Purdom, C. E. and K. F. Dyer. M.R.C. Radiobiological Research Unit, Harwell, England. Spontaneous Mutation Rates.

Spontaneous mutation frequencies from Drosophila males reflect the response of the entire cycle of spermatogenesis to whatever processes are involved. Brood patterns will not reflect any variation in the sensitivity of germ cells, as in the case of induced mutation, but should reveal the rate at which mutations arise spontaneously in the spermatogonial stem cells. Thus, it may be assumed that the difference between sperm from old as opposed to young males lies only in the length of time the cells existed as spermatogonia, and that any difference in mutation frequency may be related to this period of spermatogonial life.

During the past four years much information has been collected on IInd chromosome mutation frequencies from 3- and 21-day old male flies. The data which are shown in Table 1, show that mutation frequency is significantly higher in offspring from the older males ($X^2 = 6.00$ $p = 0.014$). If mutation rate was constant at all germ cell stages the mutation frequency from 21-day old males should be from 2 to 2.5 times the frequency from 3-day old males. This is clearly inconsistent with the data and it must be concluded that mutation rate is not constant and that the stem cells are less sensitive than the later meiotic or maturation stages. This is similar to the pattern discovered for induced mutation and raises the possibility that spontaneous mutation during spermatogenesis mirrors the differential sensitivity pattern observed for radiation induced mutations.

Further experiments were conducted to determine the spontaneous mutation rate in stored sperm in an attempt to compare this with the spermatogonial rate. Male flies derived from an F_1 from two inbred lines were allowed to inseminate Cy/Bll females. These were sampled for mutations by the standard Cy/Bll technique either immediately after insemination or after various periods of storage at 10°C. The summed data of a number of experiments are shown in Table 2.

Contrary to expectation, there was no significant evidence of an increase in mutation frequency with ageing of the male. A significant increase in mutation frequency was observed, however, following storage of spermatozoa. The data of all experiments were fitted to the model: $D(y) = \alpha + \theta_1 x_1 + \theta_2 x_2$ where y is the mutation frequency and x_1 and x_2 the age of the male and duration of sperm storage respectively. The mean value for θ_1, the assumed mutation rate for spermatogonia, was 0.034 ± 0.027% mutations per week, which does not differ significantly from zero. However, individual values showed heterogeneity between experiments and one possible explanation is that a low but positive mutation rate in spermatogonia was obscured in some experiments by exceptionally high mutation frequencies in spermatozoa from early ejaculates. Some evidence does exist for this high initial frequency (1, 2,).

Values for θ_2, the mutation rate in stored spermatozoa, showed no evidence for heterogeneity and gave a mean value of 0.040 ± 0.015% mutations per week. This is much lower than the figure quoted by Muller (3) for sex-linked recessive lethals (0.06% per week) when due allowance is made for the greater length of the IInd chromosome. It is also not significantly different from the mean value for θ_1.

Neither the mutation rate calculated for spermatogonia, nor that for mature spermatozoa was adequate to explain the initial (Brood I unstored) mutation frequency. This suggests that some intermediate germ cell stage is particularly sensitive to the processes involved in spontaneous mutation. Alternatively, the high initial rate may be due to a high incidence of "partial" or mosaic damage amongst spontaneous mutations. Recessive lethal mutations arising in this way would only be revealed after a further operation during which segregation would occur. Further tests on the stored spermatozoa groups suggested that the rate of origin of "partial" damage was about equal to that for "complete" lethals - this was still inadequate to explain the initial high mutation frequency.

References: 1. Lamy, R., J. Genet., 48:223-236, 1947
2. Ives, P. T., Genetics, 48:981-996, 1963
3. Muller, H. J., Proc. 2nd U. N. Int. Conf. Peaceful Used of Atomic Energy, 22: Geneva, 313-320, 1958

Table 1. IInd chromosome recessive lethal mutations from young and old Drosophila males mated in a 3-day brood sequence

Age of male (days)	3	21
Number of tests	10,434	8,370
Number of lethals	34	48
% ± S.E.	0.326 ± 0.056	0.573 ± 0.083

Table 2. IInd chromosome recessive lethal mutation of spontaneous origin in F_1 hybrid Drosophila

Brood		Sperm storage in weeks			
		0	4	6	8
I	Tests	13,017	4,543	927	3,750
	lethals	76	36	11	34
	%	0.58	0.79	1.19	0.91
VI	Tests	7,450	1,072	2,493	1,564
	lethals	45	9	25	15
	%	0.61	0.84	1.00	0.96
XI	Tests	1,829	-	277	1,124
	lethals	12	-	3	4
	%	0.66	-	1.08	0.34

Friedman, L. D. and W. C. Kiriazis. Hiram College, Ohio. Chemically induced viability mutants in D. melanogaster.

Studies are in progress on the relative frequency of chemically induced sex-linked lethal and detrimental mutations and their effect on the viability of D. melanogaster. Tests were made on 3925 X-chromosomes from Basc and Canton-S strains that were treated with the monofunctional alkylating agent ICR 100. The treated males were injected with 0.1% ICR 100 in 0.4% saline. Parents were discarded after 3 days so that the effects measured were on mature sperm. The experimental design and analysis is the same as used previously for similar studies with X-rays (Friedman, 1964, Genetics 49:689-699).

Estimates were made on the proportions of complete lethals and the genetic load of lethals and detrimentals induced.

(1) The complete sex-linked lethal frequency induced by this compound in our experiments has been on the average of about 4.5%. There is no significant difference between the lethal rates induced in the + and Basc chromosomes. This differs from the results obtained with X-rays.

(2) The ratio of the genetic load from non-lethal detrimental mutants to that from lethals was .390. The load is computed as the product of the frequency and the average effect on viability. It is a much higher value than any effect of the same kind that has been established for X-ray. This indicates a much higher detrimental effect in relation to lethals that has been induced by the chemical mutagen in comparison to the effect caused by X-ray.

Further studies are in progress including the determination of the induced mosaic lethal frequency. (This work is supported by U.S. Public Health Service Grant GM 11354.)