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Final Technical Report

This three year project was initiated at the end of 1987 and confirmed until recently through no-cost extension. The research involved genetic effects of alpha radiation. It was not horrible to use plutonium residue to recent stringent safety regulations and therefore the alpha source of radon gas at Oak Ridge National Laboratory and helium ions at the BEVALAC of the Lawrence Berkeley Laboratory was used.

There was no Drosophila Laboratory at the A&M . During the first year a new Drosophila laboratory was established by renovating space and by purchasing necessary equipment, as described in the first annual report.

As the equipment and supplies were purchased, the technician was trained in Drosophila methods such as medium preparation, sterilizations, dispensing, handling of flies, microscopy etc. This followed identification of mutants, and participation in actual experimentation.

Two of the several experiments described in the original proposal were performed. These were undertaken to accurately determine the spontaneous rate of mutations in the Canton-S stock of Drosophila. These data are necessary to compare the mutation frequencies with those which will be obtained in radiation experiments consisting of exposures to plutonium radionuclide and alpha beams at the BEVALAC.

Canton-S females were allowed to lay eggs for four hours in bottles. After eclosion of emerging males, 11 days after laying, males of uniform age were collected within two hours after hatching. During this period, several hundreds of virgin females

belonging to the stock Basc or M5 were collected and stored at 18°C. Six broods were cultured for spontaneous mutation experiments. This was done so that differential radiosensitivity in different germ cell stages can be compared to the comparable broods from spontaneous experiments. The males of uniform age were serialized mated individually to virgins in bottles for two days. After this 2-day period, the male was removed and the females were allowed to lay eggs for the next 6 days in the same bottles. The male removed from the first harem, was then mated to another set of 10 virgins for the next two days for the second brood. This type of brooding was continued for 12 days to obtain six successive broods which represent respectively, spermatozoa, spermatids, spermatocytes and spermatogonia.

The F1 progeny was observed to confirm that all males and females had bar eyes and that females also have red pigment. These F1 individuals were used to make several hundred pairmatings in each brood. After 16 days these pair matings were examined (scored) for the presence or absence of red dyed males. The absence of red eyed males indicated a lethal induced in the treated male due to radiation or spontaneous reasons, as the case may be. The suspected lethal mutation vial was used to make more pair matings for scoring in the third generation. As can be seen from the following data, the spontaneous mutation frequency in this stock is very low, less than 0.1%.

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Brood	Expt. I Lethal	Total	Expt. II Lethal	Total
1	1	591	1	1004
2	1	588	1	601
3	1	707	2	1254
4	0	580	1	1131
5	0	536	1	1031
6	1	528	1	920
7	0	583	0	763
8	0	482	1	586
9	0	362	0	616
10	1	212	0	174
11	0	180	0	534
12	0	388	0	233
13			0	212
14			0	208
15			1	376
Totals	5	5737	8	9643
%	0.087		0.082	

We have thus observed that, the spontaneous mutation rate in our stock is very low and does not vary from one experiment to another. The stocks are therefore quite suitable for comparisons with similar data from experiments with plutonium or alpha radiation.

Radiation induced sex linked recessive lethal mutation experiments were initiated in the second year, using the radiation source at the BEVALAC accelerator of the Lawrence Berkeley Laboratory. Six visits were made to Berkeley according to the schedule provided by the biomedical scheduling committee of the BEVALAC. Radiation energies during the visits and treatments varied from 600 MeV to 850 MeV for different experiments. Most of these visits and experiments were funded by NASA, except those

with helium ions which was the source of alpha radiation which was funded by the Department of Energy.

Drosophila males of uniform age (Canton-S type) were collected within four hours of eclosion in the genetics laboratory of the biology department of the A&M University at Huntsville. These males were carried in glass tubes to California at the BEVALAC. The males to be treated were kept in gelatin capsules for radiation exposures. the capsules were attached to the target plexiglass plate which was placed in the beam path in the biomedical cave. The middle of the plate was focused with a laser beam and the capsules were placed at the spot centered by the beam.

The exposure period was short for all the experiments. A time period of 15 seconds to 1 minute was enough to obtain a dose range of 20 to 360 R. The dosimeter was performed by the biomedical personnel of BEVALAC. The energies and the type of radiation varied at each visit as scheduled.

Immediately after the irradiation, the treated males were mated individually to six M5 (BASC) virgins collected and brought from the A&M at Huntsville. These matings were brought to Huntsville for all further experimentation. Later brooding continued with individual males and 6 females per female. Each brood was a 2-day brood. Six such broods were cultured to obtain information on mutation induction in spermatozoa, spermatids, spermatocytes and the spermatogonia. After twelve days of matings, the treated males were discarded.

The inseminated females were allowed to lay eggs for next 4 to 6 days in bottles for all broods from each individual numbered male. The F1 progeny from these females was observed to obtain red bar females and apricot bar males. Any contaminant bottle was not used. This progeny was then pair mated in vials. Nearly 2000 pair matings were made for all radiations. In addition for low doses, the number was increased to 4000 since lesser number of mutations were expected at these low doses.

The observations or the scoring of the F2 cultures was started 16 days after culturing. Each vial was observed for the presence or absence of red eyed males. Absence of such males indicated a lethal mutation induced by the radiation in the treated grandfather. All suspect mutations were confirmed by continuing the matings for one more generation. Vials without red dyed females were not counted as mutants and in total progeny scored.

The data from the different doses and different broods are given in table 1. As can be seen from the table, even the doses as low as 20 R induced significant number of mutations. Higher numbers of mutations were recovered at higher doses. The dose frequency response appears linear for spermatozoa and for spermatids. The earlier two germ cell stages, i.e., spermatocytes and spermatogonia did not show a linear response since the mutation frequencies decline at higher doses. This can be attributed to the saturation effect due to the higher radiosensitivity to cell killing for these germ cell stages. Nevertheless, significant number of mutations have been recovered even at low

In the third and last year of the project, Alpha radiation from radon gas was used. The radon facility at Oak Ridge National Laboratory was available when in-house radon experiments by laboratory scientists were not running. The facility is directed by Dr. Charles Dudney of the health research division of the ORNL, who was also responsible for the dose measurements. With the highest possible radon concentration, the maximum available dose of alpha radiation was quite low. It was 2900 counts per minute or 300 pCi/L. Therefore longer and chronic exposures were performed.

A one liter glass jar with 2 inch high medium was put in a large desiccator which was housed in the safety hood of the radon room. The radon gas passed through a measuring device before entering the desiccator and the exposure jar.

A large number of Canton-S females of Drosophila melanogaster were allowed to lay eggs for 6 hours on the medium in the exposure jar. In addition to the eggs about 0 Drosophila males were also kept in the exposure jar. The jar was then exposed to radon gas continuously for five days for the first experiment and for eight days for the second experiment. The alpha dose received by Drosophila was 30 and 43 rem respectively.

Treated individuals were brought to A&M laboratory for further experimentation. The methods for brooding, pairmatings, scoring etc. were the same as described earlier. Brooding was performed for eight broods or sixteen days after the treatment. The results are given in the table.

As can be seen from the data in this table, alpha radiation from radon gas induces significantly higher number of mutations than the control. We were unable to

estimate the RBE values since identical data from the low LET radiation are not available. However if the x-ray induced mutation frequencies as reported in the literature are used for comparison, it can be concluded that alpha radiation from radon gas induces at least two to three times more mutations to Drosophila.

TABLE I. Induced Sex-Linked Recessive Lethal Mutation by Neon - 640 MeV

Germ Cell Stage	Dose (rads) 20R			40R			80R			160R		
	L	T	%	L	T	%	L	T	%	L	T	%
B_1 (Spermatozoa)	2	1105	0.18	2	888	0.23	5	1136	0.44	3	635	0.47
B_2 (Spermatids)	10	1148	0.87	2	782	0.26	0	454	0. 00			
B_3 (Spermatids)	5	782	0.64	15	895	1.68	4	773	0.52	7	1135	0.62
B_4 (Spermatocytes)	6	914	0.66	24	871	2.76	3	694	0.43	4	440	0.91
B_5 (Spermatocytes)	2	575	0.35	10	876	1.14	12	334	4.00	4	570	0.70
B_6 (Spermatogonia)	1	632	0.16	9	725	1.24	0	560	0.00	3	519	0.58

L= lethal
T= chromosomes total

Sex-linked recessive lethal mutations induced by alpha radiation from gaseous radon at an approximate dose of 40 R.

Male No.	Brood 1		Brood 2		Brood 3		Brood 4		Brood 5		Brood 6		Brood 7		Brood 8		Total	
	L	N	L	N	L	N	L	N	L	N	L	N	L	N	L	N	L	N
1	0	31							0	88	0	67	0	11			0	197
2	1	32	0	49													1	81
3	0	29	0	65			0	36	0	73			0	56			0	259
4	0	14	0	42					0	142							0	198
5	0	70	0	50													0	120
6	0	46	0	42													0	88
7																		
8	0	44	0	43			0	33	0	62	1	98	0	36			1	316
9	0	43	1	44			0	38	0	56	2	90	0	49			3	320
10	0	96					0	24	0	36	0	97	0	23			0	276
11	0	63	0	66			0	21	0	49			0	19			0	218
12	0	25	1	62	0	79	0	6	0	67	1	65					2	304
13	0	55	0	46			0	26	0	68			0	17			0	212
14	0	55	0	73	0	9	1	18	0	54	0	67	0	62			1	338
15				0	32				0	102?	0	44					0	178
16	0	21	0	23			0	24	0	90	0	76	1	74			1	218
17	0	59	0	46			0	30	0	41	0	81	0	53			0	310
18	1	60															1	60

2 pm 7/26/94 to 3 pm 8/13/94

Male No.	Brood 1		Brood 2		Brood 3		Brood 4		Brood 5		Brood 6		Brood 7		Brood 8		Total	
	L	N	L	N	L	N	L	N	L	N	L	N	L	N	L	N	L	N
19	0	70	0	50													0	120
20	0	31			1	53			1	37	0	55	0	48			2	224
21			0	46			1	59	0	54	0	11					0	167
22																		
23			0	48			0	30	0	88	0	53	0	39			0	258
24	1	52	0	45	0	44	0	29	0	81	0	29					1	280
25	0	36			0	71	0	37	0	5							0	149
26	1	62	0	44					1	38	0	30	0	9			2	183
27			0	24	0	40	0	8	0	22	0	35	0	19			0	148
28	0	43	0	57													0	100
29	2	82															2	82
30	0	54	0	46			0	11	0	28	0	44					0	183
31	0	52	0	59			0	18	0	64	0	92	0	33			0	318
32	1	82			0	16											1	98
33	0	27	0	55			0	24									0	106
34																		
35																		
36																		

Male No.	Brood 1		Brood 2		Brood 3		Brood 4		Brood 5		Brood 6		Brood 7		Brood 8		Total	
	L	N	L	N	L	N	L	N	L	N	L	N	L	N	L	N	L	N
37																		
38			0	69													0	69
39							0	65	1	52	0	60	0	39			1	216
40	1	72	0	55			0	35					0	0			1	162
41	0	32			0	46	0	40	0	55							0	173
42	0	32			0	27	0	27			0	24	0	28			0	138
43	0	51															0	51
44																		
45	0	50			0	37											0	87
46			0	41			0	28	1	98	0	8	0	50			1	225
47	0	27	0	45			0	82	0	47	0	86	0	26			0	313
48			1	34			0	17									1	51
49			0	61			0	2	0	51	0	6	0	20			0	140
50	0	67	0	67			0	73	0	28	0	92	0	50			0	377
51	0	99	0	54			0	76									0	229
52	1	46	0	56													1	102
53																		
54																		

Male No.	Brood 1		Brood 2		Brood 3		Brood 4		Brood 5		Brood 6		Brood 7		Brood 8		Total	
	L	N	L	N	L	N	L	N	L	N	L	N	L	N	L	N	L	N
55																		
56	0	66			0	30											0	96
57			0	46			0	59									0	105
																	24	8643

0.277%