COMPARATIVE STUDIES OF CYTOCHROME C OXIDASE ACTIVITY AND MUTABILITY IN TWO STRAINS OF *DROSOPHILA*¹

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COMPARATIVE studies of spontaneous and X-ray-induced mutation rates for a number of strains of *Drosophila melanogaster* show strain differences (DEMEREK 1937; DEMEREK, KAUFMANN and HOOVER 1938). The more recent investigations by STRØMNÆS (1959) on the Oslo and Iso-Amherst strains of this species demonstrate strain differences in the mutation patterns as determined by sequential broods for dominant lethals, sex-linked recessive mutations and 2-3 translocations. STRØMNÆS also compared the differences in rate of induction of mutations in sperm of young males with sperm of older males. The differences in mutation rates and patterns of sensitivity were correlated with the developmental rates of the two strains. Since the purpose of our investigation is the delineation of genetically determined physiological differences that are related to spontaneous and induced mutability, these strains were ideal material for the study.

The experiments of KING, SCHNEIDERMAN and SAX (1952) show an increase in the induced mutation rate when *Tradescantia* is irradiated in the presence of carbon monoxide. HAAS, DUDGEON, CLAYTON and STONE (1954) demonstrated the effect of carbon monoxide on the induction of mutations by irradiation in *Drosophila*. STONE (1956) and DEBUSK, using the poky strain of *Neurospora* which lacks cytochrome oxidase and uses the alternate terminal oxidase, reported the comparative effects of carbon monoxide during irradiation of poky and the normal strain. These experiments indicate that the cytochrome oxidase system plays a role in protection against the mutagenic effects of irradiation. For this reason we chose to do a comparative study of cytochrome c oxidase levels throughout the life cycles of strains exhibiting different mutation rates.

MATERIALS AND METHODS

The Oslo and Iso-Amherst strains of *Drosophila melanogaster* used for these studies were very kindly furnished to us by DR. ØISTEIN STRØMNÆS. DR. STRØMNÆS (1959) indicated that both strains had been in laboratory culture for more than ten years.

Cultures of the Oslo and Iso-Amherst strains were maintained on the standard banana-agar-yeast medium, transfers being made at regular intervals to half-pint

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milk bottles containing fresh medium. The cultures used for these studies were maintained in an American Instrument Company Climate Maker set for a temperature of 25°C and a relative humidity in the range of 55–70 percent. The variation of temperature was no greater than $\pm 0.5^\circ\text{C}$ and usually in the range of $\pm 0.2^\circ\text{C}$. The cultures were maintained at population levels that permitted the collection of large numbers of white pupae or emerging adults at hourly intervals. Prior to enzyme analyses or irradiation, both pupae and adults were placed in small shell vials containing the banana medium and aged in the climate maker under conditions similar to the stock culture.

The determinations of the cytochrome c oxidase activity at various stages of pupal and adult life were made by a spectrophotometric technique similar to the one used by SACKTOR (1950). Ten males were used for each sample. Duplicate paired samples were analysed in each run; duplicate activity determinations were made on each homogenate. Only those runs in which the replicate activity determinations of both strains were satisfactory have been included in the data.

For the enzyme analyses male pupae which had been aged for 6, 24, 48, or 72 hours from the white pupal stage were transferred to a fine mesh screen and washed briefly in distilled water. Each sample of ten males was dried on filter paper and weighed, after which it was transferred to a 5 ml semimicro Vir-Tis homogenizing vessel containing 5 ml of cold sodium taurocholate solution (.2 mg per ml of demineralized water). Pupae were broken with jewelers forceps to facilitate complete homogenization. The samples were homogenized for four minutes and stored in the refrigerator for at least an hour before the spectrophotometric analyses were made. A modification of this procedure was used for adults. Lightly etherized flies were weighed and allowed to recover fully before being transferred to the homogenizing vessel. Adult samples were homogenized for only two minutes.

Prepared cytochrome c from beef heart (type IV) was obtained from the Sigma Chemical Company. The cytochrome c was dissolved in M/15 phosphate buffer, pH 7.4, to a final concentration of 2.86×10^{-5} M. A few crystals of sodium hydrosulfite were added to effect the reduction of the cytochrome c solution; excess sodium hydrosulfite was oxidized by aeration. To 2.5 ml of the reduced cytochrome c solution was added 0.5 ml of the homogenate. Readings of the change in optical density were taken at 15 second intervals after the addition of the homogenate (at a wavelength of 550 $m\mu$ and a slit width of .03 mm) using a Beckman model DU spectrophotometer. A drop of potassium ferricyanide was added to complete the oxidation of the system. The activity was derived from the slope of the line ($\log \text{CyFe}^{++}$) plotted against time as described by SACKTOR (1950). In Table 1 the activity is given per mg of live weight of the sample.

The Muller-5 or "Basc" method for the determination of sex-linked recessive lethal mutations was used to measure the extent of the X-ray-induced damage. Males of each strain were collected at hourly intervals for a period of three hours and irradiated at the median adult age of six hours. The flies were held in gauze capsules which were placed in a lucite chamber maintained at 25°C by circulating water during the irradiation.

TABLE 1

Comparative determinations of cytochrome c oxidase activity on the Oslo and Iso-Amherst strains

Age Stage—Age	Activity per mg		t	Age Stage—Age	Activity per mg		t
Iso-Amherst	Oslo			Iso-Amherst	Oslo		
Pupae 0 day	.145	.060	4.695	Adult 3 days	.446	.420	3.556
	.187	.028			.434	.427	
	.199	.116			.434	.459	
	.084	.031			.428	.471	
	.140	.074					
1 day	.050	.062	5.186	4 days	.434	.419	2.338
	.046	.072			.451	.440	
	.023	.054			.485	.460	
	.056	.093			.466	.425	
	.030	.044			.491	.467	
	.038	.052			.479	.459	
2 days	.054	.101	4.571	5 days	.439	.347	8.695
	.054	.090			.424	.327	
	.052	.065			.427	.350	
	.041	.073			.427	.385	
	.031	.059			.439	.395	
3 days	.149	.179	4.333		.441	.347	
	.102	.172		6 days	.372	.284	3.727
	.050	.160			.306	.260	
	.086	.188			.318	.270	
4 days	.162	.286	4.953		.350	.268	
	.105	.245			.415	.414	
	.205	.238			.424	.400	
	.133	.292			.393	.376	
	.096	.279			.396	.373	
Adult 6 hr	.359	.397	6.000	7 days	.491	.499	6.111
	.302	.371			.472	.511	
	.307	.362			.444	.529	
1 day	.466	.528	5.682		.450	.542	
	.455	.535			.424	.475	
	.375	.434			.434	.512	
	.344	.431			.390	.472	
	.405	.429			.385	.478	
2 days	.435	.491	8.007	8 days	.388	.476	3.864
	.472	.508			.419	.466	
	.466	.514			.419	.464	
					.415	.440	

A 250 kvp Picker industrial X-ray machine was used. In all experiments 250 kvp X-rays, produced by a 15 ma current, were delivered at a rate of approximately 100r per minute to give a 2000r dose. The inherent filtration was equivalent to 1.04 mm of Cu.

After irradiation the males were lightly etherized and individually placed into small vials of food, each containing a single virgin Muller-5 female. Each male was transferred without etherization to a fresh tube containing a virgin female every 24 hours for a period of 11 days. At the end of the 11th day the male was removed and discarded. F_1 heterozygous females were individually mated to Muller-5 males for each of the transfer days 1, 3, 5, 6, 7, 8, 9 and 11. F_1 matings were made from a P_1 male only if all previous tubes in the series had produced at least one offspring, thus indicating at least one copulation each day.

RESULTS

The results of the analyses of the cytochrome c oxidase activity at 24 hour intervals from the white pupal stage through the eighth day of adult life are plotted in Figure 1 and presented in Table 1 with the t value for paired experiments. There is a significant difference at the 5 percent level in all paired tests except those for the fourth day of adult life. The mean values for the activity determinations are plotted in Figure 1. The graph shows that Oslo precedes Iso-Amherst in the cycle of enzyme activity. By the fourth day of pupal life the activity of the former is approximately twice that of the latter. Because the activity is determined on the basis of live weight, it is not possible at present to relate the enzyme activity determinations on the pupae to the activity of the adult stage, except to say that the additional inert weight of the pupa case probably results in the pupal portion of the curve being much lower than it should be. The five hour difference in development time for the two strains (STRØMNÆS

FIGURE 1.—Mean values for the comparative determinations of cytochrome c oxidase activity in the Oslo and Iso-Amherst strains of *D. melanogaster*.

1959), must also be taken into consideration. It may be seen from the graph that the Oslo strain reaches a level of enzyme activity that is much higher than the Iso-Amherst during both pupal and adult life. Possibly the cytochrome oxidase activity of Iso-Amherst reaches a level as high as that of Oslo but it is missed by the determinations at 24 hour intervals.

The results of the comparative determinations of the rate of induction of sex-linked recessive lethal mutations by 2000r of X-ray in the two strains, Oslo and Iso-Amherst are presented in Table 2. Due to the fact that small samples were obtained in some of the more critical days of the period of low fertility, a second run was made some months after the first. Since, using the five percent level of significance, day 3 of Oslo and days 5, 7 and 11 of Iso-Amherst do not show homogeneity, the two runs are presented separately. The peak in the damage cycle for both runs of Oslo occurs in day 6, whereas the peak of damage in the Iso-Amherst strain occurs in the seventh day. The plot of these data is given in Figure 2 showing the five percent limits of error as calculated by the method of STEVENS (1942).

DISCUSSION

That there exist inherent differences between wild strains of *Drosophila* which affect both spontaneous mutability and response to mutagenic agents has been demonstrated by several researchers. In his investigation of *Drosophila* strains of widely separated geographic origins DEMEREC (1937) found differences in spontaneous mutability. A strain from Florida carried a second chromosome recessive factor which was shown to be responsible for its higher degree of genetic instability.

STRØMNÆS (1959) has done extensive comparative studies on the differences in mutational patterns as the result of X-radiation of the Oslo and Iso-Amherst

TABLE 2

A comparison of lethal mutations induced in broods of males of the Oslo and Iso-Amherst strains irradiated at six hours of age

Day		1	3	5	6	7	8	9	11
Oslo									
Lethals									
Total chromosomes tested	Expt. 1	27/411	26/435	37/381	31/217	34/298	15/176	17/284	15/360
	Expt. 2	24/510	17/525	32/531	61/547	50/545	30/472	26/531	10/555
Percent lethals	Expt. 1	6.57	5.98	7.09	14.29	11.41	8.52	5.99	4.17
	Expt. 2	4.71	3.24	6.03	11.15	9.17	6.36	4.90	3.42
Iso-Amherst									
Lethals									
Total chromosomes tested	Expt. 1	14/414	14/397	26/372	14/151	20/97	6/54	8/121	11/197
	Expt. 2	17/519	18/538	18/525	37/541	58/507	47/478	46/536	7/511
Percent lethals	Expt. 1	3.38	3.53	6.99	9.27	20.62	11.11	6.61	5.58
	Expt. 2	3.28	3.35	3.43	6.84	11.44	9.83	8.58	1.37

FIGURE 2.—Comparison of the percentages of sex-linked recessive lethal mutations induced in the Oslo and Iso-Amherst strains of *D. melanogaster* treated with 2000r of X-radiation at six hours of age. The broods for successive days are plotted on the abscissa. The first symbols for each strain represent the first experiment.

strains of *D. melanogaster*, the strains used in our investigation. His experiments show that Oslo has a higher mutation rate for the sperm utilized during the first six days after irradiation of the very young males than does the Iso-Amherst strain. The peak of damage occurs later in Iso-Amherst than in Oslo. Following the damage peak the earlier relationship is reversed.

The results of our investigations of the mutational pattern of sex-linked recessive lethals in the Oslo and Iso-Amherst strains compare very well with the data reported by STRØMNÆS. Since he carried out sex-linked recessive mutation tests only on the progeny from days 3, 6, and 8, we repeated the tests using days 1, 3, 5, 6, 7, 8, 9, 11 in order to obtain a clearer picture of the mutational damage and to determine whether or not it followed the dominant lethal cycle that he reported. In our experiments (Table 1) the data show a peak of damage for Oslo on the sixth day and for Iso-Amherst on day 7. The relationships that he demonstrated for the pattern of dominant lethals holds for sex-linked lethals with the Oslo strain showing the higher mutation rate before the peak of damage which precedes the Iso-Amherst peak; the relationship is reversed after the damage peaks.

The experiment was repeated because of the small sample size on the peak days, particularly in Iso-Amherst where there was an indication that the damage might actually reach a higher level than in the Oslo strain, an important factor to consider. Differences in temperature or other environmental factors may be responsible for the nonhomogeneity of the two runs, however, the patterns are

very similar. The peak of Iso-Amherst damage on the second run is lower than the first, but, day 9 is higher, possibly indicating that sperm from the period of greater sensitivity to radiation were utilized on both days 8 and 9, depressing the level of the measured damage on day 8 and raising it on day 9. Even though the peaks coincide in both experiments, the second run shows lower damage throughout, perhaps due to dosage differences.

The damage patterns are plotted without subtracting the control values which are based on STRØMNÆS' data representing only sperm from three day old males. Since Iso-Amherst has the higher spontaneous mutation rate, subtracting the control value would only serve to emphasize the differences between the mutation rate of the two strains, at least in the earlier stages. MULLER (1946) has shown that spontaneous mutation rates are higher for the first crop of sperm than for sperm from nonvirgin males a week later, thus, the possibility that the level of spontaneous mutation in later stages would be sufficiently high to alter the relationships of these curves seems remote.

In order to work out a feasible explanation of the relationships between the X-ray sensitivity patterns and the cytochrome c oxidase cycles, one must postulate that the determination of the sensitivity relative to the cytochrome system occurs at some stage previous to the time of irradiation, otherwise Oslo cells would be expected to show lower damage than Iso-Amherst throughout the cycle; such is not the case. In addition it must be assumed that the gonadal cells of each strain have the same relationship to the cytochrome c oxidase activity as determined on the whole organisms.

The behavior of the mitochondria, the bearers of the cytochrome system, in at least one form, the centuroid scorpion, is consistent with the hypothesis that the activity level of the cell is fixed in the premeiotic stages. E. B. WILSON (1931) described the division of the mitochondria during maturation in the genus. A single ring mitochondrion is systematically divided during meiosis, parcelling out two mitochondria to each sperm. COOPER (1950) describes the mitochondria of *Drosophila* as being "passively separated into two essentially equivalent groups" in the meiotic divisions. Because the cytochrome system is an integral part of the mitochondria, one would not expect change other than that resulting from the parcelling out of mitochondrial material as a result of the meiotic divisions.

Since the first broods show a higher mutability for Oslo it would seem that the sensitivity of the sperm in the earlier broods was determined at a time when the cytochrome activity was lower for Oslo than for Iso-Amherst. This could be at the time of pupation or perhaps earlier during larval life. The broods after the peak of damage show a reversal in the mutability relationships in both our data and STRØMNÆS' data. This reversal could be correlated with the cytochrome relationship during the later pupal life. One would then be inclined to relate the damage peaks on the sixth and seventh days to the low levels in the cycles in early pupal life. However, this interpretation is inconsistent with the data reported by STRØMNÆS on the comparative studies of mutability of mature sperm in very young males (one to three hours) and in three to four day old males. The irradi-

ated sperm from the young males of Oslo have a higher mutation rate than those from the young Iso-Amherst. The relationship is reversed in the older males. This leads one to the conclusion that factors other than cytochrome changes are responsible for the damage peaks. Actually, the reversal of the sensitivity relationships as determined by cytochrome levels may be occurring before the peak damage periods but be obscured by the action of the other factors.

The investigations of AUERBACH (1954) in which crossover and mutation studies were carried out in the same experiments indicate that the period of peak damage probably corresponds to the spermatid stage; however, that peak occurred in the second brood which includes days 3 through 6, in a mating system in which individual males were mated to three females and transferred at three day intervals. Since the peak of damage in our experiments comes in days 6 and 7, it could be that our mating system is resulting in a less rapid utilization of sperm or that strain and environmental differences are responsible for the later peaks. The more rapidly developing strain reaches the peak of damage before the slower strain does.

If the peak of damage does occur in irradiated spermatids, the high degree of sensitivity of this cell stage may be due to the change in the relationship of the genetic material and the cytochrome oxidase system. COOPER (1950) describes an increase in nuclear volume during the time when the mitochondria are localizing to form the mid-piece. The timing of these events may leave the genetic material without chemical protection before the decrease of volume of the nucleus and the restoration of the close proximity of the genetic material and the protective system.

SCHMID (1961a), in his discussion of possible explanations for the differences in sensitivity of spermatids and mature sperm, argues that the sperm rather than the spermatids are the exceptional cells in relation to radiation sensitivity. He concludes that the sensitivity difference between sperm and other types of cells is due to the decrease in space around the chromosomes. This decrease in space or volume of the nucleus would result in a smaller volume for the formation of the diffusing secondary radiochemical products of ionization, thus resulting in less damage in the sperm than in the spermatid. In his experiments on the effect of the presence and absence of oxygen during CO poisoning, SCHMID (1961b) shows that the protective action of the cytochrome system in *Drosophila* is due to the removal of oxygen by respiration. It would not seem plausible that volume differences would account for the strain differences in radiation sensitivity of mature sperm and for the differences in sensitivity of mature sperm of the young and older males, thus, the level of cytochrome oxidase activity must be determining these more subtle differences in radiation sensitivity.

SUMMARY

A comparative study of the cycles of cytochrome c oxidase activity during pupal and adult life of two strains of *Drosophila* shows that the cycle of enzyme activity in the more rapidly developing strain precedes the slower strain, thus alternating the relationship of the enzyme activity during the time of develop-

ment of the irradiated germ cells. The relationship of the differences in radiation sensitivity of the developing cells to the enzyme cycles is discussed. It is necessary to postulate at least two factors other than the protective effect of the cytochrome system in order to explain the entire sensitivity pattern of the two strains.

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