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Induction of mutations in D. melanogaster with Deuterium and gamma rays.

Deuterium is a non-radioactive isotope of Hydrogen resulting from a neutron, emerging from an atomic reactor, which is captured by the nucleus of a hydrogen atom. The study of effects of Deuterium interested many biologists for the reason that Hydrogen, being the most common element in biological systems, its substitution may cause drastic effects. Zamenhoff and Demerec (1943) cultured Drosophila melanogaster on food medium containing Deuterium and reported the failure of this isotope to induce lethal mutations. Hughes et al. (1963) reared flies on medium containing various concentrations of Deuterium and observed the ineffectiveness of this isotope to increase the mutation frequency. Konard (1960) failed to induce any forward mutations in Bacteriophage T_4 with 50% Deuterium. However, he did observe a few wild type revertants from r-II mutants which was attributed primarily to base pair substitution. Rosalic de Giovanni (1960) reported that the growth of several strains of E. coli and B. subtilis was inhibited by the presence of Deuterium and the degree of inhibition in each strain was specific. He thought that the mutagenic effect of Deuterium might be due to the replacement of hydrogen by Deuterium in the Deoxyribonucleic acid molecule during its synthesis which might upset the bonding and geometry of the Deoxyribonucleic acid molecule resultimg in replication error or possibly due to the effect of Deuterium on the enzymes involved in Deoxyribonucleic acid synthesis. Usha Purnima (1972) demonstrated the failure of this isotope to induce sex-linked recessive lethal mutations in D. melanogaster by feeding on a medium containing 30% Deuterium. Manohar Rao (1971) reported an increase in mutation frequency with Deuterium in Oryza sativa.

Pollard (1961) raised cultures of E. coli in Hydrogen oxide and Deuterium oxide and irradiated the two cultures simultaneously with gamma rays where no visible alterations were found in bacteria grown in Deuterium. Manohar Rao (1971) observed an increase in the frequency of chlorophyll mutations in Oryza sativa when Deuterium is used in combination with Ethylmethanesulphonate (EMS). The present investigation reports the work carried out to assess whether Deuterium would interact with gamma rays to alter the radiation damage in D. melanogaster.

Oregon-K strain flies were allowed to feed on a normal medium containing 30% Deuterium. The males developed on this medium were exposed to 3000r of gamma rays. A series of experiments as detailed below were conducted and the criteria for assessing the mutations induced was sex-linked recessive lethals.

1) Control;
2) Irradiating the flies with 3000r of gamma rays;
3) Feeding the flies on a medium containing 30% Deuterium;
4) Rearing the flies on a medium containing 30% Deuterium and irradiating the F_1 males with 3000r gamma rays.

Oregon-K males and females were allowed to feed on a medium containing 30% Deuterium. After 72 hours the flies were removed and the eggs laid were allowed to hatch. The emerging F_1 males were divided into two groups. The first group was used for studying the effect of Deuterium. The second group was exposed to a dose of 3000r gamma rays so as to study the interaction if any. The genetic damage induced was studied by screening for sex-linked recessive lethal mutations. To study the differential response of various stages of spermatogenesis, a brood pattern of three days each of six broods was employed. Each treated male was mated with three females of y sc^{S1} In49 sc^{8};bv;st stock. After three days of mating, the females were transferred to fresh vials while the male was transferred to a fresh vial along with three more females. In F_2 the absence of a wild type body colour male is an indication that a sex-linked recessive lethal has been induced.

The Chi-square test has been done to compare the following groups:

1) Control versus flies reared on Deuteriated food; 2) control versus flies exposed to 3000r gamma rays; 3) flies exposed to 3000r gamma rays versus flies reared on a medium containing 30% Deuterium and then exposed to 3000r gamma rays. If the calculated values exceed the chi-square value at 5% level for one degree of freedom the groups compared are taken to be significantly different from each other. The results are presented in Table 1. The results of the statistical analysis are presented in Table 2.

This analysis clearly indicated that Deuterium failed to interact with gamma rays broodwise, whereas there was a statistically significant increase when the total frequencies of all the six broods were considered where flies were exposed to 3000r gamma rays after culturing on Deuterium medium. The chemical basis of interaction between Deuterium and gamma rays is not clear.

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Frequency of heterozygous inversions from a Korean population of D. immigrans.

In October, 1972, a collection of Drosophila was made at Yonsei University Forest, Seoul, at 300 feet. Daily mean temperature in the area was 15°C. In the sample of 369 flies taken, D. immigrans comprised the greatest fraction (62%) and D. bizonata occurred in the next largest numbers (14%). There were eight other species of the same genus present.

The 135 larvae (one/female) of the wild D. immigrans females were examined cytologically for the types and frequencies of inversions and the data are summarized in the table. The notations A, B and C are the same as in Brncic's (1955) data. The result is very similar to that in Japanese populations (Hirumi 1961; Toyofuku 1961), but strikingly different from those reported from other widely separated geographic regions.

Larvae tested		No. of heterozygous inversions observed			No. of inversions per larva (S.E.)
		A	B	C	
135		9	14	1	0.18 ± 0.03
	%	6.7	10.4	0.7	

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References: Brncic, D. 1955, J. Hered. 46:59-63; Hirumi, H. 1961, Jap. J. Genetics 36: 297-305; Toyofuku, Y. 1961, Jap. J. Genetics 36:32-37.

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Table 1. The incidence of sex linked recessive lethals induced in various experiments conducted.

Treatment	Brood A			Brood B			Brood C					
	N	L	%	N	L	%	N	L	%	N	L	%
Control	861	2	0.23	827	4	0.48	874	2	0.23			
30% Deuterium	214	4	1.86	440	1	0.22	500	1	0.20			
3000r γ rays	763	23	3.01	240	14	5.83	348	7	2.01			
30% Deuterium & 3000r γ rays	741	31	4.01	485	28	5.77	323	7	2.1			

	Brood D			Brood E			Brood F			Total		
	891	4	0.45	764	-	-	783	2	0.25	5005	14	0.27
	212	1	0.47	99	-	-	15	-	-	1480	7	0.47
	955	11	1.15	883	5	0.57	582	1	0.27	3771	61	1.61
	519	15	2.8	557	1	0.18	338	2	0.59	2963	84	2.83

N = Total number of X-chromosomes scored
L = Total number of lethals recorded

Table 2. χ^2 values for the differences in sex linked recessive lethal frequency for the groups compared.

Group	BROODS						
	A	B	C	D	E	F	Total
Control vs 30% Deuterium	5.58	1.35	0.40	0.28	-	-	1.32
Control vs 3000r γrays	20.01	17.4	8.51	1.93	2.67	0.83	45.43
3000r γrays vs 30% Deuterium & 3000r γrays	1.48	0.0	0.21	5.28	2.33	0.23	13.40

References: Hughes, A.N., E.H. Phillip and G.C. Becker 1963, Genetics 49:715; Konard, M 1960, Ann. N.Y. Acad. Sci. 84:678; Manohar Rao, D. 1971, M.S. Disser., Osmania University; Pollard, E. 1961, Nature 192:177; Rosalic de Giovanni 1960, Ann. N.Y. Acad. Sci. 84:644; Usha Purnima 1972, M.S. Disser., Osmania University; Zamenhoff, F.C. and M. Demerec 1943, Amer. Nat. 77:380.