MUTATION STUDIES WITH *DROSOPHILA MELANOGASTER* EXPOSED TO SELECTED GASES AND IONIZING RADIATION

A DISSERTATION

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INTRODUCTION

Studies reported in this dissertation were designed to detect mutagenic effects induced by specific gases on the genome of exposed Drosophila melanogaster males. In some experiments a combination of gas treatments, and Co-60 irradiation, were investigated with respect to their effect on the Drosophila test strains.

After preliminary consideration of toxicity and other factors, the gases studied were selected as being representative chemical substances that may occur in the atmosphere of some urban environments. No reports could be found in the literature regarding studies with the hydrocarbons that were tested on Drosophila melanogaster.

The gaseous hydrocarbons chosen for this investigation were the completely fluorinated perfluorocyclobutane (Freon-C318), perfluoro-2-butene; and two partially fluorinated gases, fluoroform (Genetron-23) and 1,1-difluoroethane (Genetron-152A). Nitrous oxide and sulfur dioxide were the inorganic gases selected for study.

Numerous reports of deleterious effects of urban atmospheric environments have appeared in the literature (Stern, 1962, 1968; Hagen-Smit, 1964; Hagen-Smit and Wayne, 1968; Loeb, Svedberg, and Martin, 1967). Most research in

this area has been concerned with damage produced by air pollutants on plants, and their adverse influence on the health and well being of animals, including man (Thomas, 1961, 1965; Dubos, 1965; Edwards, 1965; Bradshaw, McNeilly, and Gregory, 1965).

Cockroft (1967) stated that biological risks due to nuclear power for industrial use are acceptable, when they are considered in relation to other hazards to life and health, such as the inhalation of sulfur dioxide resulting from the combustion of coal and oil.

At present the United States government supports programs that monitor atmospheric environments mainly to determine acute toxicity; some of the criteria used are degrees of eye irritation, respiratory symptoms, and nausea induced in humans (Stern, 1968). The only gases regularly reported on by the United States Public Health Service are carbon monoxide, sulfur dioxide, methane, olefins, and the oxides of nitrogen.

The Air Conservation Commission of the American Association for the Advancement of Science (1965) published a list of pollutants including fluorides as well as radioactive substances in the air; it included citations of their actions and a lengthy discussion. This same report suggested that exposure to air pollutants may have effects on health-that will not appear until many years later.

Goldsmith (1960) considered allergic reactions and other respiratory disorders which may be enhanced in sensitive individuals by exposure to polluted air.

The presence of nitrous oxide at about 0.5 ppm in the normal atmosphere has been reported by Kuiper (1952), and its conversion from the upper atmosphere to nitric acid in the lower layers has been described by Hagen-Smit and Wayne (1968).

Sources of air pollution due to fluorides were listed by Stokinger and Coffin (1968). They stated that fluorides in the air result from the production of phosphate fertilizers, the manufacturing of aluminum, the leaking of fluorinated hydrocarbon refrigerants into the atmosphere, and the widespread use of aerosol propellants in insecticides and hairsprays.

Brandt and Heck (1968) believe that fluorides act as cumulative poisons in plants, and Stokinger and Coffin (1968) cited the occurrence of delayed manifestations of fluoride accumulations in animals.

According to Hamilton (1963) most fluorocarbons have gained acceptance because of their apparent low toxicity. Reviewing air control criteria, Grundy (1968) stated that the gaseous fluorides and hydrocarbons along with carbon monoxide and mercaptans require special study, especially with respect to genetic damage to man.

Carr (1965) cited the radiation-mimetic effect of certain gases with free radicals found in smog. Muller (1961) suggested that certain air pollutants could be mutagenic. It is indeed surprising that in Braun's (1965) extensive list of mutagenic agents, not a single gaseous compound was included. Herskowitz (1963), in a comprehensive list of chemical substances used in mutation studies on Drosophila, lists only a few gases among the 3,305 references in the bibliography. No air pollutants are mentioned nor are any fluorinated hydrocarbons, although over 100 indexed references to insecticides and their effects on Drosophila are given.

Muller and Mott-Smith (1930) have calculated that only about 1/2,000 of the spontaneous mutations in Drosophila could be ascribed to natural background radiation; this leaves 1,999/2,000 of the spontaneous mutations to be explained. Muller (1961) later discussed the possibility that air pollutants act as mutagens. Drake (1969) speculated that the human population, with its generally low numbers of offspring, might be particularly sensitive to increases in its mutation rate, such as may result from chemical modifications of the environment.

For the first time in history man has the ability to control the hazards and benefits of his environment (Wolfe, 1963). However, the problems in setting and enforcing acceptable standards for the quality of air in urban areas are

complicated because they involve technical, political, economic, and administrative, as well as scientific factors. These various facets have been discussed by Stern (1968), Schueneman (1968), and Middleton (1968). Dubos (1969) feared human ecology may be given low scientific priority by university biological disciplines, although he stated that it should be a lively research field.

It is recognized that studies with air pollutant gases are currently taking place at numerous research centers, including Texas A. & M. University. However, the studies in this dissertation were based on the work of Landry and Fuerst (1968) showing the effects of some gaseous compounds on Escherichia coli. The results of these investigations indicated the need for further research with gases on other organisms.

Six of the gases investigated by Landry (1968) were selected for studies with Drosophila. This organism was chosen for the dissertation research because the Drosophila chromosomes have been well mapped and marker strains and specialized techniques have been developed, so that mutagenic effects may be accurately determined and compared with well established control frequencies. Inasmuch as results of single gene differences can be very complex, the use of suitable organisms for genetic studies has great importance.

Over the past fifty-five years an enormous amount of information has been gained from genetic research with Drosophila. No attempt is made here to cover this vast

literature; only publications considered pertinent will be cited.

Muller and Altenburg (1919) began experiments on mutation frequencies as early as 1918. After the discovery of the mutational effects of high energy radiation (Muller, 1927; Stadler, 1928), intensified research efforts were directed toward the production of induced genetic alterations.

Altenburg (1933) produced mutations in D. melanogaster by treating polar caps of fertilized eggs with ultra-violet rays and concluded that UV probably has less destructive effects on chromosomes than X-rays. Later on it was demonstrated that the mutagenic action of different wave lengths of UV was proportional to its adsorption by the DNA of the chromosomes (Stadler, 1942; Hollaender and Emmons, 1946).

Auerbach and Robson (1946) and Rapoport (1946) discussed for the first time chemical mutagenesis in Drosophila. The chemical mutagens reported by these workers were mustard gas and formaldehyde.

With respect to spermatogenesis in the treated male, the time that a mutagen is introduced has a bearing on the results obtained. Auerbach and Woolf (1960) found that formaldehyde, itself, had no effect on the mutation rate in the adult Drosophila, but mutations were induced if this chemical was added to the larval food. Alderson (1961) then made the discovery that the food had to contain adenosine or adenylic acid or the formaldehyde was not mutagenic.

There may also be some evidence that the cell produces its own mutations by certain of its products of metabolism reacting with its genetic material. Wyss, Stone, and Clark (1948) and Jensen et al. (1951), in experiments with catalase and inhibitors of catalase, obtained indirect evidence that hydrogen peroxide, and other peroxides produced as the result of aerobic respiration, may be a factor in determining part of the spontaneous mutation rate.

Fuerst (1948) and Wagner et al. (1950) reported that catalase, added with hydrogen peroxide to Neurospora conidia, blocked the activity of the peroxide. If catalase poisons, such as cyanide or azide, were added with hydrogen peroxide, the mutagenic activity increased in Neurospora.

Sparrow (1951) stated that over 100 chemicals had been demonstrated to influence the mutagenicity of X-rays, and Mitchell (1960) enumerated all radiosensitizers of a chemical nature. The fact that mutations occur more frequently at one stage of the life cycle of an organism than at another, indicates that the physiological state has a bearing on mutation rate. As differentiation goes on in an organism, different metabolic conditions exist, and at some stages mutagenic chemicals may be present in sufficient concentrations to effect the genetic material, while under other conditions the mutation threshold might not be reached (De Robertis, Nowinski, and Saez, 1965).

Kihlman (1958) found that if respiration is blocked by a respiratory poison in Vicia faba, small amounts of oxygen may cause a strong enhancement of radiation effects. Schmid (1961) confirmed this finding in Drosophila. He found over 50% more lethal damage produced in flies after exposure to 1,000 R X-radiation in an atmosphere of 2% oxygen in carbon monoxide, than when carbon monoxide alone was used.

Earlier studies (Haas et al., 1954; Stone et al., 1954; Chang, Wilson, and Stone, 1959) had variously investigated the modifying influence of carbon monoxide, as well as several inert gases, on the extent of radiation induced damage in Drosophila.

Schmid (1961), confirming previous work by Chang et al. (1959) and Lüning (1954), again demonstrated that if irradiation is performed in an inert gas, such as nitrogen or helium, and at room temperature, an addition of up to 3% oxygen shows almost no enhancing effect of genetic damage produced. Schmid (1961) stated that in the normal cell, oxygen is removed by respiration before it reaches the chromosomes within the nucleus, but as soon as cellular respiration is blocked, by carbon monoxide or cyanide, the cytochrome oxidase removal of oxygen stops and small amounts of oxygen, not removed by cellular respiration, may enhance the radiation effect.

Sobels et al. (1967) reported a reduction of mutation and translocation frequencies in spermatozoa when Drosophila

males were exposed to high doses of X-radiation under anoxia, and then post-treated with nitrogen. Thus, it was demonstrated for sperm, that post-radiation interaction of radicals with oxygen could not explain the reduction in mutation and translocation frequency. These results were considered an indication of enzymatic repair of potential lesions. Early spermatids, post-treated with oxygen, produced the same effect of a reduction in translocation and mutation frequencies; in spermatids, oxygen was necessary for the repair. More repair was observed for lower rather than for higher radiation doses, which suggested that the repair mechanism might become saturated at higher doses. As presently understood, this repair mechanism differs during various stages of spermatogenesis, suggesting that RNA and RNA-inhibitors are involved (Sobel et al., 1967).

Evans (1967) presented the idea that chromosomal aberations, in plants and animals, may be considered the result of a mis-repair process, which probably takes place over a period of time and in several steps. He further suggested that the actions of certain chemical agents in the cell, as well as spontaneous events of exchange at mitosis or meiosis, might all be basically similar events, and use the same pathways in the cell for repair.

Alexander (1967) found a high incidence of mosaic mutations in the F_2 generation when mature Drosophila had been treated with ethylenimine, and that these mutations

continued to arise for two generations after the chemical treatment. Paik (1968) further investigated the actions of ethylenimine on mature sperm and confirmed the finding. Earlier, Mathew (1964) and Carlson and Southin (1963) had reported similar results using different chemicals. This production of mosaic mutations is not characteristic of ionizing radiations (Altenburg and Browning, 1961, 1964).

Searle (1967) pointed out that while much information in radiation genetics is being gained by current research with mice, it is often at variance with similar data obtained in Drosophila research. This lack of uniformity makes it difficult to apply results from other studies to man, because it is not known whether unexpected results, for example in the mouse, are peculiar to this rodent, or to some, or to all mammals.

Since it is now understood that diverse chemicals can be equally effective mutagens, the testing of new compounds may lead to a better interpretation of mutagenic specificity. Auerbach (1960) recommended that search for further mutagens should be stimulated.

As more and more chemicals are used in therapeutics, food processing, and other industries, the testing of these substances will have to become a necessary protective measure (Auerbach, 1960). In the same publication, Auerbach (1960) stated her belief that Drosophila recessive lethals

are still the best tool for the detection of mutagenic ability in new compounds.

The research undertaken and described in this dissertation was designed to determine if each selected gas studied is mutagenic when administered alone to Drosophila melanogaster, and to further ascertain if each gas, under the experimental conditions delineated, is either protective or enhancing when Co-60 irradiation is given to the organism in the gaseous atmosphere.

MATERIALS AND METHODS

Strains of organisms used. Four special stocks of Drosophila melanogaster were selected for this research. Two of them, Canton-Special and Muller-5, were obtained from the Rice University Laboratory of Dr. Edgar Altenburg in Houston, Texas. The other two strains used, Muller's Maxy and Muller's Maxy-v, were from the Mid-America Stock Center, maintained by Dr. Irwin Oster, Bowling Green State University, Bowling Green, Ohio.

Care of the organism. All Drosophila cultures were maintained under standard laboratory conditions in an air-conditioned room at about 22 C. The medium used contained:

Corn meal	125.0 g
Brewer's Yeast	25.0 g
Bacto-Agar	20.0 g
Molasses (unsulphured)	125.0 ml
Propionic Acid	11.5 ml
Cold Water	1,223.0 ml

The dry ingredients were mixed and the molasses added. Water was stirred into the mixture and the medium was cooked for 30 min at moderate heat and agitated so that it did not stick. After cooking, propionic acid was added and the medium boiled, and then poured into shell vials (25 mm by 95 mm) to 13/16" depth. One drop of live Fleischmann dry yeast suspension in water, of the consistency of thick

cream, was added per vial, and the vials cotton plugged and tilted. When half-pint bottles were used the food was poured 3/4" deep, and three drops of live yeast suspension added per bottle.

Genetic techniques. Two special genetic techniques were employed in these studies, one for the detection of visible mutations, and the other one for recessive lethal mutations. The Basc technique, using Muller-5 females and wild type males (Spencer and Stern, 1948), was used for scoring recessive lethal mutations. It was designed to discover sex linked recessive lethal mutations that arise in the germ line of the treated paternal male.

In the studies described in this dissertation, the males subjected to treatment by gas and/or irradiation, were normal males of the Canton-Special stock, having all wild type characteristics. They were mated to females homozygous for Bar eye (B), apricot eye color (apr), and for a paracentric inversion (InS) of the left arm of the X chromosome. This inversion has a left breaking point at sc^{sl} and a right one at sc^{8} and is therefore called the scute inversion. The mutations mentioned are cataloged by Lindsley and Grell (1967). The stock bearing the above mentioned markers is known as the Muller-5 stock, and is phenotypically characterized by narrow Bar eyes of apricot color.

The breeding pattern followed is shown in Figure 1. It can be noted that the Muller-5 female mated to the

Figure 1--Breeding scheme used in the Basc technique when P_1 Canton-S males were treated with gas and/or radiation and mated with Muller-5 females. The F_1 heterozygous females were individually mated to two sibling males and cultured in vials.

P_1 Generation

Muller-5 ♀			Canton-Special ♂*
Basc chromosome	sc^{sl} B InS apr sc^{8}	X	+ + + + + Normal chromosome
Basc chromosome	sc^{sl} B InS apr sc^{8}		Normal Y chromosome
Phenotype: narrow Bar eyes of apricot color			Phenotype: normal

F_1 Generation

Heterozygous ♀			Basc ♂
Basc chromosome	sc^{sl} B InS apr sc^{8}	X**	sc^{sl} B InS apr sc^{8} Basc chromosome
Normal chromosome	+ + + + +		Normal Y chromosome
Phenotype: normal except wide Bar eyes			Phenotype: narrow Bar eyes of apricot color

*These were the males treated with gas alone or with gas and Co-60 irradiation while in the gaseous atmosphere.

**The F_1 females were individually cultured in glass vials when mated to the F_1 males.

Canton-S male produces females that are heterozygous for the X chromosome. If crossing over occurs, the results are acentric and dicentric X chromosomes, because of the inversion (InS) on the maternal X. Since all F_1 males are Basc, the heterozygous F_1 female, mated with her brother, produces sons which are either phenotypically normal or Basc, as shown in Figure 2. Each normal son bears the X chromosome of the male subjected to the gas and/or irradiation treatment. When a lethal mutation occurs on this X chromosome, the F_2 culture lacks normal males. Of course in this technique, any dominant visible mutations will be observed in the F_1 generation.

Muller's Maxy stock (Muller, 1954, 1955) was used for detecting recessive visible mutations in the F_1 female. This stock, later modified (Schalet, 1958; Muller and Schalet, 1961), now enables the detection of 15 well-studied mutations on the X chromosome of the F_1 female. Table 1 lists the abbreviations of the 15 gene symbols and explains their meanings. These mutations are described by Lindsley and Grell (1967).

The Maxy technique may be explained with the aid of Figure 3. When the phenotypically normal Maxy female is crossed to the treated B, v, oc male, the regular eggs and sperm combine to produce only two types of viable zygotes, because of the system of balanced recessive lethal genes (lJl or +), which may be noted in Figure 3, on the extreme

Figure 2--Genotype and phenotype of the four types of flies produced in the F_2 generation in the Basc technique. The normal F_2 male is absent from any culture, if the P_1 X chromosome of the Canton-S male, contributed to the F_1 female (shown in Figure 1), contained a recessive lethal gene.

F_2 Generation

Heterozygous ♀		Normal ♂*	
Basc chromosome	$\underline{sc^{sl}\ B\ InS\ apr\ sc^{8}}$	$\underline{+\quad +\quad +\quad +\quad +}$	Normal chromosome
Normal chromosome	$\underline{+\quad +\quad +\quad +\quad +}$	———	Normal $\underline{Y}$ chromosome
Phenotype:	normal except wide Bar eyes	Phenotype:	normal

Basc ♀		Basc ♂	
Basc chromosome	$\underline{sc^{sl}\ B\ InS\ apr\ sc^{8}}$	$\underline{sc^{sl}\ B\ InS\ apr\ sc^{8}}$	Basc chromosome
Basc chromosome	$\underline{sc^{sl}\ B\ InS\ apr\ sc^{8}}$	———	Normal $\underline{Y}$ chromosome
Phenotype:	narrow Bar eyes of apricot color	Phenotype:	narrow Bar eyes of apricot color

*These normal males are absent in the F_2 generation if a recessive lethal mutation has occurred in the gas treated or gas and Co-60 treated P_1 male.

Table 1--Gene symbols and phenotypes of 15 mutant loci on the Maxy chromosome and two marker genes on the 1J1 chromosome.

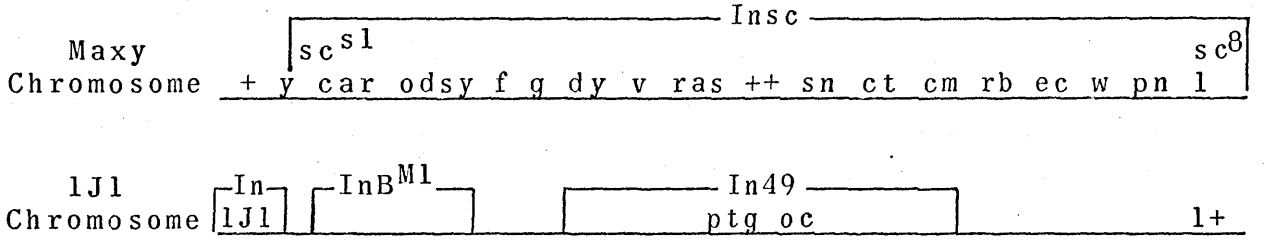
	Gene symbol	Phenotype
1.	y	yellow body
2.	car	carnation eyes
3.	odsy	outstretched wings, small eyes
4.	f	forked bristles
5.	g	garnet eyes
6.	dy	dusky wings
7.	v	vermilion
8.	ras	raspberry eyes
9.	sn	singed bristles
10.	ct	cut wings
11.	cm	carmine eyes
12.	rb	ruby eyes
13.	ec	echinus (rough eyes)
14.	w	white eyes
15.	pn	prune eyes
*	v	vermilion eyes
*	oc	ocelliless
	ptg	darker thoracic trident

*Marker genes: F_1 females have vermilion eyes unless an eye color mutation has occurred; ocelliless serves as a guard against nondisjunction as females homozygous for this gene are sterile.

Figure 3--Breeding scheme used in the Maxy technique. Genotypes and phenotypes of P_1 and F_1 generations are shown. Also listed are regular eggs, sperm and zygotes produced by the P_1 generation. The phenotypes of the F_1 generation are given.

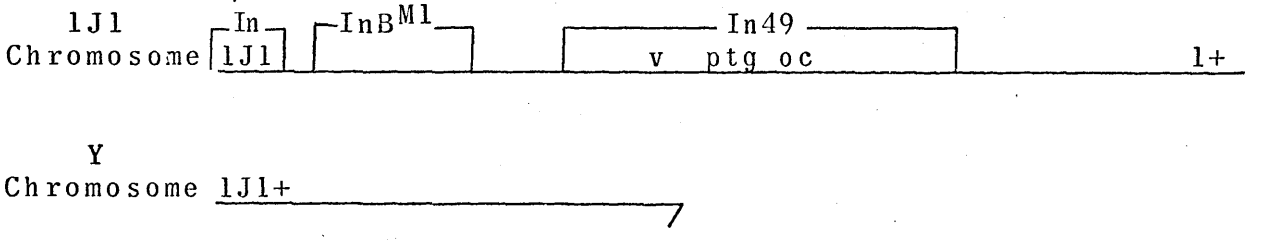
P_1 Generation

Maxy ♀



CROSSED TO

Maxy ♂



Regular eggs, sperm, and zygotes

		Eggs	
		Maxy	1J1
Sperm	Y	Maxy/Y ♂ dies	1J1/Y ♂ lives
	1J1	Maxy/1J1 ♀ lives	1j1/1j1 ♀ dies

F_1 Generation, phenotypes

Maxy ♀ v, slightly B Maxy ♂ oc, ptg, slightly B

left arm of each X or Y chromosome. An additional recessive lethal gene is located to the right of prune, on the Maxy chromosome, and has a normal allele on the other two X chromosomes. This lethal insures that no male bearing the Maxy X chromosome will survive. Crossing over is prevented by inversions on the X chromosome, which are shown in Figure 3. The Maxy chromosome carries inversions sc^{sl} and sc^{8}, with the two breakage points of the inversion at these symbols. The other X chromosome, designated the 1J1 because it carries the lethal J1, has inversions In49 and B^{Ml}, with the mutant gene ocelliless, which serves to check reproduction of non-disjunctional flies, as females homozygous for this gene are sterile.

The recessive gene pentagon, ptg, located on the 1J1 chromosome just to the left of oc, was present in the stock. The ptg phenotype has a slightly darker thoracic trident than the wild type, and its presence does not influence the Maxy technique.

All F_1 females were examined for recessive visible mutations at all 15 loci on the X chromosome. F_1 females mated to their brothers will produce no F_2 males, if a recessive lethal mutation has occurred in the treated P_1 X chromosome.

Brood I was produced by placing 15 or more treated virgin males, less than 24 hr old, with 20 or more virgin females of the same age and of the appropriate other strain,

into one-half pint culture bottles containing food. The flies were allowed to breed three days and then, if numerous eggs and larvae could be seen, brood II was made by placing the same treated males into fresh culture bottles with virgins; after the sixth day the males were discarded. In no case were more than two broods made.

Cultures to test the genome of individual F_1 females for mutations were made in shell vials containing medium. The many individual culture vials required for detecting recessive lethal mutations were placed in compartmented containers, each holding 220 cultures.

Gases used. The gases used for the studies described in this dissertation were obtained from The Matheson Co. (East Rutherford, New Jersey, and La Porte, Texas) in individual lecture bottles which contained from 1/16 pound to 1 pound of gas. Sulfur dioxide and nitrous oxide were two common gases studied. The completely fluorinated hydrocarbons used were perfluorocyclobutane, which is also called Freon-C318, and perfluoro-2-butene, another Freon. Partially fluorinated hydrocarbons also used were 1,1-difluoroethane, known as Genetron-152A, and fluoroform which is sometimes called Genetron-23. Genetron is a trade name for the General Chemical Division, Allied Chemical Corp. Freon is a trademark of the E.I. du Pont de Nemours & Co., Inc. For each gas Table 2 lists the common name, Chemical Abstracts name, empirical formula, minimum purity, and page reference in the

Table 2--Names, empirical formulae, and purity of gases used in the described studies.

Common names and synonyms of gases	Chemical Abstracts name of gas	Empirical formula of gas	Minimum purity of gas %	References to major impurities and specifications*** page
Freon-C318* (Perfluorocyclobutane) (Octafluorocyclobutane)	Octafluorocyclobutane	$CF_2CF_2CF_2CF_2$	99.5	393
Genetron-23** (Fluoroform)	Fluoroform	CHF_3	98.0	243
Genetron-152A** (1,1-Difluoroethane) (Ethylidene difluoride)	1,1-Difluoroethane	CH_3CHF_2	98.0	181
Nitrous oxide (Dinitrogen monoxide) (Laughing gas)	Nitrous oxide (Nitrogen[I]oxide)	N_2O	98.0	387
Perfluoro-2-butene (No Freon name)	Octafluoro-2-butene	$CF_3CF=CFCF_3$	99.5	403
Sulfur dioxide (Sulfurous acid anhydride)	Sulfur dioxide	SO_2	99.98	447

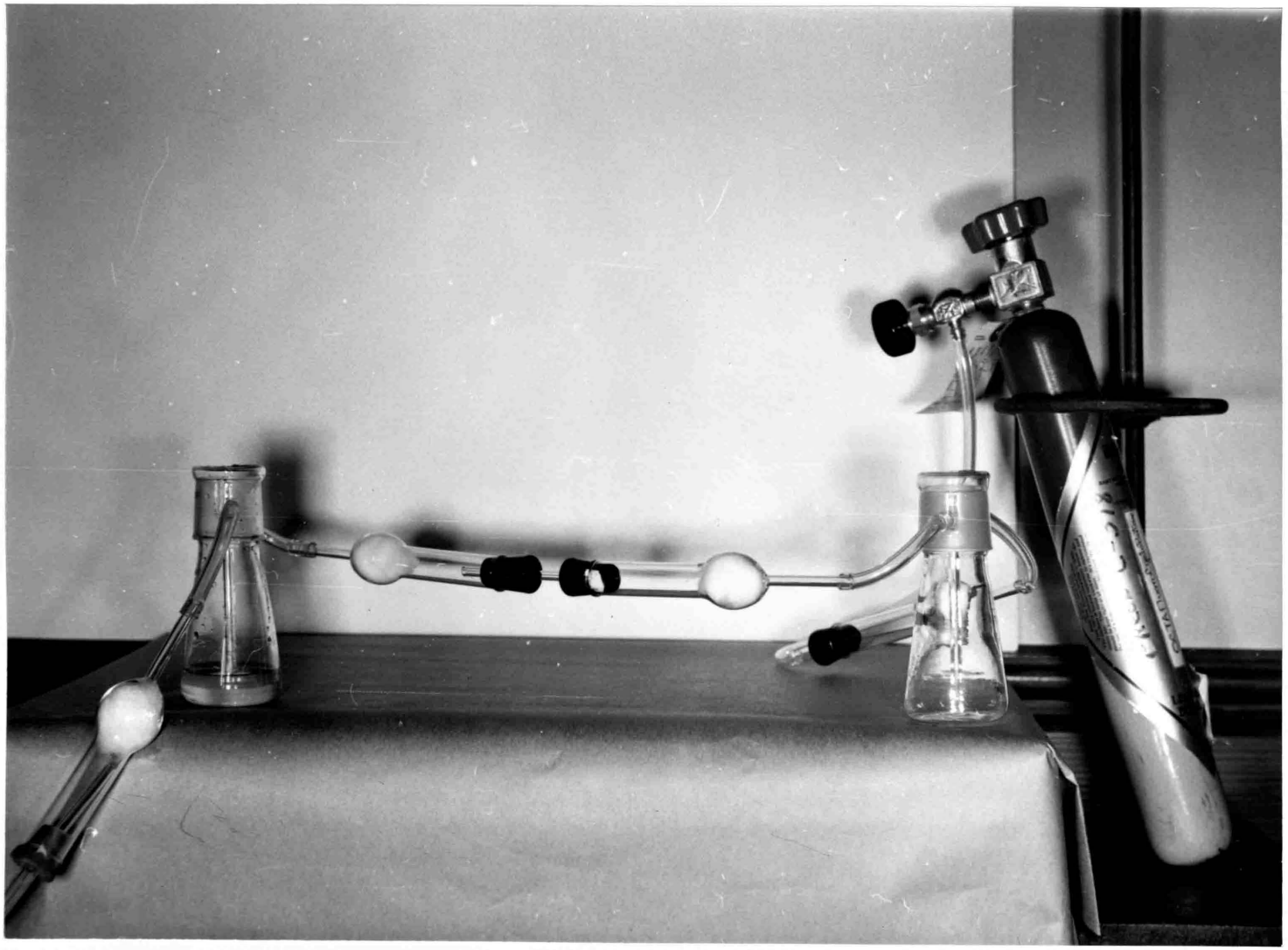
*Freon is a trade mark of the E.I. duPont de Nemours & Co., Inc.
**Genetron is the trade name for the General Chemical Division, Allied Chemical Corp.
***Matheson Gas Data Book (1966).

Matheson Gas Data Book (1966) for major impurities and specifications.

The gassing apparatus. The Turner bulb gassing apparatus was similar to the one described by Landry (1968), except for certain adaptations which were necessary to make it suitable for use with D. melanogaster. Figure 4 is a photograph of the complete assembly, as modified for gassing Drosophila. The gas was passed through a cotton trap and from there into a dry gassing chamber which contained the flies. From this Turner bulb, Tygon tubing connected with the inlet of a second Turner bulb, which contained 15 ml of water, into which the gas was bubbled. In this way 100 ± 5 bubbles per minute could be counted. The outlet arm of this second bulb was connected by Tygon tubing to the exhaust in the hood. The rate of gas flow in ml/min, as determined from bubble count, was also measured by water displacement, and was noted for each experiment.

The gassing technique. After some preliminary experimentation the following procedures were developed to prepare D. melanogaster for gassing. In order to have newly hatched flies for an experiment, the existing population was discarded from appropriate stock bottles at least 12 hr before the new study was started. At the time of the experiment, the newly eclosed organisms of both sexes, all virgins, were shaken from the stock bottle without etherizing,

Figure 4--Photograph of the Turner bulb gassing chamber. The inlet arm is shown to be connected with Tygon tubing to a lecture bottle of gas. The outlet arm of the chamber leads to the inlet arm of the second Turner bulb, which contains 15 ml of water. The position of the cotton filters in the drying tubes can be seen.



directly into the Turner bulb gassing chamber. This method eliminated anesthetizing the flies before they were gassed, and also insured that the virgin males had a fully intact sperm supply when they were treated.

The exposure period of Drosophila to the individual gases varied because of differences in toxicity. After gas treatment, all organisms remained in the gaseous atmosphere of the sealed chamber for five minutes. The treated Drosophila were then immediately placed in a clean container and observed. Upon recovery the flies were lightly etherized and the males placed in one-half pint culture bottles. The females of the same strain were discarded. As was explained earlier, it was desirable to expose females of another strain to the gassed and/or irradiated males. Details of any departure from the procedures described will be noted for specific experiments.

The irradiation source. In all experiments where radiation was required, the Co-60 irradiator, Model Gr-9 of the U.S. Nuclear Corp., was used. It is one of the standard sources available at the Texas Woman's University.

According to the method referred to as Fricke dosimetry (Spinks and Woods, 1964) each Turner bulb or shell vial used had to be individually conditioned so that the irradiation dose could be determined for the particular container exposed to Co-60. By this standardizing method it was possible to state within 8% accuracy the irradiation dose for

each experiment, with reference to time of exposure and the decay of Co-60.

Radiation technique. In all gas experiments, virgin males of D. melanogaster, up to 24 hr old, were exposed to the gamma radiation in the closed gaseous atmosphere of the Turner bulb. In experiments not requiring the use of a gas, the flies were exposed to radiation in shell vials.

Experimental procedures. Control recessive lethal mutation rates for male D. melanogaster of the Canton-S strain were established by testing over 2,500 individual F_1 female cultures. One aspect of every experimental design was repetition of all genetic crosses and statistical treatment deemed most appropriate to the experiment. The percentage of recessive lethal mutations induced in D. melanogaster was determined for each gas tested, as well as the recessive lethal mutation rate resulting from irradiation of the P_1 male in the gaseous atmosphere. This type of data was collected for every gas except the highly toxic sulfur dioxide.

The significance of the mutation rate induced by gas alone was determined by t-test comparison to the control rate (Snedecor, 1959). The t-test comparison of significance was made of the recessive lethal mutation rate induced by irradiating P_1 male Drosophila while in the closed gaseous atmospheres and the radiation only control recessive lethal

mutation rate. In some cases the Stevens (1942) method using the table of fiducial limits of expectation was followed to determine the significance of the results. For one Basc technique experiment with Genetron-23 and/or radiation, an analysis of variance was done using data obtained from a two x two factorial design, with five repetitions of each of the four experimental conditions. Each of the total of twenty repetitions was sampled by testing 200 F_1 females, cultured in individual vials, for recessive lethal mutations to be tallied among the F_2 generation.

The protocol for scoring semi-lethal (mosaic lethal) recessive mutations was to further test any F_2 culture having a ratio of at least ten heterozygous females, or eleven Basc males, to one normal male (Browning, 1961), and if this ratio continued in the F_3 generation, to score the culture as a semi-lethal mutation. Thus, it was sometimes necessary to test to the F_4 generation to assure that all recessive lethal mutations were checked to the confidence level.

EXPERIMENTAL RESULTS

Spontaneous mutation controls. Although it is acceptable standard practice to apply established control rates for extensively studied Drosophila stocks, it was thought advisable to run two control experiments for the Basc technique. The 1,300 chromosomes tested were from 240 Canton-S virgin males, less than 24 hr old at the start of the experiments.

The experimental recessive lethal mutation rate of 0.23% shown in Table 3 for the Canton-S stock was the result of combined data from the two spontaneous control studies. This table also lists five spontaneous recessive lethal mutation rates reported for the Basc technique by other workers, with all literature references cited. The recessive lethal mutation rate of 0.26% is shown as determined for the Maxy stock. This was based on a finding of six recessive lethals among 2,280 chromosomes tested. The visible spontaneous mutation rate of 0.008% for the Maxy technique, shown at the bottom of Table 3, was based on a study of 500,000 chromosomes as reported by Schalet (1958), and later confirmed by Altenburg and Browning (1961). Muller (1946) reported a spontaneous recessive lethal rate of 0.2% for several strains of D. melanogaster.

Table 3--Comparison of the spontaneous lethal mutation rate found in this study with some lethal mutation rates for unaged sperm of several strains of *D. melanogaster* as found by other workers. The visible spontaneous mutation rate for the Maxy stock is also listed.

Stock	Technique used	Lethal rate %	Literature references
Canton-Special	Basc	0.23*	This dissertation
Canton-Special	Basc	0.20	L.S. Browning, 1968
Canton-Special	Basc	0.10	A.M. Clark, 1958
Oregon-Red	Basc	0.10	G. Lefevre, Jr., 1965a
Oregon-Red	Basc	0.21	E.A. Carlson & J.L. Southin, 1963
Oregon-Red	Basc	0.30	E.A. Carlson & J.L. Southin, 1963
Several strains		0.20	H.J. Muller, 1946
Maxy**	Maxy	0.26	A. Schalet, 1958
Maxy**	Maxy	0.26	E. Altenburg & L.S. Browning, 1961

*This rate is based on a sampling of 1,300 chromosomes from first week sperm.

**The Maxy F_1 visible mutation rate is 0.008%, based on a sampling of 500,000 chromosomes (Schalet, 1958; Altenburg and Browning, 1961).

Basc technique studies using Oregon Red stock are also cited in Table 3 since mutation rates for these flies have never been considered significantly different from the Canton-S stock.

Survival studies of flies exposed to radiation. Table 4 shows the pooled results of two experiments exposing 800 Drosophila of the Maxy stock and the Canton-S stock, of both sexes, to gamma radiation doses, ranging from 1,500 R to 180,000 R. The table gives survival data in percent, for males and females, for six days following the treatment. The production of eggs, larvae, and pupae were scored. Data from doses up to 37,000 R did not differ from the controls during the six days. At 37,000 R, larvae did not appear until the fourth day; at 52,000 R, no larvae emerged. At 127,500 R and higher doses, the data indicate a sequential type survival pattern, in which females proved more resistant to Co-60 radiation than males.

Radiation induced mutation frequency. Two radiation control experiments were performed, each using the Basc technique, but differing in the irradiation dose administered to the P_1 Canton-S males. The radiation doses and resultant induced lethal mutation rates are listed in Table 5. The 4.2% rate resulted from 12 lethals, found among 287 chromosomes tested from 40 P_1 males exposed to 4,620 R of gamma radiation. In an experiment to be described later on in this dissertation,

Table 4--Pooled results of two separate experiments exposing 800 Drosophila of the Maxy stock and the Canton-S stock, of both sexes, to gamma radiation from Co-60. A series of 22 exposure periods, with radiation doses from 1,500 R to 180,000 R, were used.

Co-60 dosage 1,500 R per min R	Days after treatment with Co-60														
	1					2					3				
	Fertility			Survival		Fertility			Survival		Fertility			Survival	
	eggs	larvae	pupae	males %	females %	eggs	larvae	pupae	males %	females %	eggs	larvae	pupae	males %	females %
none	+	-	-	100	100	+	+	-	100	100	+	+	-	100	100
37,000*	+	-	-	100	100	+	-	-	100	100	+	-	-	100	100
52,500	+	-	-	100	100	+	-	-	100	100	+	-	-	100	100
82,500	-	-	-	100	100	+	-	-	100	100	+	-	-	100	100
120,000	-	-	-	100	100	-	-	-	40	100	-	-	-	20	100
127,500	-	-	-	100	100	-	-	-	40	100	-	-	-	20	80
150,000	-	-	-	50	100	-	-	-	30	80	-	-	-	10	30
165,000	-	-	-	40	90	-	-	-	25	50	-	-	-	5	20
180,000	-	-	-	20	90	-	-	-	10	30	-	-	-	2	10

*Data from eight increasing radiation exposure doses, up to this point, did not differ from control data, which are given on the line above. Only data are shown where some change occurred. Abbreviations used: + indicates the presence of eggs, larvae, or pupae; - means the absence of eggs, larvae, pupae, or flies. Table continued.

Table 4 continued--Pooled results of two separate experiments exposing 800 Drosophila of the Maxy stock and the Canton-S stock, of both sexes, to gamma radiation from Co-60. A series of 22 exposure periods, with radiation doses from 1,500 R to 180,000 R, were used.

Co-60 dosage 1,500 R per min R	Days after treatment with Co-60														
	4					5					6				
	Fertility			Survival		Fertility			Survival		Fertility			Survival	
	eggs	larvae	pupae	males %	females %	eggs	larvae	pupae	males %	females %	eggs	larvae	pupae	males %	females %
none	+	+	-	100	100	+	+	+	100	100	+	+	+	100	100
37,000*	+	+	-	100	100	+	+	-	100	100	+	+	-	100	100
52,500	+	-	-	100	100	+	-	-	100	100	+	-	-	100	100
82,500	+	-	-	100	100	+	-	-	100	100	+	-	-	100	100
120,000	-	-	-	20	100	-	-	-	20	100	-	-	-	20	100
127,500	-	-	-	20	80	-	-	-	10	40	-	-	-	-	10
150,000	-	-	-	2	10	-	-	-	-	6	-	-	-	-	-
165,000	-	-	-	1	5	-	-	-	-	2	-	-	-	-	-
180,000	-	-	-	-	3	-	-	-	-	1	-	-	-	-	-

*Data from eight increasing radiation exposure doses, up to this point, did not differ from control data, which are given on the line above. Only data are shown where some change occurred. Abbreviations used: + indicates the presence of eggs, larvae, or pupae; - means the absence of eggs, larvae, pupae, or flies.

Table 5--Co-60 gamma radiation induced lethal mutation rates found in this study. Some X-radiation induced lethal mutation rates reported by other workers for several strains of *D. melanogaster* are listed. The visible radiation induced mutation rate is reported for the Maxy stock.

Stock	Technique used	Radiation Source	Radiation Dose R	Lethal rate %	Literature references
Canton-Special*	Basc	Co-60	4,620	4.20	This dissertation
Canton-Special*	Basc	Co-60	3,000	5.80	This dissertation
Oregon-Red**	Basc	X-ray	4,000	8.20	G. Lefevre, Jr., 1966
Muller-5**	Basc	X-ray	4,000	7.44	G. Lefevre, Jr., 1966
Muller-5*** Oregon-R	Basc	X-ray	4,000	5.56	G. Lefevre, Jr., 1965b
Muller-5**** Oregon-R	Basc	X-ray	4,000	4.69	G. Lefevre, Jr., 1966
Seoul Strain* (Wild)	Basc	X-ray	1,500	7.75	Y.S. Kang et al., 1963
Maxy*****	Maxy*	X-ray	3,000	6.14	E. Altenburg & L. Browning, 1961

*The sperm tested were first week sperm from young virgin males.
**Only the maternal genome was irradiated.
***The Oregon-R males and Muller-5 females were irradiated separately.
****The inseminated Muller-5 females were irradiated.
*****A visible recessive mutation rate of 1.6% was detected in F_1 females descended from 3,000 R X-radiation exposed P_1 males.

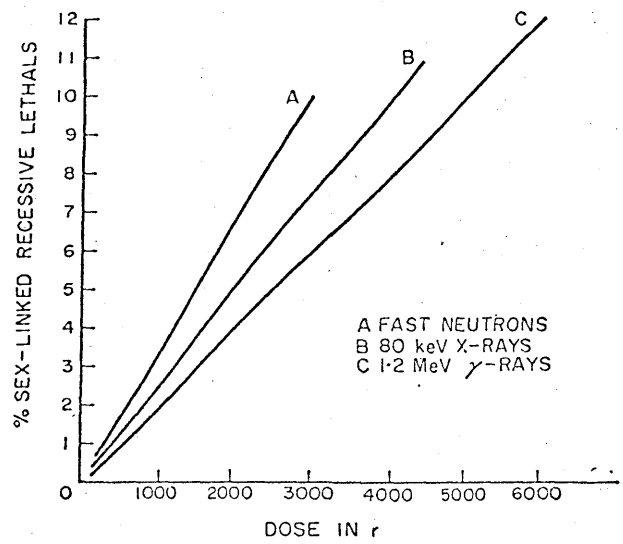
it was necessary to score 1,000 F_2 cultures to test X chromosomes from 200 P_1 males exposed to 3,000 R of gamma radiation. From the 1,000 cultures, 58 recessive lethal mutations were scored; giving a mutation rate of 5.8%.

In addition to these experimentally determined rates, Table 5 gives some values reported in the literature. These reported rates vary widely, as would be expected, since sources and radiation doses were not the same for different investigations. Lefevre (1965a), using a 4,000 R dose of X-radiation and the Basc technique, presented lethal mutation patterns which varied from 4.69% to 8.2%, depending upon whether maternal or paternal chromosomes were treated, or both, as is sometimes done when inseminated females are irradiated. Lefevre (1965b) suggested the additional criterion of using virgin males for any irradiation studies, to insure that an ample supply of mature sperm would be subjected to treatment.

One of the problems in interpreting radiation induced lethal mutation rates, such as shown in Table 5, is the discrepancy between X-radiation as compared to Co-60 gamma radiation.

Figure 5, representing a graph prepared by Purdom (1963), indicates that gamma rays, as produced by Co-60, are less mutagenic than X-rays and fast neutrons to spermatozoa of Drosophila. For this reason the induced lethal mutation control rate determined in the previous experiment (Table 5)

Figure 5--Mutagenic efficiency of different radiations on spermatozoa of Drosophila. Quantitative differences in recessive lethal mutations are shown between the effects of fast neutrons, X-rays and Co-60 gamma rays (Purdom, 1963).



MUTAGENIC EFFICIENCY OF DIFFERENT RADIATIONS IN SPERMATOZOA OF *Drosophila*

may seem low, since it is not apparent to the observer that many of the values, cited in the literature for mutation rates, are based on studies using X-radiations; these are more detrimental to the Drosophila germ cells than gamma radiation (Bacq and Alexander, 1961; Purdom, 1963; and Casarett, 1968).

Genetron-23 and gamma radiation. The results obtained from the examination of 4,558 F_2 cultures for recessive lethal mutations are presented in Tables 6 and 7. Sources and methods were as previously described and the Basc technique was followed. Table 7a shows the analysis of variance from the data in Table 7. As shown in Table 6, gas was applied to the flies for 5 min at a flow rate of 12 ml/min and the flies remained in the gaseous atmosphere another 5 min. Irradiated flies were exposed to 4,710 R of gamma radiation at a rate of 1,570 R/min.

Among the progeny of the gas treated P_1 males, seven lethal mutations and one semi-lethal, counted as 0.5 mutation, were found. This gave a total of 7.5 recessive lethal mutations in 271 cultures tested; or a frequency of 2.7%. The 0.23% spontaneous control rate was subtracted from the 2.7% induced rate, a frequency of 2.47% remained. Table 6 also shows the results from the combined treatment with Genetron-23 plus 4,710 R of gamma radiation. Six complete lethals and one semi-lethal mutation were found among 151 cultures, giving a lethal yield of 6.5/151 chromosomes.

Table 6--Lethal mutation rate found in the F_2 generation of *Drosophila melanogaster** resulting from exposure of P_1 males to 5 min of Genetron-23 at a flow rate of 12 ml/min, plus 5 min in the closed gaseous atmosphere. Also shown is the lethal mutation rate obtained as the result of exposure of the P_1 males to 5 min of Genetron-23 and 4,710 R of Co-60 irradiation while being held in the gaseous atmosphere for 5 min. The t-test results are given.

Treatment	Exposure of P_1 males		Chromosomes tested for lethals in F_1	F_2 flies examined for phenotype	Lethal(s)				Result of t-test**
	gas	Co-60			in F_2	rate	found	corrected	
	min	R	#	#			%	%	%
Control (No treatment)	-	-	1,300	29,400	3.00	3.0/1,300	0.23	-	-
Genetron-23	10	-	271	24,390	7.50	7.5/271	2.70	2.47	4.30
Co-60 treatment only	-	4,710	287	25,830	12.00	12/287	4.20	-	6.00
Genetron-23 and Co-60	10	4,710	151	13,590	6.50	6.5/151	4.30	-	0.34

*Muller's Basc Technique was used. The sperm were unaged.
**By calculations from experimental data. Interpretation: $t > 1.96$, $P = 0.05$; $t > 2.58$, $P = 0.01$.
***The lethal corrected value, 2.47%, was obtained by subtracting the control rate, 0.23% from the induced rate, 2.7%.

Table 7--Irradiation with Co-60 and treatment with Genetron-23 gas of Canton-S *Drosophila* males.*

Experiment #	Treatment	F_1 females crossed to F_1 siblings	F_2 recessive lethals	Mutation frequency %
0-1 (literature accepted value)	none (control)	-	-	0.1 - 0.3
1-1**		200****	1	
1-2	none	200	0	
1-3	(control)	200	0	
1-4		200	1	
1-5		200	0	
1-total		1,000	2	0.2
2-1	10 min	200	1½	
2-2	Genetron-23	200	3	
2-3	treatment	200	4½	
2-4	***	200	2	
2-5		200	4	
2-total		1,000	15	1.5
3-1	exposure	200	12	
3-2	to Co-60	200	14	
3-3	gamma	200	9	
3-4	irradiation	200	13	
3-5	3,000 R	200	10	
3-total		1,000	58	5.8
4-1	exposure	200	11	
4-2	to Co-60	200	6	
4-3	while in	200	10	
4-4	Genetron-23	200	9	
4-5	atmosphere	200	11	
4-total	***	1,000	47	4.7

*Muller's Basc technique was used to detect recessive lethal mutations.

**1-1, 1-2, etc. constitute replicate treatments, each applied to 40 Canton-S males, in identical but separate experimental runs.

***Sixty ml of gas was measured by water displacement, over an application period of 5 min. The flies were kept subsequently in this atmosphere for another 5 min.

****More than 200 F_1 cultures were prepared from each replicate treatment sample, but only the first 200 cultures yielding progeny were counted.

Table 7a--Analysis of variance from data listed in Table 7.

Analysis of variance				
Source of variation	Degrees of freedom	Sum of squares	Variance est. mean squares	Variance ratio F*
Total	19	21.95	-	-
Treatment:	3	17.458	5.819	20.7
1) Gas vs no gas	1	-	0.288	1.0
2) Radiation vs no rad	1	-	15.488	55.1
3) Interaction rad & gas	1	-	1.682	5.99
Within (Error)	16	4.492	0.281	-

*Interpretation: Values of F = 4.49 or greater, occur by chance less frequently than P = 0.05. Values of F = 8.53 or greater, occur by chance less frequently than P = 0.01.

This is a sex linked recessive lethal mutation rate of 4.3% for the Genetron-23 and Co-60 treated genome. This is 0.1% higher than the radiation control rate of 4.2%. The t-test showed that Genetron-23 treatment significantly increased the number of recessive lethal mutants ($P < 0.01$). The gas plus-irradiation treatment caused no statistically different results from irradiation treatment; $t = 0.34$.

A second experiment using a factorial design with five replications of each treatment was conducted. This experiment was designed so that the data obtained could be subjected to an analysis of variance, to further quantitate interactions between gas and irradiation effects. The treatments consisted of spontaneous controls, Genetron-23 gas, irradiation only, and irradiation plus gas. In the gas treatments listed in Table 7, 60 ml of gas were measured over an application period of 5 min, and the flies were kept subsequently in this atmosphere for another 5 min. In all replications using Co-60 gamma radiation, the dose administered was 3,000 R. Sources and methods were as previously described, and the Basc technique was followed. Every treatment was performed in five replications, each administered to 40 P_1 males. Their genomes were tested for recessive lethal mutations by scoring 200 F_2 individual cultures, each from the mating of an F_1 female with two or three sibs. This resulted in total sampling of 1,000 P_1 male X chromosomes for each of the four experimental

treatments applied. The mutations scored in the F_2 for each replication are listed in Table 7.

These data were subjected to an analysis of variance, which indicated that the gas alone caused an increase in the sex linked recessive lethal mutation rate when compared to the controls ($P < 0.05$). When radiation was applied to the flies, in air and in Genetron-23; radiation damage was reduced in the presence of the gas ($P = 0.05$). When the effect of gas, with and without radiation, was compared to the effect of radiation and no radiation, in air, it was not significant ($P \neq 0.05$). This comparison, while statistically valid, is not experimentally valid. The effect of gas, without radiation, was compensated for by the protective effect of the gas in the presence of radiation.

It appears from these data that the effects of Co-60 irradiation and gas treatment are not additive with respect to the numbers of recessive lethal mutations induced in Drosophila.

Both Genetron-23 experiments showed that treatment with the gas alone caused an increase in the sex linked recessive lethal mutation rate. Genetron-23, in the first experiment, demonstrated neither a protective nor an additive effect in the presence of radiation. Perhaps the sample size of this earlier experiment was not adequate. In the second experiment, Genetron-23 present at radiation reduced radiation sensitivity of the Drosophila genome.

Observations were made in the previously described Genetron-23 experiment concerning some deviant phenotypes among the 38,000 F_2 progeny of both sexes. Table 8 lists these flies according to phenotypes and loci. It shows that eye color, tumor, and wing mutations were frequent types. Although not noted in the table, the nature and extent of deviant flies did not differ to any marked degree between the offspring of the Genetron-23 treated flies, and the gassed and irradiated flies. Figure 6 shows a melanotic wing area in an F_2 fly. This illustration was selected because it was characteristic of the type of tumor found.

Genetron-152A and gamma radiation. Table 9 gives the results from 528 chromosomes tested for recessive lethal mutations by the Basc technique. The gassing time was 5 min with a flow rate of 10 ml/min, with the flies remaining in the gaseous atmosphere another 5 min. Irradiated flies were exposed to 4,710 R of Co-60 gamma radiation. From the progeny of 40 P_1 males exposed to Genetron-152A, individual lethal tests of 276 F_1 females gave one lethal and eight semi-lethals, each scored as 0.5 lethal, according to described protocol, giving a lethal count of 5/276 chromosomes or a lethal mutation frequency of 1.8%. After subtraction of the spontaneous control rate of 0.23%, a gas-induced rate of 1.5% remained.

Table 8--A tabulation of deviant types of flies observed among the F_2 generation following treatment of the P_1 paternal genome with exposure to Genetron-23 gas and to 4,710 R radiation with Co-60 in an atmosphere of Genetron-23. Transmissibility to the F_3 is shown.

Phenotype	Loci	Whole body mutations		Fractional mutations		F_2, non-fertile females
		Transmitted	Untransmitted	Transmitted	Untransmitted	
		#	#	#	#	#
Eye color	2*	7****	0	0	0	0
Tumor	4**	0	0	14	20	10
Wing	4***	6	0	18	15	0

*The apricot eye color was probably at the white loci.
**Tumors were classified as abdominal, head-eye, wing and proboscis.
***Wing mutations noted were curly, outstretched, wingless, and balloon.
****The deep red color, Bar eye flies, all in one culture, were probably representative of mosaic gonadal tissue in the F_1 female.

Figure 6--Photographs of (a) melanotic wing area in an F_2 ♀ (the P_1 ♂ had been treated with Genetron-23) and (b) range of eye colors in the progeny from the cross of a "tomato" eye color ♀ with her brothers (the P_1 ♂ had been treated with perfluoro-2-butene).

Table 9--Lethal mutation rate found in the F_2 generation of *D. melanogaster** resulting from exposure of P_1 males to 5 min of Genetron-152A at a flow rate of 10 ml/min, plus 5 min in the closed gaseous atmosphere. Also shown is the lethal mutation rate when the P_1 males were exposed to 5 min of Genetron-152A and 4,710 R of Co-60 radiation while being held in the gaseous atmosphere for 5 min. The t-test results are given.

Treatment	Exposure of P_1 males		Chromosomes tested for lethals in F_1	F_2 flies examined for phenotype	Lethal(s)				Result of t-test**
	gas	Co-60			in F_2	rate	found	corrected	
	min	R	#	#			%	%	%
Control (No treatment)	-	-	1,300	29,400	3	3/1,300	0.23	-	-
Genetron-152A	10	-	276	22,630	5	5/276	1.80	1.5***	2.5
Co-60 treatment only	-	4,710	287	25,830	12	12/287	4.20	-	6.0
Co-60 irradiation in Genetron-152A	10	4,710	252	18,900	12.5	12.5/252	5.00	0.8	0.4

*Muller's Basc Technique was used. The sperm were unaged.

**By calculations from experimental data. Interpretation: $t > 1.96$, $P = 0.05$; $t > 2.58$, $P = 0.01$.

***The lethal corrected value, 1.5%, was obtained by subtracting the control rate, 0.23%, from the induced rate, 1.8%.

Also given in Table 9 are results derived from the combined treatment of P_1 males with Genetron-152A and 4,710 R of gamma radiation. Nine lethals and seven semi-lethals, counted as 3.5 lethals, made a total of 12.5 lethals/252 chromosomes tested, or a recessive lethal mutation rate of 5.0% induced in the F_2 generation. As is apparent from Table 9 this is 0.8% above the radiation control rate.

Results from the t-test showed a significant increase in the number of recessive lethal mutants as a result of Genetron-152A treatment ($t = 2.5$, $t > 1.96$, $P = 0.05$). Gas plus radiation showed no significant difference from radiation in air. Thus, the gas demonstrated neither a protective effect, nor an additive effect, in the presence of irradiation.

Some differing phenotypes which were found upon observation of over 30,000 F_2 progeny, from Genetron-152A gas treated flies, with and without Co-60 treated P_1 males, are tabulated in Table 10. The mutants were classified as to eye color, tumor, or wing. These are phenotypically whole body or fractional (mosaic), and each was transmitted or not transmitted. The flies judged mutant were fertile or non-fertile. Eye color mutants transmitted were white, apricot, and a deep orange. A number of developmental type abnormalities were noted. One heterozygous female had ocelli in place of the proboscis and no proboscis was present; the eyes, arista, and antennae were normally oriented. In a

Table 10--A tabulation of deviant types of flies observed among the F_2 generation following treatment of the P_1 paternal genome with exposure to Genetron-152A gas and to 4,710 R radiation with Co-60 in an atmosphere of Genetron-152A. Transmissibility to the F_3 is shown.

Phenotype	Loci	Whole body mutations		Fractional mutations		F_2, non-fertile females
		Transmitted	Untransmitted	Transmitted	Untransmitted	
		#	#	#	#	#
Eye color	3*	7	0	0	0	0
Tumor	3**	0	0	14	20	10
Wing	3***	6	0	18	15	

*Eye colors found were white, apricot, and orange.
**Tumors were classed as abdominal, head, and wing.
***Wing mutations noted were "ropy" type, curly, and a modified, less curly type.

semi-lethal culture from Genetron-152A and Co-60 treatment the lethal took effect at the start of pupation. On the sides of the vial, pupae cases were observed which had turned dark inside and become soft. It was also noted that cultures using Genetron-152A and Co-60 treated males were slower in developing than those from gas only treated P_1 males. In the F_1 generation the males eclosed earlier than the females and the setting up of the F_2 cultures was delayed for lack of females.

Perfluoro-2-butene and gamma radiation. Table 11 presents results of Basc technique studies with perfluoro-2-butene, performed as previously described. Because of the toxicity of this gas to Drosophila, a gassing time of 1 min was used, with a flow rate of 13 ml/min, followed by an additional 5 min in the closed gaseous atmosphere. Two lethal mutations and four semi-lethal mutations, counted as two lethals, were found in the 312 gas exposed chromosomes tested, giving a lethal mutation rate of 1.3%. This increase was significant at the 1% level of probability. A dose of 3,140 R was administered in the radiation study with perfluoro-2-butene. One lethal mutation and eight semi-lethals, counted as four lethals, gave a total of 5/320 chromosomes tested, or a lethal frequency of 1.6%.

The gas plus radiation mutation rate was significantly less than radiation in air ($t = 3.07$, $P < 0.01$). Thus, the

Table 11--Lethal mutation rate found in the F_2 generation of *D. melanogaster** resulting from exposure of P_1 males to 1 min of perfluoro-2-butene at a flow rate of 13 ml/min, plus 5 min in the gaseous atmosphere. Also shown is the lethal mutation rate when the P_1 males were exposed to 1 min of perfluoro-2-butene and 3,140 R of Co-60 radiation while being held in the gaseous atmosphere for 5 min. The t-test results are given.

Treatment	Exposure of P_1 males		Chromosomes tested for lethals in F_1	F_2 flies examined for phenotype	Lethal(s)				Result of t-test**
	gas	Co-60			in F_2	rate	found	corrected	
	min	R	#	#			%	%	
Control (No treatment)	-	-	1,300	-	3	3/1,300	0.23	-	-
Perfluoro-2-butene	6	-	312	21,000	4	4/312	1.30	1.07***	2.60
Co-60 treatment only	-	3,140	1,000	-	58	58/1,000	5.80	5.57	8.80
Co-60 irradiation in Perfluoro-2-butene	6	3,140	320	22,300	5	5/320	1.60	1.37	3.07

*Muller's Basc Technique was used. The sperm were unaged.

**By calculations from experimental data. Interpretation: $t > 1.96$, $P = 0.05$; $t > 2.58$, $P = 0.01$.

***The lethal corrected value, 1.07%, was obtained by subtracting the control rate, 0.23%, from the induced rate, 1.30%.

gas treatment during the time of radiation produced a protective effect.

As shown in Table 12, certain changes in phenotype seemed to predominate among the 45,000 F_2 flies examined. More morphological changes were observed in the progeny of P_1 males exposed to both perfluoro-2-butene and Co-60 irradiation, although these changes were of the same types as those observed among the F_2 population from the gas only exposed P_1 males. Some flies with "tomato" colored eyes were observed in the F_2 generation. When these flies were cultured, by inbreeding, eye colors varying from a dark dull red, through several color ranges to the "tomato" color, were among the progeny, as well as some normal eye colors. Figure 6 shows flies that resulted from one F_2 female, crossed with her brothers. The other common transmissible change was a blister type wing. One or both wings of many flies were filled with a clear fluid, the fluid filled area varied from the entire wing to only a small wing area. When the flies aged, these blisters burst and left a crater-like depression in the otherwise intact wing. Figure 7 illustrates this phenotype. Many of the wings were curled upward at the tips. The degree of curl varied, some flies had only one wing curled, some had both curled, and the degree of curl ranged from slight to pronounced. Although not shown in the table, 55 cultures were

Table 12--A tabulation of deviant types of flies observed among the F_2 generation following treatment of the P_1 paternal genome with exposure to perfluoro-2-butene gas and to 3,140 R radiation with Co-60 while in an atmosphere of perfluoro-2-butene. Transmissibility to the F_3 is shown.

Phenotype	Loci	Whole body mutations		Fractional mutations		F_2, non-fertile females
		Transmitted	Untransmitted	Transmitted	Untransmitted	
		#	#	#	#	#
Eye color	5*	3	0	18	14	0
Tumor	2**	0	0	0	0	2
Wing	3***	0	0	20	3	0

*Eye colors fully transmitted were white, apricot, and orange. The tomato color variations were at possibly 2 loci, and were judged gonadal mosaics, although the phenotypes were whole body.

**One melanotic tumor was under the proboscis and the other was abdominal.

***Wing mutations were blister, slightly curly, and one "stump" type wing.

Figure 7--Photographs of (a) F_2 flies showing fluid filled blister wing as well as crater-like depression remaining after blister breaks (the P_1 ♂ had been treated with perfluoro-2-butene gas and Co-60 radiation while in the gaseous atmosphere) and (b) ♀ fly with fused unicorn antenna found in the orange eye color stock (the P_1 ♂ had been treated with Freon-C318).

made from these deviant flies to the F_3 and beyond, in an effort to establish mutant stocks.

Freon-C318 and gamma radiation. Both the Maxy and the Basc techniques were used in testing the completely fluorinated hydrocarbon Freon-C318 on the genome of D. melanogaster. All flies were prepared for the gassing in the usual way. The gas was administered to the Drosophila at a flow rate of 11.6 ml/min for a period of 5 min and then the flies were held in the gaseous atmosphere for 5 min more. The Freon-C318 acted as an impressively rapid anesthetic, and the flies on contact with this gas assumed a typical "lethal" position. On being removed from the perfluorocyclobutane atmosphere they quickly resumed full activity, with no signs of abnormal or disoriented behavior. Table 13 shows data from the F_1 female visible mutation study with the Maxy technique. Five mutations of the Maxy type were scored among the 1,300 F_1 females tested. Three were eye color mutants and two were cut wing mutants. The frequency of 5/1,300 sex chromosomes tested gave an induced visible mutation rate of 0.38% for Freon-C318 as compared with a spontaneous control rate of 0.008%.

The results of the Maxy technique recessive lethal mutation study are shown in Table 14. From Freon-C318 treated P_1 males, the recessive mutation rate was 1.3%; three lethals were among 212 tested chromosomes.

Table 13--Visible mutations* found among Maxy strain F_1 female D. melanogaster resulting from exposure of Maxy P_1 males to Freon-C318 for 5 min at a flow rate of 11.6 ml/min, plus 5 min in the closed gaseous atmosphere.

F_1 females examined for visibles	Phenotype	Loci	Whole body		Fractional		Induced visible mutation rate	Spontaneous visible mutation rate***
			Trans-mitted	Untrans-mitted	Trans-mitted	Untrans-mitted	%	%
1,300	Eye color	2**	2	1	0	0		
1,300	Wing	1	1	1	0	0		
Total****		3	3	2	0	0	0.38	0.008
Fiducial limits*****							0.17	0.016

*Only Maxy type mutants (Table 1) are scored in this technique. The sperm were unaged.

**Two females had apricot color eyes and one had prune color eyes.

***Spontaneous visible mutation rate as given in Table 3.

****A total of five Maxy type visible mutations were scored in 1,300 F_1 females.

*****Stevens' (1942) method was used. Interpretation: the lower limit of the induced rate, 0.17, and the upper limit of the spontaneous rate, 0.016, do not overlap. The results are significant, P = 0.05.

Table 14--Lethal mutation rate found in the F_2 generation of *D. melanogaster** resulting from exposure of P_1 Maxy strain males to 5 min of Freon-C318 at a flow rate of 11.6 ml/min, plus 5 min in the closed gaseous atmosphere. Also shown is the lethal mutation rate when the P_1 males were exposed to 5 min of Freon-C318 and 4,710 R of Co-60 irradiation while being held in the gaseous atmosphere for 5 min. The t-test results are given.

Treatment	Exposure of P_1 males		Chromosomes tested for lethals in F_1	F_2 flies examined for phenotype	Lethal(s)				Result of t-test**
	gas	Co-60			in F_2	rate	found	corrected	
	min	R	#	#			%	%	
Control (No treatment)	-	-	2,280	-	6	6/2,280	0.26	-	-
Freon-C318	10	-	212	21,100	3	3/212	1.30	1.04***	1.2
Co-60 treatment only	-	4,710	287	25,830	12	12/287	4.20	-	6.0
Co-60 irradiation in Freon-C318	10	4,710	231	20,400	8	8/231	3.40	-	0.9

*Muller's Maxy Technique was used. The sperm were unaged.

**By calculations from experimental data. Interpretation: $t > 1.96$, $P = 0.05$; $t > 2.58$, $P = 0.01$.

***The lethal corrected value, 1.05% was obtained by subtracting the control rate, 0.26%, from the induced rate, 1.30%.

When P_1 males were exposed to 4,710 R of gamma radiation in a Freon-C318 atmosphere, eight recessive sex linked lethal mutations were found in 231 chromosomes tested, giving a rate of 3.4%.

Table 15 presents results of Basc technique studies with Freon-C318, performed as previously described. The gas was administered to the Canton-S males for 5 min, at a flow rate of 11.6 ml/min, followed by 5 min in the gaseous atmosphere. Two semi-lethals, scored as one lethal, and one lethal mutation were found in the 285 gas exposed chromosomes tested; a lethal mutation rate of 0.54%.

Also shown in Table 15 are the results obtained from exposure of P_1 males to 4,710 R gamma radiation while in an atmosphere of Freon-C318. Nine lethal mutations were found from testing 246 chromosomes. This gave a rate of 3.7%, compared with a rate of 4.2% for Co-60 radiation in a normal atmosphere.

Determinations from statistical analysis of the data shown in Tables 13, 14, and 15 were interesting in that visible mutations were significantly increased ($P = 0.05$) from treatment of P_1 Maxy males by Freon-C318 (Table 13), while in both Maxy and Basc sex linked recessive lethal mutation studies, the increases in recessive lethal mutation rates were not statistically significant. However, this could well be a function of small sample size, since the spontaneous rate, by the Basc technique study, increased

Table 15--Lethal mutation rate found in the F_2 generation of *D. melanogaster** resulting from exposure of P_1 males to 5 min of Freon-C318 at a flow rate of 11.6 ml/min, plus 5 min in the closed gaseous atmosphere. Also shown is the lethal mutation rate when the P_1 males were exposed to 5 min of Freon-C318 and 4,710 R of Co-60 irradiation while being held in the gaseous atmosphere for 5 min. The t-test results are given.

Treatment	Exposure of P_1 males		Chromosomes tested for lethals in F_1	F_2 flies examined for phenotype	Lethal(s)				Result of t-test**
	gas	Co-60			in F_2	rate	found	corrected	
	min	R	#	#			%	%	
Control (No treatment)	-	-	1,300	-	3	3/1,300	0.23	-	-
Freon-C318	10	-	285	21,000	2	2/285	0.77	0.54***	0.21
Co-60 treatment only	-	4,710	287	25,830	12	12/287	4.20	-	6.00
Co-60 irradiation in Freon-C318	10	4,710	246	18,900	9	9/246	3.70	-	0.80

*Muller's Basc Technique was used. The sperm were unaged.

**By calculations from experimental data. Interpretation: $t > 1.96$, $P = 0.05$; $t > 2.58$, $P = 0.01$.

***The lethal corrected value, 0.54%, was obtained by subtracting the control rate, 0.23%, from the induced rate, 0.77%.

from 0.23 to 1.3 (five fold) and in the Maxy study it increased to 0.54 (over two fold). If this increase is real, it would be significant with a larger sample size.

Stevens' (1942) method was used for the analysis of the Maxy F_1 visible mutation study, and the t-test was used for the analysis of both Basc and Maxy lethal mutation studies. From both techniques the Freon-C318 plus radiation induced rates showed no significant difference from the radiation control rate. The gas demonstrated neither a protective nor additive effect in presence of gamma radiation.

A total of 39,000 flies of both sexes from the F_2 generation, by the Basc technique, were examined for phenotypic effects. Table 16 gives a tabulation of the phenotypes observed in the F_2 generation. In addition to eye color, tumor, and wing mutations, abnormalities were noticed about the antennae and aristae. The fly with the unicorn fused antenna, pictured with a sib in Figure 7 was found in the deep apricot eye color stock, which was established by inbreeding from the F_2 eye color mutant.

A number of melanotic tumors were observed in later sub-cultures, as well as developmental type defects involving the scutellum and the arrangement of bristles. Most wing mutations involved some degree of curliness, from slight to quite pronounced. An odd type of wing observed was outstretched and curly. The phenotypic effects found

Table 16--A tabulation of deviant types of flies observed among the F_2 generation following treatment of the P_1 paternal genome with exposure to Freon-C318 gas and to 4,710 R radiation with Co-60 in an atmosphere of Freon-C318 gas. The Basc technique was used. Transmissibility to the F_3 is shown.

Phenotype	Loci	Whole body mutations		Fractional mutations		F_2, non-fertile females
		Transmitted	Untransmitted	Transmitted	Untransmitted	
	#	#	#	#	#	#
Eye color	3*	14	0	0	0	0
Tumor	5**	0	0	3	9	6
Wing	2***	0	0	12*****	10	0
Antenna	1****	0	0	0	0	0

*The eye colors were apricot, deep orange, and bright red.
**Tumors were ventral abdominal (melanotic), ventral abdominal (non-melanotic), dorsal abdominal, head, antenna, and thorax.
***The wing mutants were predominately curly and curly-outstretched.
****One male had a black tumorus antenna with the arista missing, another male had no proboscis and abnormal antennae.
*****Some of the wing mutants appeared whole body but because of method of transmission they represented mosaic gonadal tissue and so were classified as fractional.

in the F_2 and later generations support the conclusions of the visible mutation experiment that Freon-C318 treatment produced changes in the genetic material of the Drosophila P_1 males.

Nitrous oxide and gamma radiation. Preparations were made for the Basc technique and the gassing and irradiation experiments. As shown in Table 17, the gassing time was five min with a flow rate of 11.6 ml/min, with the flies remaining an additional five min in the gaseous atmosphere. Ten lethal mutations were found among 380 chromosomes tested, giving a sex linked recessive lethal mutation rate of 2.6%. In the nitrous oxide plus 3,160 R gamma radiation study, 12 lethals were tallied from 375 chromosomes tested, to give a recessive lethal mutation rate of 3.5%. Irradiation in air produced a mutation rate of 5.8%.

Results of the t-test showed that nitrous oxide caused an increase in recessive lethal mutations in Drosophila ($P = 0.01$) when compared to no gas treatment. Gas and radiation produced significantly less mutations than radiation in air ($P = 0.01$). A nitrous oxide atmosphere during radiation resulted in fewer recessive lethal mutations than radiation in air.

Some phenotypic effects observed among the F_2 progeny of P_1 males treated with nitrous oxide and/or 3,000 R gamma radiation are tabulated in Table 18. Eye mutations were

Table 17--Lethal mutation rate found in the F_2 generation of D. melanogaster* resulting from exposure of P_1 males to 5 min of nitrous oxide at a flow rate of 11.6 ml/min, plus 5 min in the closed gaseous atmosphere. Also shown is the lethal mutation rate when the P_1 males were exposed to 5 min of nitrous oxide and 3,160 R of Co-60 irradiation while being held in the gaseous atmosphere for 5 min.

Treatment	Exposure of P_1 males		Chromosomes tested for lethals in F_1	F_2 flies examined for phenotype	Lethal(s)				Result of t-test**
	gas	Co-60			in F_2	rate	found	corrected	
	min	R	#	#			%	%	
Control (No treatment)	-	-	1,300	-	3	3/1,300	0.23	-	-
Nitrous oxide	10	-	380	27,600	10	10/380	2.60	2.37***	4.2
Co-60 treatment only	-	3,000	1,000	-	58	58/1,000	5.80	-	8.8
Co-60 irradiation in Nitrous oxide	10	3,160	336	23,200	12	12/336	3.50	-	3.8

*Muller's Basc Technique was used. The sperm were unaged.
**By calculations from experimental data. Interpretation: $t > 1.96$, $P = 0.05$; $t > 2.58$, $P = 0.01$.
***The lethal corrected value, 2.37%, was obtained by subtracting the control rate, 0.23%, from the induced rate, 2.60%.

Table 18--A tabulation of deviant types of flies observed among the F_2 generation following treatment of the P_1 paternal genome with exposure to nitrous oxide gas and to 3,160 R radiation with Co-60 while in an atmosphere of nitrous oxide. Transmissibility to the F_3 is shown.

Phenotype	Loci	Whole body mutations		Fractional mutations		F_2, non-fertile females
		Transmitted	Untransmitted	Transmitted	Untransmitted	
		#	#	#	#	#
Eye color	1*	10	0	0	0	0
Tumor	2**	0	0	1	1	1
Wing	1***	0	0	1	0	0
Antenna	1****	0	0	1	0	0

*Apricot was the eye color mutant.
**One tumor was abdominal, the other was at the base of the proboscis.
***The wing mutant was curly.
****Two females without antennae or arista were in one F_2 culture, so were from one P_1 event; transmission was mosaic.

apricot and were transmitted; a stock was established. Two melanotic tumors were found, and the abdominal tumor was transmitted in a mosaic pattern. Two antennaeless females were in one F_2 culture and in some subsequent cultures antennae and aristae abnormalities were noted after nitrous oxide treatment of P_1 males.

Sulfur dioxide study. Because of the importance of sulfur dioxide as an environmental pollutant it was planned to use it in extensive Maxy technique studies. This was not possible because all the flies died before they could be placed in the radiation chamber. This confirmed the extreme toxicity of the gas.

The sulfur dioxide treatment tolerated by the flies consisted of about 2.5 ml administered in the shortest possible time. Unless the flies were immediately removed to another container, none survived.

Table 19 presents the results of the Maxy technique visible mutation studies. Three eye mutations of the Maxy type were found in the 1,208 F_1 females examined, giving a mutation rate of 0.25%, which may be compared with a visible spontaneous control rate of 0.008% for the Maxy stock. Since the total number of visible mutations was small, a calculation designed for analysis of small numbers derived from large populations (Stevens, 1942) shows that the induced mutation frequency has a lower limit of 0.05% ($P = 0.05$),

Table 19--A tabulation, using the Maxy technique, of visible mutations* found among F_1 female D. melanogaster resulting from exposure of P_1 males to about 2.5 ml of sulfur dioxide.

Treatment	Exposure of P_1 males: gas ml	Exposure of P_1 males: Co-60 R	F_1 ♀ flies examined for visibles	Phenotype	Loci	Whole body mutations transmitted #	Visible mutation rate %	Statistical results*** Fiducial limits: Lower	Upper
Sulfur dioxide	2.5	-	1,208	Eye color	2**	3/1,208	0.25	0.05%	-
Control (No treatment)	-	-	500,000	-	-	-	0.008	-	0.01%

*Only Maxy type mutants (Table 1) are scored in this technique. The sperm were unaged.
**One female had apricot eyes and two females had garnet eyes.
***Stevens' (1942) method was used. Interpretation: the lower limit of the induced rate, 0.05, and the upper limit of the control rate, 0.01, do not overlap; P = 0.05.

as compared with the upper limit 0.01% (P = 0.05) of the control rate. Since these two limits do not overlap, it may be concluded that the sulfur dioxide was mutagenic under the experimental conditions.

As shown in Table 20, a recessive lethal mutation study testing 604 F_1 Maxy females was performed. Two cultures in the F_2 lacked males, giving a frequency of 0.33%. The lethal corrected value, 0.07%, was obtained by subtracting the control rate, 0.26%, from the induced rate, 0.33%. The induced frequency was not significantly different from the control rate as based on t-test comparisons.

Table 20--Recessive lethal mutation rate found in the F_2 generation of *D. melanogaster** resulting from exposure of P_1 males to 2.5 ml of sulfur dioxide gas. The t-test result is given.

Treatment	Exposure of P_1 males		Chromosomes tested for lethals in F_1	F_2 flies examined for phenotype	Lethal(s)				Result of t-test**
	gas	Co-60			in F_2	rate	found	corrected	
	ml	R	#	#			%	%	
Sulfur dioxide	2.5	-	604	24,160	2	1/302	0.33	0.07***	0.33
Control (No treatment)	-	-	2,280	-	-	-	0.26	-	-

*Muller's Maxy technique was used. The sperm were unaged.

**By calculation from experimental data. Interpretation: $t > 1.96$, $P = 0.05$; $t > 2.58$, $P = 0.01$.

***The lethal corrected value, 0.07%, was obtained by subtracting the control rate, 0.26%, from the induced rate, 0.33%.

DISCUSSION

Evidence has been presented that six gases, which may be air contaminants, are mutagenic to marker strains of Drosophila melanogaster using the frequency of sex-linked recessive lethals as a measure of activity. However, natural populations are subject to varying concentrations of mixtures of air pollutant gases, throughout the lifetime of the organisms. Thus, even well documented experimental results obtained with Drosophila warrant further testing in other organisms. Only then can the reproducibility of genetic hazards be properly evaluated, with an understanding of broad biological implications.

From the studies that were conducted with fluorine hydrocarbon atmospheres, it is not directly apparent what effects these gases would have on organisms in mixtures with other gases. These investigations are at the present time being continued by other workers in the laboratories at the Texas Woman's University as a direct consequence of the findings presented in this dissertation.

While a summation of data from the gas studies is given in Table 21, it should be pointed out that only general comparisons of mutagenicity among the gases can be made,

Table 21--Summation of recessive lethal mutation frequencies for Drosophila treated with one of six gases and/or Co-60 gamma radiation. Control data are also included. Confidence levels are given.

Strains tested	Treatment gas	Co-60 R	Mutation rate %	Confidence level
Canton-S	air	-	0.23	
Canton-S	air	4,710	4.20	
Canton-S	air	3,000	5.80	
Maxy	air	-	0.26	
Canton-S	Genetron-23	-	2.70	P = 0.01*
Canton-S	Genetron-23	4,710	4.30	P ≠ 0.05**
Canton-S	Genetron-23	-	1.50	P = 0.05*
Canton-S	Genetron-23	3,000	4.70	P ≠ 0.05**
Canton-S	Genetron-152A	-	1.80	P = 0.05*
Canton-S	Genetron-152A	4,710	5.00	P ≠ 0.05**
Canton-S	Nitrous oxide	-	2.60	P = 0.01*
Canton-S	Nitrous oxide	3,160	3.50	P = 0.01***
Canton-S	Perfluoro-2-butene	-	1.30	P = 0.05*
Canton-S	Perfluoro-2-butene	3,140	1.60	P = 0.01***

*The recessive lethal mutation rate was increased.
**The effects of gas and radiation were not additive; neither an enhancing nor protective effect was shown.
***A protective effect was shown by the gaseous atmosphere during radiation.
****The induced recessive lethal rate was not significantly increased.
*****The visible recessive mutation rate in F_1 Maxy females was increased; the control rate is 0.003%.
Table continued.

Table 21 continued--Summation of recessive lethal mutation frequencies for Drosophila treated with one of six gases and/or Co-60 gamma radiation. Control data are also included. Confidence levels are given.

Strains tested	Treatment gas	Co-60 R	Mutation rate %	Confidence level
Canton-S	Freon-C318	-	0.54	P ≠ 0.05****
Canton-S	Freon-C318	4,710	3.70	P ≠ 0.05**
Maxy	Freon-C318	-	1.30	P ≠ 0.05****
Maxy	Freon-C318	4,710	3.40	P ≠ 0.05**
Maxy	Freon-C318	-	0.38	P = 0.05*****
Maxy	Sulfur dioxide	-	0.25	P = 0.05*****
Maxy	Sulfur dioxide	-	0.33	P ≠ 0.05****

*The recessive lethal mutation rate was increased.
**The effects of gas and radiation were not additive; neither an enhancing nor protective effect was shown.
***A protective effect was shown by the gaseous atmosphere during radiation.
****The induced recessive lethal rate was not significantly increased.
*****The visible recessive mutation rate in F_1 Maxy females was increased; the control rate is 0.008%.

because of variation in the exposure time of the organisms to the gases, as well as some differences in the radiation dose given to Drosophila while they were confined to the gaseous atmospheres.

The exposure time of Drosophila to perfluoro-2-butene was shortened to seven min because of gas toxicity, and only a minimal treatment could be used with sulfur dioxide. It may well be that these two gases are potentially more mutagenic under longer exposure periods at low concentrations. Drosophila could not be subjected to radiation while being held in an atmosphere of sulfur dioxide because of the toxicity of the gas under experimental conditions for this study.

From the data presented in Table 7, the conclusion was that radiation administered in the presence of Genetron-23 significantly decreased the number of recessive lethal mutations from the number induced when radiation was given in air. However, there is no way to determine if less genetic damage resulted from radio-protection by the gas or was due to decreased oxygen tension, or the functioning of a repair process in the mature sperm, which may respond selectively to different environments present before, during, and after radiation (Sobels et al., 1967; Alexander, 1962).

Data from four studies in which Drosophila were irradiated in atmospheres of individual gases, in general, show the presence of each gas during irradiation to lessen

the genetic damage to Drosophila, as determined by decreased production of recessive lethal mutations. Such radioprotection has generally been considered a reversal of the oxygen effect (Chang et al., 1959; Sobels, 1965; Elequin, 1966), due to anoxia at the time of irradiation. Studies in vivo with both animals (Wright, 1957) and bacteria (Deschner and Gray, 1959) have shown that cells can reach their lowest level of sensitivity (anoxia) in four seconds for animals and 1/50 second for bacteria. Cells which are anoxic with respect to the usual cellular environment have a certain amount of oxygen present before, during, and after radiation (Schmid, 1961).

While exact oxygen tensions in individual cells are not often known, the ability of Drosophila to withstand extremely high doses of radiation may be due in part to low oxygen tension within the tissues, which are supplied with oxygen by tracheae (Altman and Dittmer, 1966). Also, insects maintain their osmotic pressure by means of amino acids, and some amino acids have a protective action against radiation damage (Camien et al., 1951).

Nitrous oxide has been listed (Ebert and Hornsey, 1958) as one of the most effective gases for reversing the oxygen effect. In these studies perfluoro-2-butene was found more effective than nitrous oxide in decreasing the recessive lethal mutation damage to Drosophila (Table 21). Again comparisons can not be exact because these flies were

subjected to a shorter time of exposure to perfluoro-2-butene (2 min) than to nitrous oxide (5 min) before radiation. The level of radiation in the two experiments was comparable, and the numbers of chromosomes tested were within the same range in both studies. The data indicate that a larger study of the protective effects of perfluoro-2-butene as an atmosphere during irradiation should be warranted.

Although the nature of the initial chemical lesion for which oxygen seems to have such a high affinity is not understood, inert gases present at irradiation result in less cell damage (Ebert and Howard, 1957; Alexander, 1962; Elequin, 1966; Sobels, 1965). Ebert et al. (1958) suggested that an oxygen sensitive site could be protected by a layer of inert gas. By this concept, gases could be considered as in the realm of physical protection; a gaseous shield over active sites. Simons (1950) listed the fluorinated hydrocarbons as intermediaries in metabolism, which would seem to cast doubt on the inert shield theory for these gases. Hamilton (1963) and Taylor (1965) cited evidence of the ability of fluorine to alter biological activity. Furthermore it was observed that various gases protected organisms from ionizing radiations with varying degrees of efficiency. This would seem to indicate a mechanism beyond the forming of a protective layer.

Further evidence of differential molecular mechanisms of protective action comes from observations of deviant

phenotypes, which resulted from exposure of parental Drosophila to six gases during the course of studies for this dissertation. The same genetic material subjected to differing experimental gaseous treatments in later generations gave rise to repeatedly recurring phenotypic expressions, which differ from one gas to another. This would indicate that anoxia alone cannot be the answer to the radio-protective effect of these gasses.

Melanotic tumors were most numerous among the later sub-cultures from males treated with Genetron-23 alone and Genetron-23 and irradiation. Evidently these tumors, at least, are not the result of irradiation activating a latent virus, unless the gas alone served to activate the same virus. The tumors could be the end result of an incompleted lethal event, which resulted in an altered enzyme somewhere in the pathway to melanin production. These tumors were often multiple, with one fly having several melanotic areas. Some melanotic tumors were found among the progeny of all the gas treated flies. Among the later offspring of Genetron-152A treated males a ropy black tumor involving the whole wing, and with a yellow pus-like congealed discharge at the distal base, was common. This type of tumor was not observed in later generations from any other gas treatment. The observed tumors from irradiated and/or gas treated flies are not a phenomenon that is not observed in normal populations, except that the frequency of such tumors

is definitely higher among the experimental flies.

The phenotypes observed after perfluoro-2-butene treatment were characterized by a blister wing filled with clear fluid at the time of eclosion. The blister later broke, leaving a crater lesion in the otherwise intact wing. The penetrance of this varied, sometimes both wings were affected and in other flies one wing, while some flies had only very small fluid filled areas in one or both wings.

After Freon-C318 treatment of the paternal male a unicorn type antenna development was found. This phenotype had not been listed by Lindsley and Grell (1967). A subculture obtained by selective breeding later produced five flies with this unusual type of antenna. The penetrance of the trait evidently varies, as the first instability at the antenna was noticed in the F_2. Many degrees of expression of a curly type wing were also observed after the Freon-C318 treatment.

Table 22 gives a summary of some phenotypes observed after treatment of Drosophila melanogaster with gases and/or gamma radiation. While it is realized that data from these studies are not sufficient to form conclusions concerning mutagenic specificity, results would warrant further investigation of this problem, using the fluorinated hydrocarbons as mutagens.

Unusual phenotypic effects induced by any new mutagen are interesting and should be noted, but under present

Table 22--Summary of deviant phenotypes obtained after treatment of Drosophila melanogaster with gases or gases and Co-60 irradiation.

Experimental treatment			Mutant types repeatedly observed*	Mutants	
gas exposure	Co-60 and gas			sex	description**
Genetron-23	+	+***	eye color	M F	apricot
Genetron-23	+	+	eye color	M F	orange
Genetron-23	+	+	eye color	M F	deep red Bar
Genetron-23	+	+	tumor	M F	black dot medial to eye, other tumors present****
Genetron-23	+	+	tumor	M F	abdominal
Genetron-23	+	+	tumor	M F	melanotic spots on wings
Genetron-152A	0	+	eye color	M -	white
Genetron-152A	+	+	eye color	M F	apricot
Genetron-152A	+	+	eye color	M F	orange
Genetron-152A	+	+	tumor	M F	black ropy wing with yellow exudate in clump****

*Outstretched, taxi, bent, cut, and slightly curly wings were too frequent among progeny of gas treated and gas and Co-60 treated P_1 Drosophila to enumerate.

**Based on literature (Lindsley and Grell, 1967).

***A + + indicates found in cultures from both radiated and non-radiated P_1 males.

****Not found in the literature.

Table continued.

Table 22 continued--Summary of deviant phenotypes obtained after treatment of Drosophila melanogaster with gases or gases and Co-60 irradiation.

Experimental treatment			Mutant types repeatedly observed*	Mutants	
gas exposure	Co-60 and gas			sex	description**
Perfluoro-2-butene	+	+***	eye color	M -	white
Perfluoro-2-butene	+	+	eye color	M F	orange
Perfluoro-2-butene	+	+	eye color	M F	apricot
Perfluoro-2-butene	+	+	eye color	M F	tomato; unusual eye color range among progeny
Perfluoro-2-butene	+	+	wing	M F	blister, burst, left crater; more ♀♀ than ♂♂ affected
Freon-C318	+	+	eye color	M F	deep red
Freon-C318	+	+	eye color	M F	apricot, dark
Freon-C318	+	+	eye color	M -	deep orange
Freon-C318	0	+	antenna	M F	antenna abnormalities fused unicorn type****
Freon-C318	+	+	tumors	M F	multiple

*Outstretched, taxi, bent, cut, and slightly curly wings were too frequent among progeny of gas treated and gas and Co-60 treated P_1 Drosophila to enumerate.

**Based on literature (Lindsley and Grell, 1967).

***A + + indicates found in cultures from both radiated and non-radiated P_1 males.

****Not found in the literature.

Table continued.

Table 22 continued--Summary of deviant phenotypes obtained after treatment of Drosophila melanogaster with gases or gases and Co-60 irradiation.

Experimental treatment		Mutant types repeatedly observed*	Mutants	
gas exposure	Co-60 and gas		sex	description**
Sulfur dioxide	0 +***	eye color	- F	apricot
Sulfur dioxide	0 +	tumors	M F	melanotic tumors on proboscis tip
Sulfur dioxide	0 +	eye color	- F	garnet
Nitrous oxide	0 +	eye color	M F	apricot, deep
Nitrous oxide	0 +	tumor	- F	proboscis base
Nitrous oxide	0 +	antenna	M F	antennaless

*Outstretched, taxi, bent, cut, and slightly curly wings were too frequent among progeny of gas treated and gas and Co-60 treated P_1 Drosophila to enumerate.
**Based on literature (Lindsley and Grell, 1967).
***A + + indicates found in cultures from both radiated and non-radiated P_1 males.
****Not found in the literature.

circumstances it cannot be determined if they have a genetic origin. It is thus impossible to apply any meaningful trend to them or to tell what is happening at the molecular level. This seems to be a newly recognized area of chemically induced instability, which makes an analysis of the mode of inheritance or transmissibility on a predictable basis most uncertain at present (Southin, 1966).

Browning (1968) stated that she has also encountered similar situations but that it is not possible to determine the genetic basis of these phenotypic differences within practical limitations. Similarly other workers have not yet explained the inheritance of tumor susceptibility and/or induction. Mathew (1964) interpreted the finding of mosaic daughters from a mosaic mother as the replication of an induced instability which cannot readily be explained on the basis of our present knowledge of mutagenesis.

Fahmy and Fahmy (1959) first claimed to have found Drosophila mutants specifically induced by certain chemicals, and that these mutants were different than previously observed phenotypes. A more moderate explanation suggested by Auerbach (1960) is that certain chemicals may produce a characteristic frequency distribution of mutations at different loci, and that these "new" mutants form the extreme end of this distribution. This may be the case with the unicorn antenna, as the mutations of antennae-less, arista-less and one antenna are known to occur. It would not be

surprising if the genes of Drosophila like those of bacteriophage and micro-organisms did show some degree of mutagen specificity.

In studies with gamma radiation effects on grain weevils Bull and Cornwell (1965) concluded that irradiation clearly differs in its effects on males and females, and that the susceptibility of both sexes to lethal doses is considerably modified by the method of culture. They found females more resistant to killing by gamma radiation than males. This is in agreement with the findings reported in Table 5. Not in the table, but observed, was a typical syndrome, of several days duration, which terminated in the death of these heavily irradiated Drosophila.

There is a growing awareness among geneticists that many diverse environmental factors, whether heat, cold, ionizing radiations, chemicals or gases, may produce mutations, sometimes acting selectively at one developmental stage or another. In Drosophila the mean lethal dose of radiation at the most sensitive state in the early fertilized egg may be as small as 100 R, while in the adult it may be as high as 100,000 R. As soon as environmental changes are made in the laboratory under experimental conditions and using special genetic techniques, it becomes possible to observe the exquisite response of the genetic material in many diverse organisms. Perhaps the outward species stability is the result of a meta-stable selective pressure reaction

between the DNA of any organism and its environment. A mutation might be considered a primary genetic level response state for DNA, rather than a rare inducible event.

From the data presented in this dissertation it is apparent that the frequency of melanotic tumors increased among the progeny of Drosophila exposed to certain Genetron and Freon gases, furthermore these deviant types became more frequent in later generations. Developmental abnormalities which could be pleiotropic effects of genetic change were also more common than in control populations; these abnormalities became more frequent in the fourth and fifth generation offspring of the treated flies.

All four of the fluorinated gases were found to be mutagenic to D. melanogaster under the experimental conditions employed. With the ever increasing use of industrial Freons and Genetrons it would seem that the indications of carcinogenesis and genetic damage in Drosophila exposed to these gases should be sufficient cause to place air pollution control high on the list of preventive medicine.

Chambers (1968), after citing obvious physiological effects of air pollution on animals and humans, suggested others from laboratory observations of specific enzyme inhibitions, and changes in blood chemistry. Bradshaw et al. (1965) provided evidence, accumulated over the past twenty years, indicating that the immediate process of evolution is rapid in observable animal and plant species. Dobzhansky

et al. (1966) cited changes in the chromosomal composition of natural populations of D. pseudoobscura over the last quarter of the century in the Pacific states. Oshima and Watanabe (1965) told of similar findings from Tokyo. Anderson et al. (1968) reviewed the evidence and found no meaningful difference between insecticide treated populations and controls, but cited new evidence of the sensitivity of adaptive values to even slight environmental variations. They offered no explanation for the rapid gene shift observed in these natural populations. Patterson and Stone (1952) provided well documented evidence that evolution occurs today in Drosophila. Much of the data are based on numerous laboratory studies cited in their book.

While it may take many generations for mutations and resulting selective pressures due to air pollution to be determined in humans, the research with Drosophila clearly indicates the necessity of adequate atmospheric controls.

A small increase in human mutation rates induced by air pollutants would not normally be detected in a short period of time by any direct observation. Large numbers of individuals could be exposed in metropolitan areas before danger is realized, resulting in increased frequency of genetic diseases in the future, as well as somatic damage in the current population.

The experiments conducted for this dissertation present evidence for this concept. It should be remembered that

changes produced by some of the gases tested were often not apparent until the F_3 generation.

For while modern medicine is lessening the selective pressures of infectious diseases and controllable genetic defects, it may be that chemical air pollutants and man made radiations are now inducing widespread and unknown adaptions, in the true evolutionary meaning, on every aspect of terrestrial life.

SUMMARY

1. The research described in this dissertation was designed to determine if selected gases would be mutagenic when administered alone to Drosophila melanogaster and to elucidate the effect of each gas, under experimental conditions delineated, when Co-60 irradiation was given to the organism in the gaseous atmosphere.
2. The genetic procedures employed in these studies were the Basc technique, generally used to detect recessive lethal mutations in Drosophila that arise in the genome of the treated male in the X chromosome loci which are hemizygous, and Muller's Maxy technique, which detects recessive mutations at specific loci on the X chromosome of the treated Maxy male. The latter method has the advantage that recessive mutations are viable and visible in the F_1 female.
3. Two completely fluorinated hydrocarbons, Freon-C318 (perfluorocyclobutane) and perfluoro-2-butene; two partially fluorinated gases, Genetron-23 (fluoroform) and Genetron-152A (1,1-difluoroethane); and two inorganic gases, nitrous oxide and sulfur dioxide, were used in these studies.

4. All data were subjected to applicable statistical treatment, using one or more of the following methods: t-test analysis, analysis of variance, and Stevens' test. The fluorinated hydrocarbons were found to affect the genome of exposed D. melanogaster, significantly increasing mutation rates in progeny Drosophila over control levels.
5. For each gas tested, pronounced phenotypic effects were observed among progeny of males exposed to the gas alone or to Co-60 radiation in the gaseous atmosphere. While most of the deviant types found after such treatment had been previously described in the literature, two of the tumors and the fused medial "unicorn" antenna phenotype were not described by Lindsley and Grell (1967). If these phenotypes have a genetic origin it was not determined.
6. After exposure to acute doses of gamma radiation, up to about 150,000 R, it was observed that females survived longer than males. A typical mode of death resulted, which was preceded by a progressive loss of coordination.
7. It is difficult to establish the relative sex linked recessive mutagenic effectiveness of the various gases studied, since the time of exposure of the flies to the gases and/or irradiation varied from experiment to experiment. Nevertheless, Genetron-23 and nitrous oxide appear to be more mutagenic than Genetron-152A or

Freon-C318. However, with radiation Genetron-152A produced the highest sex linked recessive mutation rate. Since Genetron-152A induced the most semi-lethals of any of the gases tested and it was least protective, its mode of action appears to be different than Genetron-23. Perfluoro-2-butene was more effective than nitrous oxide in decreasing the sex linked recessive lethal mutation damage to Drosophila. It is also recognized that an undetermined part of the observed mutagenic effects of the gases may be due to anoxia, and this aspect warrants further investigation.

Gas and radiation induced recessive sex linked lethal mutation rates were, by observation, not additive in effect. In general, the gas environment reduced mutation yield when compared to air.

8. Implications of this research may not be easily dismissed. The simple observation that melanotic tumors increased in frequency in the gas exposed Drosophila strains would be more than sufficient reason to expand these studies to numerous other gases, to other forms of living organisms, and in general to the establishment of further research into the long neglected but vitally important field of "mutagenic gas ecology."

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APPENDIX

Part A

Sample calculations of statistical analyses applied to this dissertation:

The t-test used for the data listed in Table 6 was calculated on the following basis:

Treatment	Lethals found %	Lethals induced in the number of chromosomes tested
Genetron-23	2.7	7.5/271
Control (no treatment)	0.23	3.0/1,300

The t-test was used for testing the significance of the difference between two means (gas vs. no gas).

p represents the mutant portion of the population.

q represents the non-mutant portion of the population.

$$p + q = 1.$$

$$1 - p = q.$$

The subscript g refers to gas treated samples.

The subscript o refers to untreated controls.

N_o = number of control chromosomes tested.

N_g = number of gas treated chromosomes tested.

N_{o+g} = total number of chromosomes tested.

S = standard deviation of the sample.

$$S^2 = \frac{pq}{N_{o+g}}$$

$$S = \sqrt{\frac{pq}{N_{o+g}}}$$

$$p = \frac{p_o + p_g}{N_o + N_g}$$

For the Data listed in Table 6:

$$p = \frac{3 + 7.5}{1,300+271} = 0.00732 \qquad q = 1 - 0.00732 \quad q = 0.99268$$

$$S^2_{p_o} = \frac{0.00732 \times 0.99268}{1,300}$$

$$S^2_{p_g} = \frac{0.00732 \times 0.99269}{271}$$

$$S^2_{p_{go}} = 0.00732 \times 0.99268 \ (1/1,300 + 1/271)$$

$$= 0.007266 \times 0.00445$$

$$= 0.000032335$$

$$S_{p_{go}} = \sqrt{0.00003233548}$$

$$= 0.0057$$

$$t = \frac{\%p_g - \%p_o}{S_{p_{go}}}$$

$$t = \frac{0.027 - 0.0023}{0.0057} = \frac{0.0247}{0.0057} = 4.3$$

Interpretation:

The probability is read from the t chart entering at infinity degrees of freedom because of the large number of samples (N_{g+o}). The confidence levels at ∞ degrees of freedom (df).

$$t = >1.96 \quad P = 0.05$$

$$t = >2.58 \quad P = 0.01$$

If the probability is less than 0.05 the means are regarded as significantly different. If the probability is less than 0.01 the difference is highly significant. In this experiment

$$t = 4.3 \text{ which is } >2.58 \therefore P = 0.01.$$

This indicates that 4.3 as shown in the sample calculation for Table 6 is highly significant. The conclusion is that Genetron-23 gas treatment caused an increase in recessive mutations over the control (no treatment) in the genome of P_1 treated male Drosophila melanogaster. Genetron-23 was mutagenic.

Part B

Calculations for the statistical analysis of the Genetron-23 gas experiment by the $\sqrt{x + 1}$ method:

The statistical method applied was an analysis of variance. A 2 x 2 factorial design was used to detect interactions. Two factors were tested at two levels each, with five observations (of 200 samples each) per combination. The data for all treatments are given in Table 7.

The square root transformation of data, $(\sqrt{x + 1})$, was determined according to Snedecor (1959, p. 315). The preliminary calculations are shown below:

	Treatment			
Replication	Control	Gas	Radiation	Radiation plus gas
1	1.4	1.6	3.6	3.4
2	1.0	2.0	3.8	2.6
3	1.0	2.4	3.1	3.3
4	1.4	1.7	3.7	3.1
5	1.0	2.2	3.3	3.4
Total	5.8	9.9	17.5	15.8
Mean	1.16	1.9	3.5	3.16

The F-test was used to find the ratio of each Mean Square to the within (error) Mean Square. Critical values of the variance ratio, F, are given in Table 7 (Goldstein, 1967).

Calculations for Analysis of Variance

1. Mean of Control $= \dfrac{\Sigma \text{ (of totals from transformed data table)}^2}{\text{Number of replications}}$

$= \dfrac{(5.8 + 9.9 + 17.5 + 15.8)^2}{5}$

$= \dfrac{(49)^2}{5}$

$= \underline{\underline{120.05}}$ (1)

2. Total Sum of Squares (Total SS) $=$ Σ (number of lethals from each treatment + number of replications added to each) (From Table 7) $-$ mean of control (1)

$= (2+5)+(15+5)+(58+5)+(47+5)-120.05$

$= 7 + 20 + 63 + 52 - 120.05$

$= 142 - 120.05$

$= \underline{\underline{21.95}}$ (2)

3. Sum of Squares of Means $= \dfrac{\Sigma \text{ of each of the 4 totals squared (From Transformed Data)}}{\text{Number of replications}}$

$= \dfrac{(5.8)^2 + (9.9)^2 + (17.5)^2 + (15.8)^2}{5}$

$= \dfrac{687}{5}$

$= \underline{\underline{137.508}}$ (3)

4. Treatment Sum of Squares = Sum of squares of means (3) - Mean of control (1)

= 137.508 - 120.05

= 17.458 (4)

5. Natural variability within Sum of Squares (SS) = Total SS (2) - Treatment SS (4)

= 21.95 - 17.458

= 4.492 (5)

6. Mean Squares (for 4 treatments = 3 degrees of freedom = $\frac{\text{Treatment SS (4)}}{\text{3 degrees of freedom}}$

= $\frac{17.458}{3}$

= 5.819 (6)

7. Calculation of Total Degrees of Freedom (df).

Total df = Total number of replications - 1 (n-1)

= 20 - 1

= 19 (7)

8. Calculation of Degrees of Freedom for Treatments:

Treatments given = 4 = 1. Gas vs. no gas
2. Radiation vs no radiation
3. Interaction radiation and gas.

$$\text{Degrees of freedom for treatments} = \text{number of treatments} - 1$$

$$= 4 - 1$$

$$= \underline{3} \qquad (\underline{8})$$

9. Calculation of Source of Variation Within (Error)

$$\text{Within (Error)} = \text{Total degrees of freedom } (\underline{7}) - \text{Treatment df } (\underline{8})$$

$$= 19 - 3$$

$$= \underline{16} \qquad (\underline{9})$$

10. Calculation of Mean Square of the Error (variability within)

$$\text{Mean Square of the Within (Error)} = \frac{\text{SS } (\underline{5})}{\text{df of error } (\underline{9})}$$

$$= \frac{4.492}{16}$$

$$= \underline{0.281} \qquad (\underline{10})$$

11. Mean Square of Gas (in presence of irrad) vs. no gas (in presence of irrad) (From transformed data)

$$= \frac{\left(\text{total of control} + \text{total of radiation} - \text{total of gas} - \text{total of radiation with gas}\right)^2}{\left(\text{number of treatments}\right) \times \left(\text{number of replications for each treatment}\right)}$$

$$= \frac{(5.8 + 17.5 - 9.9 - 15.8)^2}{4 \times 5}$$

$$= \frac{5.76}{20}$$

$$= \underline{0.288} \qquad (\underline{11})$$

12. F value for mean square of gas vs no gas (both in presence of irradiation (11)

$$F \text{ value} = \frac{\text{mean square of gas vs no gas (both in irradiation) (11)}}{\text{mean square of error (10)}}$$

$$= \frac{0.288}{0.281}$$

$$= \underline{\underline{1.00}} \qquad (12)$$

13. Mean square of radiation vs no radiation (from transformed data)

$$= \frac{\left(\begin{matrix}\text{total of} \\ \text{control}\end{matrix} + \begin{matrix}\text{total of} \\ \text{gas}\end{matrix} - \begin{matrix}\text{total for} \\ \text{radiation}\end{matrix} + \begin{matrix}\text{total of radiation} \\ \text{with gas}\end{matrix}\right)^2}{\left(\begin{matrix}\text{number of} \\ \text{treatments}\end{matrix}\right) \times \left(\begin{matrix}\text{number of replications} \\ \text{for each treatment}\end{matrix}\right)}$$

$$\text{Mean square} = \frac{(5.8 + 9.9 - 17.5 + 15.8)^2}{4 \times 5}$$

$$= \frac{(15.7 - 33.3)^2}{20}$$

$$= \frac{(17.6)^2}{20}$$

$$= \underline{\underline{15.488}} \qquad (13)$$

14. F value for radiation vs no radiation

$$F = \frac{\text{mean square of radiation vs no radiation (13)}}{\text{mean square of error (10)}}$$

$$= \frac{15.488}{0.281}$$

$$= \underline{\underline{55.1}} \qquad (14)$$

Further analysis of treatments:

15. Calculations for interaction of gas and radiation

Mean square of interaction of gas and radiation

$$= \text{SS of Treatment } (\underline{4}) - \text{Mean square of rad. vs no rad. } (\underline{13}) - \text{Mean square gas vs no gas (in rad) } (\underline{11})$$

$$= 17.458 - 15.488 - 0.288$$

$$= \underline{\underline{1.682}} \qquad (\underline{15})$$

16. F value for interaction of gas and radiation

$$F = \frac{\text{mean square for interaction of gas and irradiation } (\underline{15})}{\text{mean square of error } (\underline{10})}$$

$$= \frac{1.682}{0.281}$$

$$= \underline{\underline{5.99}} \qquad (\underline{16})$$

17. F value for treatments

$$F = \frac{\text{mean square for treatments } (\underline{6})}{\text{within (error) mean square } (\underline{10})}$$

$$= \frac{5.819}{0.0281}$$

$$= \underline{\underline{20.7}} \qquad (\underline{17})$$

For the analysis of variance derived from "Preliminary Calculations" see the listed numbers at the beginning of Part B. The method of arriving at each number shown below has been demonstrated in the calculations.

Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Variance est. mean squares	Variance ratio F
Total	19	21.95	-	-
Treatment:	3	17.458	5.819	20.7
1) Gas vs no gas	1	-	0.288	1.0
2) Radiation vs no rad	1	-	15.488	55.1
3) Interaction rad & gas	1	-	1.682	5.99
Within (Error)	16	4.492	0.281	-

By entering Table 7 (Goldstein, 1967) with the F values shown above the critical values of the variance ratios are determined. The table is entered with DF associated with the greater of the two variance ratios and entered at DF' with the degrees of freedom associated with the smaller of the two variance ratios. In each of the above cases the Within (Error) degrees of freedom, 16, DF' would be entered at 16 because its mean ratio of 0.281 is the smaller mean variance ratio.

The interpretations of the above data are given in the text of the dissertation, following Table 7.

The following listing gives the upper and lower fiducial limits of the expectation of a Poisson Distribution and is more accurate than the standard error test (Stevens, 1942). Sample calculations using the listings follow: 7 mutations were found among 3,708 chromosomes tested, what are the limits to this mutation rate? $n = 7$, $N = 3{,}708$, $p = n/N = 0.189\%$. Enter the listing at 7 and the lower limit is 2.0; the upper limit is 17.1, dividing each of these by 3,708, the limits to the mutation rate are 0.05% and 0.46%. The result contradicts rates outside these limits at the 0.01 confidence level. If a control mutation rate is found by the method to have an upper limit of 0.02%, which does not overlap the lower limit of the induced 0.05% rate, findings are significant.

CONFIDENCE LIMITS FOR THE EXPECTED VALUE OF A POISSON DISTRIBUTION

Total observed count $x' = \Sigma x_i$	Significance level				Total observed count $x' = \Sigma x_i$	Significance level			
	$\alpha = 0.01$		$\alpha = 0.05$			$\alpha = 0.01$		$\alpha = 0.05$	
	Lower Limit	*Upper Limit*	*Lower Limit*	*Upper Limit*		*Lower Limit*	*Upper Limit*	*Lower Limit*	*Upper Limit*
0	0.0	5.3	0.0	3.7					
1	0.0	7.4	0.1	5.6	26	14.7	42.2	17.0	38.0
2	0.1	9.3	0.2	7.2	27	15.4	43.5	17.8	39.2
3	0.3	11.0	0.6	8.8	28	16.2	44.8	18.6	40.4
4	0.6	12.6	1.0	10.2	29	17.0	46.0	19.4	41.6
5	1.0	14.1	1.6	11.7	30	17.7	47.2	20.2	42.8
6	1.5	15.6	2.2	13.1	31	18.5	48.4	21.0	44.0
7	2.0	17.1	2.8	14.4	32	19.3	49.6	21.8	45.1
8	2.5	18.5	3.4	15.8	33	20.0	50.8	22.7	46.3
9	3.1	20.0	4.0	17.1	34	20.8	52.1	23.5	47.5
10	3.7	21.3	4.7	18.4	35	21.6	53.3	24.3	48.7
11	4.3	22.6	5.4	19.7	36	22.4	54.5	25.1	49.8
12	4.9	24.0	6.2	21.0	37	23.2	55.7	26.0	51.0
13	5.5	25.4	6.9	22.3	38	24.0	56.9	26.8	52.2
14	6.2	26.7	7.7	23.5	39	24.8	58.1	27.7	53.3
15	6.8	28.1	8.4	24.8	40	25.6	59.3	28.6	54.5
16	7.5	29.4	9.4	26.0	41	26.4	60.5	29.4	55.6
17	8.2	30.7	9.9	27.2	42	27.2	61.7	30.3	56.8
18	8.9	32.0	10.7	28.4	43	28.0	62.9	31.1	57.9
19	9.6	33.3	11.5	29.6	44	28.8	64.1	32.0	59.0
20	10.3	34.6	12.2	30.8	45	29.6	65.3	32.8	60.2
21	11.0	35.9	13.0	32.0	46	30.4	66.5	33.6	61.3
22	11.8	37.2	13.8	33.2	47	31.2	67.7	34.5	62.5
23	12.5	38.4	14.6	34.4	48	32.0	68.9	35.3	63.6
24	13.2	39.7	15.4	35.6	49	32.8	70.1	36.1	64.8
25	14.0	41.0	16.2	36.8	50	33.6	71.3	37.0	65.9

VITA

Virginia Campbell Foltz, the daughter of Grace Griffey Campbell and the late Hosea W. Campbell, was born in Ashtabula, Ohio, and was graduated from Ashtabula High School. Married to Daniel Shannon Foltz, she has two sons, James and Richard; a daughter-in-law, Margarita Orozco Foltz; and two grandsons, Heinrich and Werner.

Mrs. Foltz received the B.S. degree, with honors, from Baldwin-Wallace College. Her major was biology, with minors in chemistry and French. Later she took graduate courses from the University of Vermont and Boston University.

After completing her work toward the M.S. degree, with a major in biology and a minor in biochemistry, at the University of Houston, she continued graduate studies in the new doctoral program at the Texas Woman's University.

Among the positions held by Mrs. Foltz were editor and indexer for a publishing firm, professional work with the hard of hearing, and teacher in the public schools in Vermont. While a graduate student at the University of Houston she held a Teaching Assistantship and taught biology, genetics, and comparative anatomy laboratories, 1960-1963, and was a substitute lecturer in genetics, Spring 1963. In 1963-1964

she was lecturer in freshman biology. She did her research for the master's degree in the Rice University laboratory of Dr. Edgar Altenburg, 1961-1963.

At the Texas Woman's University, Mrs. Foltz performed the following duties: Graduate Assistant, 1964; Lecturer and Laboratory Instructor in heredity, Spring 1965; Teaching Assistant, mammalian physiology laboratory and freshman biology laboratory, Summer 1965; Research Assistant in histochemistry, 1965-1967; Teaching Assistant, freshman biology laboratories, 1966-67; and genetics research, 1968 to 1969.

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Mrs. Foltz is a member of Beta Beta Beta, the Texas Woman's University Club of the Society of the Sigma Xi, the North Texas Biological Society, and The Genetics Society of America.

This dissertation was typed by Mrs. Carole Normile.