutagens to have received attention, since many of them are carcinogenic, carcinostatic and mutagenic. Also, the alkylating agents have been classified as 'radiomimetic' compounds because of the striking similarities between radiation-induced and alkylation-induced mutagenesis. Studies on the relative mutagenic efficiencies of the ethylation and methylation reactions (using diethyl sulphate and dimethyl sulphate) following treatment of *Drosophila* spermatozoa by adult feeding method, have shown that ethylation is a better mutagenic agency than methylation.[1] The present study extends these observations on *Drosophila melanogaster* (fruit fly) for the monofunctional ethylating and methylating agents (ethyl methylsulphonate, methyl methanesulphonate and diethyl sulphate) and it will be seen that methylation appears to be distinctly more mutagenic than ethylation, following the treatment of *Drosophila* spermatozoa by an adult feeding method. These results indicate that the relative efficiency of the ethyl and methyl groupings in mutagenesis depends to some extent on the type of chemical carrier *via* which these alkyl groups are introduced; the ethyl group being apparently more efficiently contributed *via* diethyl sulphate than methyl group *via* dimethyl sulphate, whilst the opposite appears to be the case when these alkyl groupings are introduced *via* the methane sulphonates.

Since most of the chemical agents show a delayed mutagenic activity,[3,9] the relative mutagenic effectiveness of the ethylation and methylation reactions is determined from the F1 complete and F1 mosaic sex-linked recessive lethal mutation frequencies generated from *Drosophila* spermatozoa after adult feeding treatments with monofunctional alkylating agents: diethyl sulphate (DES), ethyl methanesulphonate (EMS), and methyl methanesulphonate (MMS).

Materials and Methods

The bottoms of several one-pint milk bottles were covered with a double thickness of filter paper saturated with a solution containing 2 parts of distilled water to 1 part of absolute alcohol, 5% glucose, and the desired concentration of the alkylating agent. Since neither of these alkylating agents is miscible with water, alcohol was present in the treatment solution to disperse the globules otherwise formed. The filter paper was kept saturated during the period of treatment by adding 3 drops of the treatment solution 4 times daily.

Fifty newly-emerged wild-type Oregon-K *Drosophila* adult males were starved for 24 hr and placed for 24 hr in one of the treatment bottles; the treatment solution was the only source of nourishment during this time. After treatment, the males were examined for complete and F1 mosaic sex-linked recessive lethal mutations by the Muller-5 (Basc) method.

Results

Table 1 illustrates the complete and F1 mosaic sex-linked recessive lethal mutation frequencies generated from *Drosophila* spermatozoa after adult feeding treatments with diethyl sulphate (DES), ethyl methanesulphonate (EMS), and methyl methanesulphonate (MMS). In DES-treatment, survival was very good, sterility was 4% and 27 lethals were detected in F2 giving a F1 complete sex-linked recessive lethal mutation frequency of 8.9%. No lethal was found in the control treatment. EMS was toxic giving a low survival rate of 64%, sterility was 4% but the mutation rate was very high with 43.6% F1 complete lethals. MMS produced very high sterility which can be judged

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from the precentage of sterile F1 cultures (17%), and the average number of chromosomes it has been possible to examine per male, since all females recovered from F1 cultures were examined. Out of 333 chromosomes examined after MMS-treatment, 53 lethals were found giving a lethal frequency of 15.9%.

These experiments were extended for a further generation to detect F1 mosaic sex-linked recessive lethal mutations in the F3 generation. Details are listed in Table 1 of the number of non-lethal F2 cultures examined and the number of males from which these are derived, the average number of females examined from each non-lethal F2 culture, and the number of F2 cultures which later show mosaicism for a lethal in the F3 generation. The percentage of F1 lethalmosaics is calculated from the number of non-lethal F2 cultures which yield one or more lethal cultures in their F3 sets out of the total number of F3 sets examined. DES-treatment gave 7.3% lethals against 1.5% F1 mosaic sex-linked recessive lethals in control.

The average number of lethal cultures defining the F1 lethal-mosaics after each treatment can be estimated from the number of F2 lethal-bearing females (detected in the F3 generation). For example, DES gives 5 F1 lethal-mosaics and there are 15 lethal-bearing F2 females distributed among them. Consequently, the average number of lethal cultures from each F1 lethal-mosaic is 15/5 or 3 from an average of 5 females from each non-lethal F2 culture (see Table 1). This figure allows an estimation of 60% ($3/5 \times 100$) as the average mutated fraction, of F1 lethallymosaic gonads in the DES-treatment. In EMS-treatment, a sample of F2 females from each of several non-lethal cultures was tested to detect the F1 lethal-mosaics: an average of 9 cultures per non-lethal F2 culture were examined. Treatment of spermatozoa with EMS is found to produce 10% lethally mosaic F1 gonad. MMS-treatment gave 24.1% F1 mosaic sex-linked recessive lethals in F3.

Table 2 summarises further experiments on the frequency of complete sex-linked recessive lethal mutations generated from *Drosophila* spermatozoa after adult feeding treatments with three concentrations of MMS (0.075, 0.05, and 0.025%). There is an increase in sterility and mutation

TABLE 1.—THE F1 COMPLETE AND F1 MOSAIC SEX-LINKED RECESSIVE LETHAL MUTATION FREQUENCIES GENERATED FROM *Drosophila* SPERMATOZOA AFTER ADULT FEEDING TREATMENTS WITH DIETHYL SULPHATE, ETHYL METHANESULPHATE AND METHYL METHANESULPHONATE.

Mutagen	Control	Diethyl sulphate	Ethyl methane-sulphonate	Methyl methane-sulphonate
Concentration (%)	—	0.5 (3.2×10^{-2}M)	0.15 (1.2×10^{-2}M)	0.015 (1.36×10^{-3}M)
Duration of treatment		24 hr		
Survivors (%)	100	96	64	85
Sterile F1 culture (%)	0.0	4.0	4.0	17
No. males examined	40	85	78	36
No. chromosomes tested	398	303	465	333
Average No. chromosomes examined/male	10	4	6	9
No. lethals	0	27	203	53
Complete F1 lethals (%)	0.0	8.9	43.6	15.9
No. non-lethal F2 cultures examined	80 (arising from 40 males)	68 (arising from 68 males)	70 (arising from 70 males)	29 (arising from 29 males)
Average No. F2 females examined/non-lethal F2 culture	10	5	9	13
No. F2 cultures yielding at least one lethal in the F3 set	1	5	7	7
FI lethal-mosaicism (%)	1.5	7.3	10.0	24.1
Total No. F2 females examined	802	317	652	372
No. lethal-bearing F2 females	1	15	63	19
Lethals in F3 (%)	0.12	4.7	9.7	5.1

TABLE 2.—THE F1 COMPLETE SEX-LINKED RECESSIVE LETHAL MUTATION FREQUENCIES GENERATED FROM *Drosophila* SPERMATOZOA AFTER ADULT FEEDING TREATMENTS WITH METHYL METHANESULPHONATE (THREE EXPERIMENTS).

Concentration (%)	Control	0.075 (6.8×10^{-3}M)	0.05 (4.5×10^{-3}M)	0.025 (2.3×10^{-3}M)
Duration of treatment		24 hrs		
Survivors (%)	100	63	78	85
Sterile F1 culture (%)	0	50	48	35
No. males examined	34	32	18	26
No. chromosomes tested	308	191	137	260
Average No. chromosomes examined/male	9	6	8	10
No. lethals	0	59	41	50
F1 complete lethals (%)	0.0	90.9	29.9	19.2

rate from 0.025% of MMS-treatment to two higher concentrations of MMS-treatment i.e. 0.05 and 0.075%; but no significant difference was found in the sterility and mutation rate between the two higher concentrations of MMS-treatments.

Discussion

The object of the present investigation was to compare the mutagenic effectiveness of ethylation and methylation reactions in *Drosophila*. All the monofunctional alkylating agents tested here are mutagenic towards *Drosophila* when treated by an adult feeding method. EMS produced the highest number of lethals giving a 43.6% complete sex-linked recessive lethals. These results confirm the previous observations where these alkylating agents have been tested for their mutagenic activity towards *Drosophila*.[1,3,4,11,12]

During the early period of research into chemically-induced mutagenesis, it was recognised that one notable difference between the genetic effects of chemical mutagens and X-irradiation in *Drosophila* was the relatively high frequency of mosaicism produced after chemical treatment.[5] Alderson[2] has discussed this phenomenon of mosaicism as the co-existence of somatic and/or germinal cells of different genetical constitution (for a mutation) in one and the same organism. Since mosaicism is a well known phenomenon among the progeny from chemically treated germ cells of *Drosophila*,[3,9] a proportion of lethals (mosaics) will be revealed in F3. The result of lethal-mosaicism obtained in the present investigations agree with Alderson's[2] findings where he has reported the induction of germinal mosaicism for sex-linked recessive lethal mutations after treatment of spermatozoa of *Drosophila* by adult feeding of two monofunctional ethylating agents.

The higher efficiency of the ethylation reaction is observed when introduced *via* ethyl methanesulphonate compared with its efficiency when introduced *via* diethyl sulphate (Table 1); and this is particularly noticeable when it is considered that the concentrations used for treatment are 0.15 and 0.5%, respectively. The effect is not at all marked, however, for the actual frequencies of F1 lethal-mosaics generated from these treatments. It should be noted that the F1 lethal-mosaics arising after diethyl sulphate treatment have an average of 3 lethal cultures in F3 sets, whereas the ethyl methanesulphonate treatment has corresponding F3 sets with an average of 7.5 lethal cultures.

The methylation reaction (*via* methyl methanesulphonate) is found to be as good, or better an inducer of mutation than the ethylation reaction (*via* ethyl methanesulphonate, and particularly so when introduced *via* diethyl sulphate). This is particularly clear when it is realised (Tables 1 and 2) that the treatment concentrations used in these experiments are lower for methyl methanesulphonate. In contrast Alderson's[1] experiments on the relative mutagenic efficiencies of the ethylation and methylation reactions on *Drosophila* spermatozoa when introduced *via* diethyl sulphate and dimethyl sulphate, showed ehthylation to be more effective. These two differing results suggest that the methanesulphonate carrier can donate either alkyl group efficiently, whereas the sulphate carrier appears to donate an ethyl group more efficiently than a methyl group.

Administration of the ethylating and methylating agents by an adult male feeding method as used here appears to be more effective than administration by injection into the adult male. For example, the Fahmy's data[11] for adult injection of (1.6×10^{-2}M) ethyl methanesulphonate

yields 18.3% sex-linked recessive lethals in a first brood, whereas adult feeding of 1.2×10^{-2}M EMS yields a first brood with 43.6% sex-linked recessive lethals (Table 1): direct comparison between these results is obviously complicated, since it is not known how much of the reagent is introduced relatively by the two methods. A similar comparison for methyl methanesulphonate can be made with Bateman and Chandley's[12] data for injection of 1.5×10^{-4} mg methanesulphonate (combination of their first 3 single-day broods) which shows a 9.2% sex-linked recessive lethal mutation rate compared with the adult feeding of methyl methanesulphonate which yields a first brood of over 30% sex-linked recessive lethals for 6.8×10^{-3}M concentrations (Table 2): the Fahmy's[11] data for adult injection of 4.5×10^{-1}M methyl methanesulphonate yields 11.6% sex-linked recessive lethals in the first brood.

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