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Two chemical mutagens, ethyl methanesulfonate (EMS) and quinacrine mustard (ICR-100), when injected separately into Oregon-R males in our laboratory, have each induced fourth chromosome

recessive lethal mutations at a frequency approximating 4%. This mutational response of the microchromosome is about four times that produced by X-ray doses of 3 or 4 Kr (Hochman, Gloor and Green, 1964).

The experiments utilizing ICR-100 were performed by Thomas B. McCune (1965, unpub. thesis). When an ICR-100 solution of 0.5% in saline was used, 10 lethal-containing fourth chromosomes were found out of 266 examined (3.75%). The same treatment induced sex-linked lethals at a 12% rate (112 per 924 X chromosomes tested).

Allelism tests of the ten chromosome 4 lethals show that they are situated at only four loci. There were four occurrences at two of the loci and a single mutation at each of the other two sites. The possibility of "clusters" was avoided by discarding the treated males after three days of mating with tester stock females. Moreover, eight injected males each "contributed" one lethal chromosome, while a ninth male was responsible for two of the ten lethals. Since we now know of the existence of more than 25 microchromosomal loci, these findings suggest that the mutational sites of chromosome 4 may vary widely in their sensitivity to ICR-100.

Experiments involving EMS were conducted on a much larger scale than those described above. Thirteen temporally distinct series of Oregon-R males were injected with a 0.05 M solution of EMS in saline. Of 1,640 fourth chromosomes analyzed, 65 (4%) were lethal in the homozygous state. As table I reveals, rather dissimilar mutation frequencies characterize some of the series.

Table I. Chromosome 4 lethals induced by 0.05 M EMS on saline.

Series	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
No. chromosomes tested	248	121	57	82	40	25	120	50	305	83	98	187	224	1,640
Lethal chromosomes	5	3	3	2	0	1	6	0	14	7	5	10	9	65
% lethal	2.0	2.5	5.3	2.4	0	4.0	5.0	0	4.6	8.4	5.1	5.3	4.0	4.0

Although the data cannot be subjected to rigorous statistical treatment, we are confident that much of the variation seen above is due to chance. Nevertheless, mention can be made of other factors that might be responsible for some of the variability noted. Since EMS breaks down rapidly in aqueous surroundings, there may have been differences in the efficacy of the solutions injected at the various times. Secondly, no attempt was made to measure precisely the amount of fluid received by each treated male; injected flies were merely given a dose sufficient to cause a slight distention of the abdomen. Dr. J. L. Epler (pers. comm.) has observed this swelling effect following injections of about 0.5µl. Despite these methodological limitations, it is felt that the 4% mutation rate obtained, being based on 1,640 chromosomes tested, rests on solid ground.

The EMS lethals are currently being tested for allelism inter se and with previously obtained lethal and visible mutants. Results to date indicate that this chemical mutagen has induced genetic changes at some twenty chromosome 4 loci.

References: Hochman, B., H. Gloor and M. M. Green 1964, *Genetica* 35:109-126. McCune, T. B. 1965, M. S. Thesis, The University of Tennessee, Knoxville. (Research supported in part by NSF Research Grant GB 5144.)