The Oxygen Effect in Irradiated Mature and Meiotic Germ Cells of *Drosophila melanogaster*

Anoxia has been regarded as a radiation protector[1]. This finding started a series of investigations still in process which have revised the very nature of mutations. Previous workers[2,3] had noticed that irradiated water produces peroxides. It was argued that the cell, being a colloid in water, where the solutes are in small concentrations, should produce peroxides which cause mutations. Soon radiomimetic substances of this type were found[4].

By studying the different stages of spermatogenesis, from meiotic to mature sperm cells, and their mutability under different atmospheric conditions with X rays, we are able to: a) analyze the chromosome behaviour under these conditions as condensed and full with water in meiotic stages, and as uncondensed elements in the resting stage. At these two general periods of development, the chromosomes behaved differently with respect to the mutations induced by the irradiation. b) Furthermore, our data demonstrate the presence of an oxygen effect in meiotic stages only when lower doses are given. This differential response to oxygen is discussed in the light of a chromosome rejoining hypothesis.

Materials and methods. 0–10 h old *D. melanogaster* Oregon R males were irradiated with doses of 150, 300, 600, 1200, and 2400 r an intensity of 200 r/min and 12 mA with a Ghilardoni Therapy X-ray machine using a 4 mm Al. inherent filtration and a Siemens dosimeter. Three days old Basc females were used to facilitate the count of recessive lethals. The males irradiated were the laboratory stock of Oregon R. The F_1 females from the cross of Basc females and wild type males were bred singly with Basc males from the stock cultures, not with their own brothers because of the low fertility of males with irradiated chromosomes[5]. The absence of the wild type males in the F_2 indicated the presence of a lethal in the paternal X chromosome.

The mating scheme was daily crosses of the irradiated males to the virgin females for a period of 10 days. Thus, the males copulate daily to fresh 3 days old Basc females while the female is left another period of several days to lay eggs. The results of the first copulations represent the most mature and old samples of sperms, while the last samples represent the stages which were irradiated as meiotic stages. AUERBACH[6], on the other hand, changed her flies every 3 days in which one male copulated with 3 females, thus disregarding crowding effects upon the developing zygotes; with her method she reaches the stage of highest mutation rate in her second brood. With our method that stage is reached only after the 6th day and even the 9th day after irradiation. This fact points to a perfect or near perfect agreement between ours and AUERBACH's stage of highest mutation rate, as one of her males mating with 3 females releases the amount of sperm in one day that one of ours with a single female releases in 3 days. Since she kept her males mating for 3 consecutive days, in her first brood she reaches the equivalent of our 6th–9th days of mating depending upon a regular sperm ejaculation.

The gases given (O_2 and N_2) are passed directly to a bottle from the gas source. The bottle containing the males treated with the gas is then closed with a plastic cover top which maintains the gas in the bottle without loss during the irradiation time. The gases are given for 45 min before and after the X-ray treatment. The flies are in the same gas during the irradiation. During the pre and post treatment, the gas is allowed to flow through a cylindrical container, where the males remain, into an Erlenmeyer flask where it bubbles at a constant rate. No combination of gases was tried in the present work. The O_2 used was 99.6% pure, the rest were traces of water, N_2 was 99.9% pure and free of oils.

Results. Our results should be considered in the light of previous investigations which attempted to discover the sensitivity to X-rays in *Drosophila* spermatogenesis. The present preliminary report, as well as others here referred to, can get from mature spermatozoa down to the earlier stages of sperm development by using the sperm release successively in a series of copulations of the irradiated males. Thus, we may get to the stage of highest sensitivity (more mutability), amply demonstrated by others to correspond to the meiotic stage. With our daily mating scheme we are able to demonstrate, as shown in Tables I–III, that it is the late meiotic stage which is most sensitive to the different doses of X-rays. That is, the most sensitive period is the one immediately after the mature sperm supply is exhausted. The mature sperm are represented by the first sperm batches which are utilized, and which, according to the extend of the sperm ejaculations (which, by the way, are not uniform), are in store for the first 4–5 days of copulations in the mating conditions here established. The spectrum of mutation rate which we got can be divided in two clear zones: one formed by the most resistant spermatozoa and a second zone, immediately before the last one, formed by the most sensitive products of late meiosis.

Our second type of results consists in the different behaviour which spermatogenesis displayed in the presence of different gases. While oxygen increases the mutation rate, nitrogen serves as a protector from X-rays. The effect of oxygen on the two zones, genetically distinguished by their X-ray sensitivity, was the same. However, the results force us to make a distinction between the oxygen effect at lower doses (150 and 300 r) and its effects at higher doses (600, 1200, and 2400 r). While at lower doses there is no apparent oxygen effect for the stage of mature spermatozoa (the first 5 days of sperm release), although there is a clear oxygen effect in meiotic germ cells (50% + greater mutation rate in O_2 than in the corresponding stage in air or N_2), there is, at higher doses, an oxygen effect in both spermatozoa and meiotic stages.

Discussion. When we irradiate *D. melanogaster* germ cells which are undergoing spermatogenesis, we induce mutations at different stages of sperm development, the nature of which can be analyzed by the results we get from the mutability under certain conditions.

From our results we may discuss the following points concerning the incidence of mutations under certain conditions: a) the sensitivity spectrum observed during the first 10 days after irradiation; b) the effect of oxygen in the mutation frequencies.

The sensitivity spectrum corresponded, counting for the differences in the mating procedures, to the spectrum observed by AUERBACH[6] using crossing over and mutational data. Her peak of highest mutation rate was in the second brood. With a mating scheme of 1 male to 3 females, she quickly reached the sensitive stage because more copulations brought about a greater amount of sperm release than if 1 male is coupled with 1 female every

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Tab. I. Spectrum sensitivity of recessive lethals over a 10 day period of *Drosophila melanogaster* irradiated in air with 150, 300, 600, 1200, and 2400 r at 200 r/min

Dose		Days 1	2	3	4	5	6	7	8	9	10
150 r	X Chromosomes	249	124	252	236	248	270	346	243	337	302
	% Mutations	0.8	0.0	1.2	0.8	0.4	0.0	0.8	0.8	1.8	0.6
300 r	X Chromosomes	212	142	201	218	249	253	283	274	338	266
	% Mutations	1.4	0.0	0.9	1.4	1.2	2.4	2.1	1.4	2.9	1.9
600 r	X Chromosomes	100	126	244	216	253	218	205	222	200	222
	% Mutations	5.0	2.4	1.2	0.9	1.2	3.2	3.9	2.2	3.5	3.1
1200 r	X Chromosomes	219	205	239	216	217	236	246	369	314	276
	% Mutations	2.7	3.9	2.9	6.5	2.3	5.9	5.7	8.1	7.0	8.3
2400 r	X Chromosomes	155	232	276	263	239	142	150	209	124	152
	% Mutations	7.1	7.7	6.1	4.6	7.5	10.5	11.3	12.9	16.9	6.6

Tab. II. Spectrum sensitivity of recessive lethals over a 10 day period of *Drosophila melanogaster* irradiated in N_2 with 150, 300, 600 1200, and 2400 r at 200 r/min

Dose		Days 1	2	3	4	5	6	7	8	9	10
150 r	X Chromosomes	260	273	258	261	268	224	256	241	209	223
	% Mutations	0.0	0.0	0.0	0.0	0.0	0.44	0.4	0.4	0.9	0.4
300 r	X Chromosomes	175	137	181	171	179	188	193	171	226	152
	% Mutations	0.6	1.4	1.1	0.6	1.7	0.0	0.5	0.6	1.3	0.0
600 r	X Chromosomes	291	256	266	272	271	267	266	269	257	190
	% Mutations	1.0	1.2	0.7	1.8	1.1	1.5	2.6	2.6	2.3	1.1
1200 r	X Chromosomes	198	227	263	146	223	179	284	200	249	239
	% Mutations	1.5	1.7	2.6	2.1	2.2	2.2	2.1	4.5	3.2	2.1
2400 r	X Chromosomes	148	261	252	250	224	238	225	154	123	182
	% Mutations	2.7	4.6	5.5	6.8	6.7	4.2	4.8	3.9	12.2	5.5

Tab. III. Spectrum sensitivity of recessive lethals over a 10 day period of *Drosophila melanogaster* irradiated in O_2 with 150, 300, 600, 1200, and 2400 r at 200 r/min

Dose		Days 1	2	3	4	5	6	7	8	9	10
150 r	X Chromosomes	219	215	212	178	231	326	339	209	182	299
	% Mutations	0.0	0.5	1.0	1.0	0.4	2.7	1.7	1.9	4.4	1.3
300 r	X Chromosomes	56	203	214	211	168	135	241	216	142	196
	% Mutations	0.0	0.5	0.9	1.4	2.9	2.2	2.9	3.7	3.5	3.5
600 r	X Chromosomes	90	71	64	37	123	64	63	50	42	24
	% Mutations	1.1	4.2	1.5	8.1	1.6	3.1	3.1	2.0	4.7	0.0
1200 r	X Chromosomes	108	125	115	102	86	92	104	97	27	21
	% Mutations	6.5	5.6	6.9	5.9	5.8	7.6	9.6	4.1	3.7	19.4
2400 r	X Chromosomes	29	93	120	104	90	26	32	33	16	6
	% Mutations	3.4	12.9	10.0	11.6	10.0	0.0	9.4	18.2	6.2	0.0

day, as in our present mating system. With our procedure we reach the highest mutation rate on the 6–9th day. That peak is present in air, N_2 and O_2. Furthermore, we are able to notice that the sensitive zone is exhausted in 2–3 days. From AUERBACH[6], AUERBACH and MOSER[7], and KHISHIN[8] we learn that the meiotic product extends over several days of sperm release, as they used a male to three females for three days per brood, i.e. at least 6–7 days by our system of mating. Therefore, we are inclined to believe that our highest mutation period involved only late meiosis.

It is unnecessary to discuss the negligible influence which germinal selection may have in the sensitivity spectrum. For this purpose we refer the reader to PONTECORVO[9], who relegates the role played by germinal selection to immature spermatogonia.

The effect of oxygen augmenting the effectiveness of X-rays mutagenicity is well established[10]. However, the mechanism by which the oxygen effect takes place is still unknown. Some believe the effect of oxygen is on the primary breakage of the chromosomes at the time of irradiation. This hypothesis is well grounded in experiments by THODAY and READ[11]. Nevertheless, recent results[12–14] of oxygen metabolism acting on the rejoining mechanism of broken ends, reopen the problem.

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In our data on the oxygen-exposed males, before, during and after X irradiation at various doses, we found the oxygen effect present in the lower doses 150 and 300 r but only in the highest mutation rate stage (Table III). We found for the 600 r dose an oxygen effect spread here and there in the mature sperm zone and in the meiotic zone. At highest doses (1200 and 2400 r) the effect is spread throughout the whole spectrum of spermatogenesis studied.

The explanation of this result through the formation of peroxides is untenable, since there was oxygen effect in the anoxic zone of mature spermatozoa at higher doses. On the other hand, if the effect of oxygen was due to differential rejoining of affected chromosomes, then it would be subjected to the dose[12] although it could be independent of the number of breaks induced. Therefore, oxygen affects not the primary breakage but some mechanism which controls the rejoining of broken ends. Work is in process which will attempt to clarify the nature of such a mechanism of damage.

Riassunto. Gli esperimenti qui riferiti sono di due tipi: a) per la determinazione dello spettro di sensibilità di cellule germinali maschili durante le fasi meiotiche e mature (spermatozoi) di *Drosophila melanogaster*, irradiate con 5 differenti dosi di raggi X in condizioni differenti; b) per lo studio dell'effetto dell'ossigeno. Il risultato fu una aumentata mutabilità in O_2, ma non in tutti gli stadi della spermatogenesis, poiche le fasi meiotiche diedero maggiori frequenze di mutazioni recessive che non gli spermatozoi maturi. Quando l'effetto dell'ossigeno si combina con basse dosi di raggi X la mutabilità aumenta solo nelle cellule che si trovano in fasi meiotiche. Questa nuova osservazione viene interpretata come effetto dell'O_2 sulla possibilità di riattacco fra roture piuttosto che come effetto primario sulle rotture stesse.

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The Effect of Fusaric Acid on the Oxidative Phosphorylation of Plant Mitochondria

Fusaric acid (5-butyl-2-picolinic acid) is a toxin produced by *Fusarium lycopersici* Sacc. *in vitro*[1]. It has also been identified in diseased plants infected with this fungus[2] and, therefore, fulfils all the criteria laid down for a vivotoxin[3]. Fusaric acid also shows vivotoxicity in the cotton wilt disease[4].

Fusaric acid exhibits a pleiotropic mode of action[5,6], i.e. it affects the host cells in more than one way. Thus the toxin causes a disturbance in the permeability of the host cells[5,7] and at higher concentrations (10^{-3} *M* to 10^{-2} *M*) inhibits respiration[8]. The inhibition of respiration at higher concentrations is no doubt caused by an inhibition of the cytochrome oxidase system[9]. In diseased plants, however, the concentration of fusaric acid[6] probably does not reach 10^{-4} *M* and there is no significant decrease in the oxygen uptake of the diseased tomato leaves[8,10].

ALLEN[11] had earlier postulated the role of phytotoxins in general as uncouplers of oxidative phosphorylation. Perhaps some evidence in favour of this hypothesis has come from studies where it has been demonstrated that diseased tissues do not respond to the uncoupling effect of 2,4-dinitrophenol to the same extent as the healthy tissues[11–13]. In some instances it has also been demonstrated that inorganic phosphate accumulates during disease development[11]. BACHMANN[14], studying the effect of fusaric acid on the permeability of plant cells, came to the conclusion that the pyridine ring in the fusaric acid molecule is responsible for the inhibition of oxidative phosphorylation and consequently the non-osmotic water uptake mediated by this system.

We have now been able to demonstrate the inhibition of oxidative phosphorylation in isolated plant mitochondria by fusaric acid. Mitochondria were isolated from both tomato hypocotyls[9] and cauliflower buds[15]. For the isolation of tomato mitochondria, surface sterilized seeds of Bonny Best variety were grown on sterilized Vermiculite trays in the dark at 25°C for four days. The etiolated seedlings were harvested and the hypocotyls separated from the cotyledons and the roots. The chilled material was macerated at 2°C and extracted with 5 vol of a buffer

Effect of different concentrations of Fusaric acid on the oxidative phosphorylation and respiration of mitochondria isolated from tomato hypocotyls.

Mitochondria isolated in 0.5 *M* sucrose + 0.066 *M* phosphate + 10^{-3} *M* EDTA, pH 7.0. Warburg vessels contained 20 μM ATP, 100 μM glucose, 2 mg hexokinase, 25 μM K_2HPO_4, 20 μM EDTA, 5 μM $MgSO_4$, 20 μM NaF, 0.33 mg DPN, 0.1 mg cytochrome C, 0.5 ml mitochondria, Fusaric iacid and distilled water to make up a total of 2.8 ml, pH 6.8. Respiration measured for 30 min.

Concentration of inhibitor	Q_{O_2}	μM P esterified/ mg *N*/h	P:O	% inhibition
Control	236	41.6	1.98	—
1×10^{-3} *M* Fusaric acid	152	18.2	1.35	31.2
5×10^{-4} *M* Fusaric acid	200	25.8	1.45	26.0
2.5×10^{-5} *M* Fusaric acid	232	35.0	1.70	13.4

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