USE OF 5-BROMODEOXYURIDINE AS A MUTAGENIC AGENT IN *DROSOPHILA MELANOGASTER*

A Thesis

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Virginia Campbell Foltz

August 1966

ACKNOWLEDGMENT

The author wishes to express appreciation to Dr. Luolin Browning for guidance during this research and to Dr. Sara Huggins for advice and encouragement. Completion of this work would not have been possible without the cooperation and understanding of her family.

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ABSTRACT

1. Young males of Muller's "Maxy" stock were administered BUDR by abdominal injection and then mated with virgin Drosophila melanogaster of the "Maxy" stock. The resulting F1 females were examined for visible mutations at 14 selected loci on the paternally derived X-chromosome.

2. Normal appearing virgin F1 females were individually mated with special male stock according to Muller's "Maxy" technique to detect lethal mutations which arose in the paternally derived X-chromosome of the F1 female.

3. Experimental methods and criteria for classifying both visible and lethal mutations were described.

4. Among 6,598 F1 females examined 12.5 mutations arising on the X-chromosome of the BUDR treated male were scored. This would be 1 mutation per 528 chromosomes tested or a .19% rate. This may be compared with a spontaneous mutation rate of 1 mutation per 12,564 chromosomes or .008% found by previous workers with the "Maxy" stock. Visible mutations among F1 females carrying the X-chromosome from the BUDR treated male were found to be about 24 fold the spontaneous rate.

5. Lethal mutations were found among 5 of 1,231 chromosomes tested or one lethal mutation per 246

chromosomes, a rate of .41%. In previous spontaneous lethal mutation studies in "Maxy" stock 14 lethal mutations in 4,082 paternally derived X-chromosomes were reported. This would be a .34% spontaneous rate. Among the BUDR treated paternal chromosomes 17 semi-lethal mutations per 1,231 chromosomes were found. One chromosome out of 72 would bear a semi-lethal mutation; this would be a rate of about 1.4%.

6. Many of the visible whole body mutations were untransmitted because the females bearing them did not reproduce. Some of these may actually have been fractional mutations. The high rate of semi-lethal mutations could be an indication that at least some of the mutations scored as whole body may actually have been mosaics.

7. BUDR, as used in this research, was found to be a potent mutagenic agent in Drosophila melanogaster.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Research in the science of genetics was started shortly after DeVries, Correns, and von Tschermak rediscovered the papers of Mendel in 1900. This research began with the study of genes which behaved as unit factors in sexual inheritance in higher plants and animals. The unit observed was not the gene itself but its effect upon the organism. Inasmuch as the results of single gene differences can be very complex, the selection of suitable organisms for genetic study has great importance.

Over the past fifty-five years an enormous amount of information has been gained from genetic research with Drosophila melanogaster. Muller and Altenburg began studies on mutation frequency as early as 1918. Many workers contributed to the improvement and standardization of genetic techniques so mutation rates could be better studied.

In 1921 Muller and Altenburg had evidence on the borderline of significance that increase in temperature caused mutation. However, Muller (1928) had definite evidence of the effect of temperature on the mutation rate. 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 mutations.

Altenburg (1933) induced mutations in Drosophila melanogaster by treating polar caps of fertilized eggs with ultra-violet rays and concluded that ultra-violet rays probably had less destructive effects than X-rays on chromosomes. Later it was demonstrated that the mutational effect of different U. V. wave lengths was proportional to the absorption of the U. V. light by the DNA of the chromosomes. (Stradler, 1942 and Hollaender and Emmons, 1946).

Next came the discovery that certain chemicals had powerful mutagenic effects (Auerbach and Robson, 1946; Rapoport, 1946). Herskowitz (1951) gives a very complete list of chemical mutagens studied in the next few years. The requirements for a chemical mutagenic agent are the ability to penetrate the cells and the ability to alter the chemical structure of the genetic material of the cell. Muller and Mott-Smith (1930) have calculated that only about 1/2000 of the spontaneous mutations in the Drosophila could be ascribed to natural background irradiation; this leaves 1999/2000 of the spontaneous mutations to be explained. Chemical mutagens could account for some of these.

A fairly recent trend in experimental work is to use the analogs of purine and pyrimidine bases as mutagenic

agents. In 1956 Litman and Pardee reported production of bacteriophage mutants by a disturbance of deoxyribonucleic acid metabolism. Freese, Benzer, and Freese in 1958 reported on the induction of specific mutations with 5-bromouracil. Under the feeding techniques they used, the 5-bromouracil was incorporated into the DNA of phage instead of thymine. They concluded that these induced mutants showed a lower reversion index than do spontaneous mutations, and that the analog has a specific effect rather than general, indicating change in specific places in genetic structure. Small alterations rather than gross changes were found.

Hsu and Somers (1962) working with cells of LM (a subline of strain L mouse fibroblasts) were able to incorporate into its DNA 5-bromodeoxyuridine (BUDR) to replace thymidine. They found extensive cellular damage, manifested as polyploidization, chromosome breakage, and cellular death as the result of BUDR treatment. After a period of time the surviving cells became BUDR resistant, although they still maintained a high proportion of the analog in the DNA. The growth capacity resumed and the frequency of chromosome breakage was reduced to barely above the control value. They concluded that the reduction of growth power by BUDR treatment appeared to be the result of indirect cellular damage, namely, the production of numerous chromosomal lesions and mutations which in turn inhibited growth. The

BUDR resistant populations were found to be more resistant to radiation damage than the original LM cells treated with BUDR for a short period (Humphrey, Somers, and Dewey, 1961).

The time that a mutagen is introduced into the organism 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 to induce mutations it is necessary to add formaldehyde to the larval food. Alderson (1961) then made the interesting discovery that formaldehyde added to larval food which does not contain adenosine or adenylic acid is not mutagenic, and that the addition of either of these compounds in the presence of formaldehyde recreates the mutagenic environment. These results can be interpreted to mean that formaldehyde reacts with these compounds to produce a metabolite which may be introduced into the germ cells' DNA. In other words, an analog of a purine or pyrimidine may form within the cell which could be similar to BUDR in function.

The fact that mutation is under genetic control is evidence that the genetic material must react with other genetic materials to bring about mutation, as shown by the fact that there are genes that can increase the mutation rate. There is also some evidence that the cell may provide for its own mutations by certain of its products of metabolism reacting with its genetic material. Jensen et al

(1951), and Wyss et al (1948) in experiments with catalase and inhibitors of catalase obtained indirect evidence that hydrogen peroxide produced as the result of aerobic respiration may be a factor in determining part of the spontaneous mutation rate. If catalase is added together with hydrogen peroxide in the treatment of Neurospora conidia or bacteria, the mutagenic activity of the hydrogen peroxide is stopped. If catalase poisons are added with hydrogen peroxide, the mutagenic activity is increased in Neurospora.

Sparrow (1961) has stated that the number of chemicals which have been demonstrated to influence the mutagenicity of x-rays is now over one hundred. The fact that mutations may occur more frequently at one stage of the life cycle 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.

At the time this research was undertaken no report could be found in the literature concerning the use of 5-bromodeoxyuridine (BUDR) as a mutagenic agent in studies with Drosophila melanogaster. This paper reports the results of such a study.

CHAPTER II

MATERIALS AND METHODS

The halodeoxyuridine used in this research was 5-bromodeoxyuridine (BUDR), a nucleoside of uracil. Its structural formula is shown in Fig. 1. The BUDR was obtained from the Nutritional Biochemical Corporation of Cleveland, Ohio.

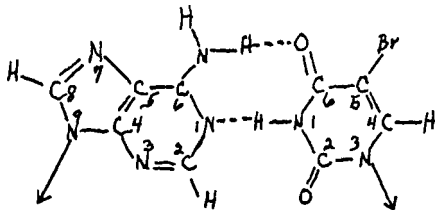
After some preliminary experimentation the technique decided upon involved injecting the analog directly into the abdomen of the newly hatched Drosophila melanogaster male. A .001 M solution was used in all three series of experiments. Injection pipettes were made by drawing tips of medicine droppers to very fine points in the flame of a blow torch. Flies were lightly etherized, a few at a time, and magnification 150X under a dissecting scope used.

Genetic Technique

A special stock of Drosophila melanogaster, constructed by Muller (1954, 1955), and designated by him as the "Maxy" stock was used. This stock enables the detection of well-studied and commonly occurring visible mutations at 14 selected loci on the X chromosome. This chromosome bearing the mutant alleles is called the "Maxy" chromosome,

(a) Normal keto state, with a hydrogen atom in N_1 position. Bond is to adenine.

Adenine

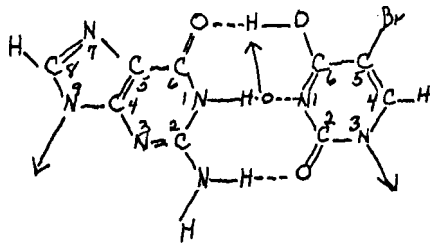


5-Bromodeoxyuridine (normal keto state)

deoxyribose

(b) In enol state, a tautomeric shift of the hydrogen atom determines specific pairing with guanine.

Guanine



5-bromodeoxyuridine (enol state)

deoxyribose

Fig. 1

PAIRING POSSIBILITIES OF 5-BROMODEOXYURIDINE IN ITS TAUTOMERIC STATES

each of the other two X chromosomes is called the 1J1 chromosome, one bears the mutant allele of vermilion while the other does not.

The operation of this specific locus method may be explained with the aid of Fig. 2, which shows the genotypic construction of parents (P_n) and offspring (P_{n+1}) in any two successive generations. Fig. 3 shows the possible gametes and zygotes produced by the P_n generation. This "Maxy" technique is a means of detecting any mutation which may occur at one of the 14 selected loci in the X chromosome of the male. "Maxy" females have the "Maxy" chromosome which carries the recessive alleles at these 14 loci. These loci were selected because, as before mentioned, they have been well studied as to their mutagenicity under a variety of agents, are known to mutate spontaneously, and are sharply defined phenotypically. This latter characteristic is especially valuable in reducing personal observational bias, which is inevitable if all types of aberrant organisms, genetic or otherwise, are observed. There is no reason for believing that other loci are different in their mutagenic capabilities from the 14 selected, nor has evidence been produced that any agents act differently on these loci compared to other loci. In addition this "Maxy" X allows the recessive vermilion eye marker to be expressed when present in the 1J1 chromosome. That is, the P_n male is vermilion,

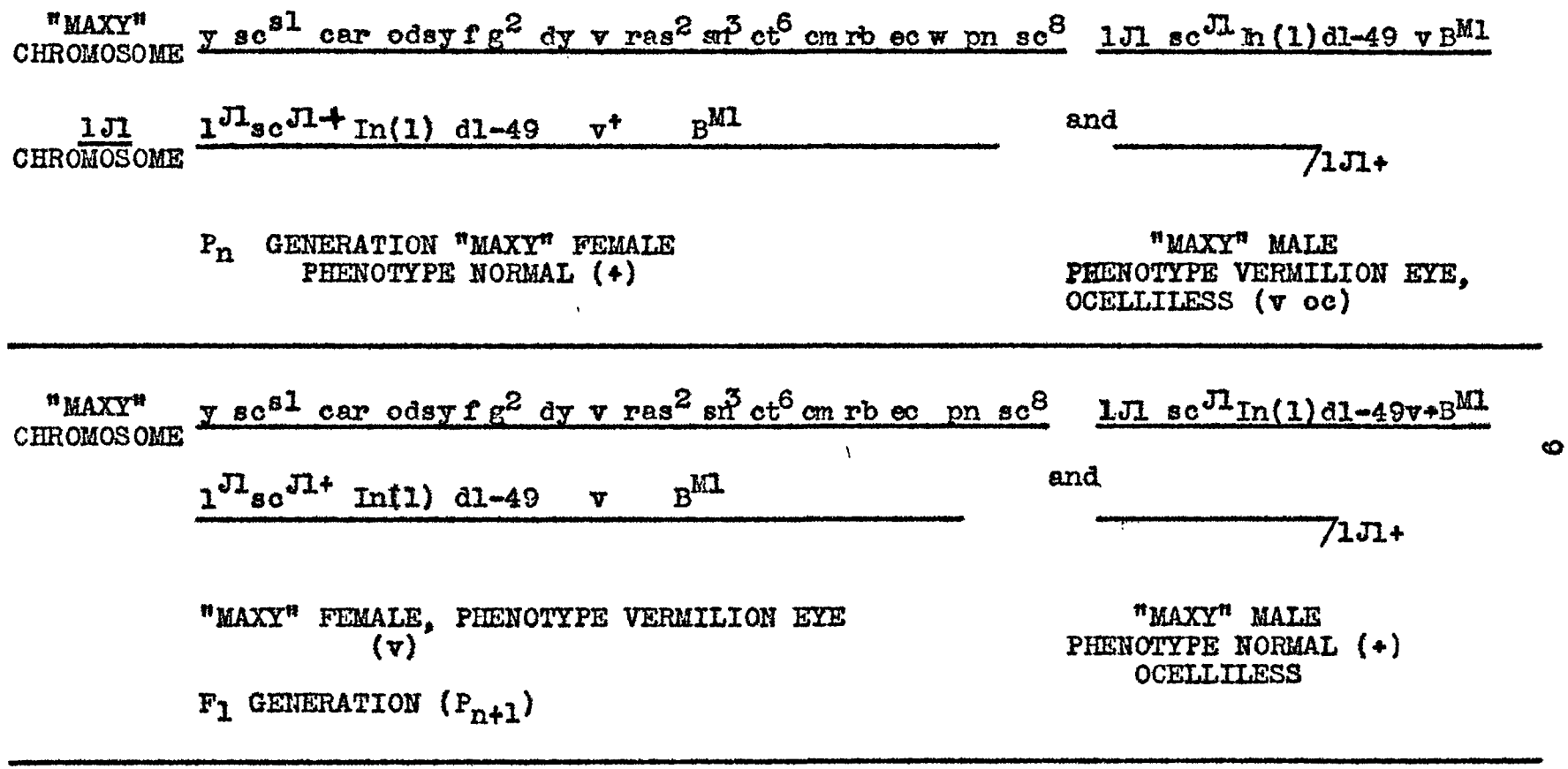


Fig. 2

GENOTYPIC CONSTRUCTION OF PARENTS (P_n) AND OFFSPRING IN F_1 (P_{n+1}) IN ANY TWO SUCCESSIVE GENERATIONS. PHENOTYPES ARE GIVEN IN PARENTHESIS.

		REGULAR EGGS		NON-DISJUNCTIONAL EGGS	
		1J1	"MAXY"	1J1 "MAXY"	NO-X
REGULAR SPERM	1J1	(1J1) FEMALE DIES	(v) FEMALE	(XXX) DIES OR STERILE	(1J1) MALE DIES
	Y	(1J1) MALE	(1 MAXY) MALE DIES	(+) FEMALE	(NO-X) DIES
NON-DISJUNCTIONAL SPERM	1J1 / y	(sc^{J1}) FEMALE	(v) FEMALE	(XXX) DIES OR STERILE	($sc^{J1}{}_{v}$) MALE
	NO X OR Y	(sc^{J1}) FEMALE	(1 MAXY) MALE DIES	(+) FEMALE	J1(NO X) DIES

Fig. 3

GAMETES AND ZYGOTES PRODUCED BY P_n GENERATION. PHENOTYPES OF ADULTS DERIVED FROM ANY ZYGOTE ARE SHOWN IN PARENTHESIS.

his daughters (F_1) vermilion and so on. The loaded "Maxy" chromosome carries Inversions $\underline{sc}^{sl}$ and $\underline{sc}^{8}$ with the two breakage points of the inversion just to the right of these symbols. The recessive lethal mutation, l, is located to the right of prune. The other X chromosome, carrying the normal alleles, and designated the 1J1 because it carries the lethal J1 also carries moderate-sized inversions In49 and B^{M1} with the mutant gene ocelliless inserted. This serves as a check on the reproduction of non-disjunctional flies as females homozygous for this gene are sterile. The visible effect of this gene does not interfere with the detection of any phenotype brought about by a mutation at any of the specific loci. See Table 1 for a list of the 14 loci with an interpretation of the gene symbols.

Detection of Visible Mutations

Any recessive mutation produced by the BUDR in the male 1J1 chromosome may be expressed in the P_{n+1} female if it occurs at any one of the 14 loci mutant in the "Maxy" chromosome. Of course any dominant viable mutation, not allelic to any of the specific loci may also be detected in the daughter (F_1). If they are dominant, even though lethal when homozygous, they may be detected in the female. The lethal gene in the "Maxy" chromosome kills the "Maxy" bearing male either in the egg or at a very early larval stage. This

TABLE 1

GENE SYMBOLS AND PHENOTYPES OF 14 MUTANT LOCI
ON "MAXY" CHROMOSOME

	GENE SYMBOL	PHENOTYPE
1.	y	yellow body
2.	car	carnation eyes
3.	odsy	outstretched wings, small eyes
4.	f	forked bristles
5.	g^2	garnet eyes
6.	dy	dusky wings
7.	ras^2	raspberry eyes
8.	sn^3	singed bristles
9.	ct^6	cut wings
10.	cm	carmine eyes
11.	rb	ruby eyes
12.	ec	echinus (rough eyes)
13.	w	white eyes
14.	pn	prune eyes
	*v	vermilion eyes
	*oc	ocelliless (lack 3 "simple" eyes)

*MARKERS

lethal makes scoring easier as it eliminates one class of males and it also makes more food available to individuals who will be scored as adults.

The "Maxy" male has a special Y chromosome which contains attached to the long arm of the Y a duplication of the left end of the X designated as $\underline{lJl^{+}}$. This duplication allows the males to live even though they have the lethal $\underline{Jl}$ in their X. As a result of the balanced recessive lethals the Maxy stock normally produces only two types of flies. See Fig. 2. The F_1 individuals are genotypically like their parents except that the vermilion eye marker has criss-crossed between the sexes. The males are now $\underline{v^{+}}$ and the females $\underline{v}$. Individuals produced from non-disjunctional gametes may be recognized because of the wrong eye color. In Fig. 3 the phenotypes of adults derived from any zygote are shown in parenthesis.

Any mutant females found have to be crossed to male stock having the normal alleles of lethal $\underline{Jl}$ in both the X and Y chromosomes in order to incorporate the mutant chromosome into a stock. The special stock used for this purpose has been designated the f41 stock of Muller. The X chromosome of the male carries two markers, yellow body and forked bristle plus an Inversion c2. The Y chromosome may be written $sc^{8}Y$. The sc^{8} contains the normal allele of $\underline{lJl}$ as well as the normal allele of yellow, and thus appears

non-yellow. For the next generation, young non-yellow daughters were crossed to males like their father. These daughters carry the X chromosome from their mother which is the treated X chromosome from the BUDR injected P_1 male.

Detection of Lethal and Semi-Lethal Mutations

The frequency of lethal mutations arising as a result of the BUDR treatment can also be detected by the "Maxy" stock. From the F_1 females being scored for visibles, normal flies young enough to be virgins were mass mated to f41 males and then placed in individual vials with two f41 males. The absence of sons in a vial with an otherwise normal population is presumed evidence of a lethal arising in the 1J1 treated chromosome of the P_1 male. Semi-lethal mutations can be detected by a high ratio of females to males. A ratio of eleven females to one male is considered evidence of a semi-lethal mutation. Whenever a sex ratio of four females to one male was observed further tests were carried out.

Breeding Methods--First Series of Experiments

During the first series of experiments all flies were cultured in bottles containing a standard food medium. The temperature of the laboratory was maintained at about 24-25° C. After injection males were allowed a recovery period before being mated. It was found that because of the

high mortality rate about 150 males would have to be injected in order to have 60 or 75 males to start an experiment. Table 2 shows shortening of the life span of the injected males as well as fertility information regarding surviving males. Figures for an equal number of controls injected with distilled water are also shown. The survival rate depends on the skill of the operator to a certain extent. No buffer was used because long experience with chemical injections into the abdomen of Drosophila has shown that distilled water is satisfactory.

Fig. 4 shows the brooding pattern followed. For brood 1, five injected males were placed in each culture bottle with fifteen virgin "Maxy" females. After two days with the first group of females, the flies were lightly etherized and the males placed in a fresh culture bottle with another fifteen "Maxy" virgins. This was designated brood 2. The females from the first culture bottles were placed in fresh food bottles and labeled brood 1A. At the end of the next two days the flies in brood 2 bottles were etherized and the males placed with fifteen Maxy virgins in fresh food bottles and designated brood 3. Females from brood 1A culture were placed in fresh food bottles and called brood 1B. Successive broods were made according to the above pattern until all males were dead, usually at the end of brood 3. Females, however, were placed

TABLE 2

RESULTS OF SURVIVAL CHECK FOR BUDR INJECTED MALES
CONTROLS INJECTED WITH DISTILLED WATER

TEST	BUDR INJECTED	CONTROLS*	24 HOUR SURVIVAL		3 DAY SURVIVAL	6 DAY SURVIVAL
			BUDR	CONTROLS		
1	50	50	46	45	14	4
2	30	30	27	28	9	3
	80	80	73	73	23	7
% SURVIVAL			91%	91%	29%	9%

*In controls injury from injection with distilled water caused death within 24 hours. After 24 hours survival for controls did not differ from uninjected flies and so was not listed.

MALE FLIES		FEMALE FLIES	BROOD NO.	DAY
BUDR Injected males	+	Virgin females	1	0-2
		↓ transfer		
		Inseminated females	1A	2-4
		↓ transfer		
	transfer	Inseminated females	1B	4-6
		↓ transfer		
		Inseminated females	1C	6-8
BUDR Injected males	+	Virgin females	2	2-4
		↓ transfer		
		Inseminated females	2A	4-6
		↓ transfer		
	transfer	Inseminated females	2B	6-8
		↓ transfer		
		Inseminated females	2C	8-10
BUDR Injected males	+	Virgin females	3	4-6
		↓ transfer		
		Inseminated females	3A	6-8
		↓ transfer		
		Inseminated females	3B	8-10
		↓ transfer		
		Inseminated females	3C	10-12

Fig. 4

GENERAL METHOD FOLLOWED IN MAKING BROODS

Series 1 results were given as totals for each experiment.
Series 2 results were tallied by broods.

in fresh culture bottles every two days and the broods labeled until the yield per bottle became small. The aim was to fractionate the germ line of each injected male to the greatest extent possible.

Breeding Methods--Second Series of Experiments

During the next series of experiments it was decided to breed the flies in vats so each active male would have maximum chance of encountering females. Each vat has the food capacity of about seven of the usual half-pint culture bottles. Again it was necessary to inject about 150 males in order to have 60 to 75 males to mass mate to 300 to 400 "Maxy" virgins in a vat. Food and laboratory conditions were as described for the first series of experiments. A similar method of designating broods was also used, with the later broods again being made in bottles when the surviving males became too few to justify use of a vat. Lethal checks were made as before with one "Maxy" female mated to several f#1 males in each vial. Several hundred lethal checks were made for each experiment.

Since most injected males were dead within a week, the sperm tested were ejaculated within this week. Sperm held in the reproductive tracts of females and used in later broods (when inseminated females were placed in culture bottles) were aged somewhat. Muller (1946) has shown that

spermatozoa expended during the first week of adult life contain a frequency of naturally occurring mutations two to three times as high as sperm expended in following weeks. This factor will have to be considered in discussion of the results.

Method of Scoring Mutations

The total population in a vat or bottle were etherized, and the flies were lined up in rows on the stage of the dissecting scope. During the first scaning the flies were sexed with the females brushed into separate rows. This was done quite rapidly and the F_1 females were observed for mutations. It was necessary to look at eyes, bristles, wings, and body color for Maxy type mutations. In order to check for partial mutations both eyes as well as the abdomen of each female had to be observed. At this point normal appearing F_1 females, young enough to be virgins were set aside for lethal checks. Flies of both sexes from bottle cultures were counted and discarded. To estimate the number of flies in vat populations, the flies were not counted but dehydrated and then weighed. This method has been found accurate to within 3%.

Three counts of the F_1 generation were made from each culture, with the first count made about two days after the first flies eclosed and the third and last count made

on the ninth or tenth day with the culture then being discarded.

Scoring of Lethal Checks

In preliminary scoring of lethal checks, vials in which about equal numbers of males and females could be observed were discarded without further attention. Vials in which no males or few males could be observed were individually checked and young females from each one mated to males of f41 stock to make an F_3 check. Checks of a number of lethal and semi-lethals were carried out to the F_4 generation with interesting results.

Identification of Mutant Phenotypes

A sudden phenotypic change which proves to be hereditary indicates that a mutation has occurred. When a mutant F_1 female was found, every effort was made to incorporate the mutant gene into a stock by breeding her to the f41 male. If the sons of this cross expressed the mutation, then the gene was known to be sex-linked and the testing of this mutation could end at this point.

In a number of cases the mutant female proved to be either not fertile or died before she could be mated. If the mutation was of the "Maxy" type and appeared whole body it was scored as such. Partial mutations were scored as right-left or front-rear depending on the distribution of the

mutant tissue. The number of partial mutants encountered greatly outnumbered whole body mutants observed.

Where more than one F_1 female expressing a similar "Maxy" type mutation was found in a single culture, it was scored as one mutation and considered to be of premeiotic origin in the germ line of the P_1 male.

White eye mutations show as such among F_1 females. However, the vermilion allele on the "Maxy" chromosome and on the 1J1 chromosome "sensitizes" a dark eye mutation and causes it to be expressed as a bright apricot which is easily detected. An F_1 female showing apricot eye color (which means a mutation has occurred at one of the dark eye loci) must be first bred to an f41 male, so that a stock bearing the mutation can be established. Non-yellow daughters of this cross (which bear the gene for the eye mutation on their 1J1 X chromosome) are then crossed to males of the "tester" stocks, each stock bearing a mutant gene at one of the loci being considered. Each of the seven "tester" stocks expresses one of the dark eye mutations. When the F_1 daughters of one of these crosses has the eye color of the "tester" stock, that eye mutation has been identified.

Many individuals were found which differed from the normal, and because it was often impossible to tell by inspection whether these flies were "Maxy" type mutants

they were bred at least to the F_1 generation. Flies with abnormalities of one wing were numerous. In many flies one eye differed from the other in size and shape. Some had only one eye while others had either a divided-type eye or even the appearance of having three eyes. Many pigmentation differences were observed.

Some "Maxy" type mutations may be mimicked by mutations occurring at other loci than the 14 under consideration but these would have to be dominants if they are to show in the F_1 females and hence would be very rare. The fact that the "Maxy" female is heterozygous at so many loci on the X chromosome may create an internal environment which favors expression of other genetic variations (also unlikely to be detected). Some of the variations may have been influenced by external environmental conditions. In all cases clear cut "Maxy" type mutations could be detected. The evaluation of odsy and dusky tends to become somewhat subjective.

CHAPTER III

RESULTS

Results of the first series of experiments are summarized in Table 3. There were eight experiments in the series, with three broods being made in each experiment. A total of 327 "Maxy" males were injected with BUDR; 289 (88%) survived for 24 hours to be mated. A total of 2,706 F_1 females, by count, were examined for mutations. In experiment 1, brood 1, an eyeless female was found. This is not a "Maxy" type mutation and so is not counted in the total which will later be compared with spontaneous "Maxy" mutation rates. Two very dark eye mutations were found in experiment 2, brood 1. These were considered a cluster and counted as one mutational event. An apricot eye mutation was found in experiment 5, brood 1. In experiment 7, brood 3, a white eyed female was found. In experiment 4, brood 2, a partial mutant was found of the left-right type. This was counted as $\frac{1}{2}$ mutation. In series one experiments $3\frac{1}{2}$ mutations were found among 2,706 F_1 female flies.

During this series of experiments the laboratory was moved to a new location. This is mentioned because an insecticide had been used on the floor of the new laboratory

TABLE 3

SUMMARY OF RESULTS OF FIRST SERIES OF EXPERIMENTS

EXP.	NO. VERMILION INJECTED ♂♂	SURVIVAL 24 HOUR	NO. F1 ♀♀ EXAMINED FOR VISIBLES	VISIBLES FOUND WHOLE PARTIAL BODY L-R F-B	NO. F2 CULTURES CHECKED FOR LETHALS	NO. LETHALS & SEMI-LETHALS F2 F3 F4 F5
1.	50	46	250	1(Br.1)#	80	
2.	63	55	750	2c(Br.2)+	150	S-L (1-7)=
3.	13	11	120		70	
4.	40	36	417	1(Br.2)ℓ	90*	
5.	45	40	437	1apr(Br.1)	220*	S-L (1-11)=
6.	35	29	255		55*	
7.	31	26	227	1w(Br.3)	81	
8.	50	46	450		290	
TOTAL	327 (88%)	289	2,706	3 ½	1,036 - 361 675	2 S-L

No eyes (not "Maxy" type mutation).
+ Cluster, 2 very dark eye mutations, counted as 1 event.
ℓ Left wing short, right wing normal, counted as ½ event.
* Killed by insecticide except 4 cultures in exp. 5.
= Ratios of males to females are given in parentheses.

resulting in the loss of cultures stored on shelves near the floor. This accounts for the small F_1 yield in experiment 6. A total of 1,036 lethal checks were set up; 361 of these were killed by the insecticide leaving a total of 675 cultures to be scored. Two semi-lethals were found, one with a ratio of one male to seven females and the other one male to eleven females. No lethal mutations were found. Breeding was not continued beyond the F_2 generation.

Table 4 gives results found in the first half of the series 2 experiments. These experiments, called Vat 1, are tallied by broods. One hundred seventy-five males were treated with BUDR, with a 24 hour survival of 148 males. These were mated with virgin females for broods 1, 2, and 3. Broods designated 1A, 1B, 1C, 2A, 2B, etc., were made by transferring inseminated females to fresh culture bottles. From the 11 broods made 2,705 F_1 females were examined. Three whole-body mutations were found. In brood 2 a forked bristle mutation was found. In brood 3 a white eyed fly was found; this fly was fertile. Also in brood 3 an apricot eyed female was found and by tester stock was determined to be garnet. In brood 1, a yellow, forked female was found but was not counted because of the possibility it may have been a contaminant. Three partial mutations were found. In brood 1 a female with one very dark eye was found; she lived twelve days and was not fertile. In brood 1B a non-fertile

TABLE 4

SUMMARY OF RESULTS OF VAT 1 SECOND SERIES OF EXPERIMENTS

BROOD	NO. VERMILION INJECTED ♂♂	SURVIVAL 24 HOUR	NO. F1 ♀♀ EXAMINED FOR VISIBLES	VISIBLES FOUND WHOLE BODY	VISIBLES FOUND PARTIAL L-R F-B	NO. F2 CULTURES CHECKED FOR LETHALS	NO. LETHALS & SEMI-LETHALS F2	F3	F4		
1.	175	148		1yf=	1+	240 checks	0-14	5-10	1-4	S-L&	
1A						set up for	0-6	1-5	0-17	S-L	
1B					1y	Br. 1 Lost	0-4	0-1			L
1C						16	2-20	3-31		S-L	
			1,178			224	2-23	1-15		S-L	
2.				1f		55 set up	2-21	---		S-L	
2A						for Br.2	0-2	1-10	0-17	S-L	
2B						14 lost	1-6	0-12	2-9	S-L*	
2C					1f	41	0-4	---			L*
			1,311				3-21	---		S-L	
3.				1apr,1w		No checks	1-11			S-L	
3A						made on	1-9	2-6		S-L	
3B						Br. 3.	0-7	---			L
3C			216				0-6				L
TOTAL			2,705#	3	1½	265				10	4

= Not counted, hatched early, may have been contaminant.
+ One very dark eye. Each partial counted as ½.
* Semi-lethals and lethals will be discussed.
# Calculated by weight.

yellow mosaic was found. In brood 2C there was a mosaic female with forked bristles on the left side and the left wing short. In brood 3B there were 16 females which developed a tumor of the left abdominal area; after these flies developed the tumors they were not fertile, and they lived for less than two days. However brood 3B was continued; that is, all flies hatching from the 3B culture bottle were examined and then placed in fresh culture bottles. Stock from the 3B culture was kept going for two years with interesting results which will be discussed later.

A total of 265 lethal checks were examined in this experiment. Four lethals were found and 10 semi-lethals were recorded. As shown in the chart a few of the lethal checks were carried out to the F_4 generation. It was interesting that checks which appeared to be lethal in the F_2 generation often were semi-lethal in the F_3 generation. Of the four lethals counted, only one bred as a lethal in the F_3 generation; there was no F_3 yield from the other three checks which scored as F_2 lethals.

The results of the second half of the series 2 experiments are given in Table 5. One hundred seventy five vermilion males were injected with BUDR and 145 survived to be mated. Brood 1 had to be discarded; the hatch was three days early, which indicated the vat had been contaminated before the experiment started. One thousand one hundred and eighty seven

TABLE 5

SUMMARY OF RESULTS OF VAT 2 SECOND SERIES OF EXPERIMENTS

BROOD	NO. VERMILION INJECTED ♂♂	SURVIVAL 24 HOUR	NO. F1 ♀♀ EXAMINED FOR VISIBLES	VISIBLES FOUND WHOLE BODY	VISIBLES FOUND PARTIAL L-R F-B	NO. F2 CULTURES CHECKED FOR LETHALS	NO. LETHALS & SEMI-LETHALS F2	F3		
	175	145								
1.=			--							
1A						132 checks	2-16	3-16*	S-L*	
1B						set up.	1-3	0-5	S-L*	
1C						17 lost	1-5	2-32	S-L*	
			734			115	1-		S-L	
2.				2w (1)⁴			2-20	3-23	S-L	
2A				1 apr		240 checks	0-3	0-36		L*
2B						set up for	0-4	---		L?
2C			206	1w		Brds.2&3				
						64 lost				
3.						176				
3A				lysn♂ lapr(½)						
3B			247							
3C										
TOTAL			1,187#	4	½	291			5	1

= Brood 1 discarded, hatched three days early.
Calculated by weight.
* White rose eyes, lived four days, not fertile. Counted as 1 event.
♂ Yellow, singed bristle, left eye missing, lived 13 days, not fertile.
* Will be discussed.

F1 females were checked for mutations, 4 whole body and 1 partial left-right "Maxy" type mutations were found. A number of partial wing and eye mutations were found in the F3 generation of the lethal checks. A total of 372 lethal checks were set up; 81 failed to yield, 291 were checked for lethals and semi-lethals. Five semi-lethals and 1 lethal were found. However in the F2 generation 10 cultures appeared to be lethals but on going to the F3 generation 5 of these cultures had viable males and so did not qualify as lethals. Two of these false lethals had flies with visible mutations, and these were checked through the F4 generation.

The combined data of the experiments are compiled in Table 6. All F1 flies from the second series of experiments were dessicated and weighed. The dried weights were multiplied by 3,220 (the number of Drosophila equivalent to 1 gram of dessicated Drosophila melanogaster) to get the number of F1 progeny. The number of F1 flies was multiplied by .545 to determine the number of F1 females. For example in arriving at 1,178 as the number of F1 females examined in Broods 1, 1A, 1B, and 1C of Vat 1 experiment, the F1 were dessicated and found to weigh .6715 grams. This weight was multiplied by 3,220 to give an F1 yield of 2,162 females and males; this figure was multiplied by .545 to give 1,178 females and 983 F1 males. Altenburg and Browning (1961) reported this method to be accurate to 3%.

TABLE 6

COMBINED DATA FOR SERIES 1 AND SERIES 2, VAT 1 AND VAT 2

EXP. SERIES	NO. F1 EXAMINED	NO. F1 FEMALES	"MAXY" TYPE MUTATIONS WHOLE BODY SINGLE	CL.	PARTIAL L.R.	TOTAL	%	LETHAL CHECKS NO. F2 CULTURES CHECKED	L.	Semi-L.
1.	4,989	2,706	2	1	$\frac{1}{2}$	$3\frac{1}{2}$		675	-	2
2. Vat 1.	4,907	2,705	3		$1\frac{1}{2}$	$4\frac{1}{2}$		265	4	10
Vat 2.	2,206	1,187	3	1	$\frac{1}{2}$	$4\frac{1}{2}$		291	1	5
	12,102	6,598	8	2	$2\frac{1}{2}$	$12\frac{1}{2}$	.19%	1,231	5	17

Among 6,598 F1 females examined 12.5 mutations arising on the X-chromosome of the BUDR treated male were scored. This would be 1 mutation per 528 chromosomes tested (.19%). Lethal mutations were found among 5 of 1,231 crhomosomes tested or 1 mutation per 246 chromosomes (.41%). Semi-lethals were scored at 17 or 1 per 72 chromosomes tested for lethals (1.4%).

The visible mutation rates from both series of experiments are significantly different from the spontaneous or control rate of .008% found in the same kind of genetic material by Schalet (1958), based on observation of over 500,000 F_1 females. This rate was confirmed by Altenburg and Browning (1961), based on observation of 150,000 F_1 females.

CHAPTER IV

DISCUSSION

Before the detailed discussion of specific mutations found in this research some consideration should first be given to possible mechanisms for the mutagenic action of BUDR.

BUDR has been found to replace thymine in DNA but the fact that all genes do not mutate even when a great deal of BUDR has been incorporated implies that the incorporation itself does not constitute the mutation. Fig. 5 shows a speculation on the effect of substituting BUDR for thymine in replicating DNA. This hypothesis of incorporation and copy-error assumes as in case 2 that a mistake might come about by the tautomeric shift of one of the bases as shown in Fig. 1. This might lead to pairing between BUDR and guanine. Litman and Pardee (1956) have shown that mutations are not necessarily in proportion to the amount of BUDR incorporated as mistakes in replication may vary.

BUDR apparently undergoes a higher frequency of pairing mistakes than thymine. Szybalski (1961) suggests that because Br and P are electron negative, there may be a strong electrostatic repulsion between these atoms.

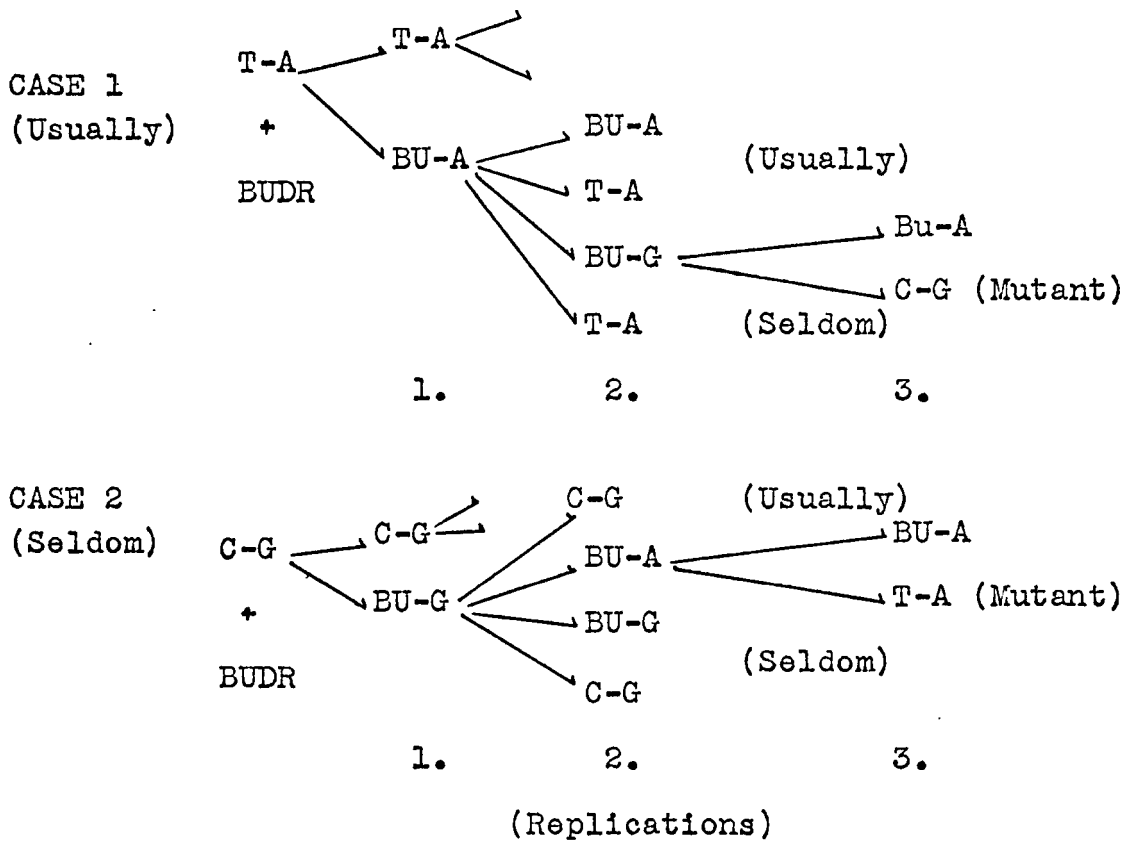


Fig. 5

A SPECULATION ON THE EFFECT OF SUBSTITUTING BUDR FOR THYMINE IN REPLICATING DNA

Case 1. Mistake in replication.

Case 2. Mistake in incorporation.

However, when BUDR is already a part of a DNA strand its electronegativity may be reduced by electron-donating neighboring groups (Freese, 1963). When DNA contains BUDR it is more sensitive to chemical attack. (Litman and Pardee, 1960)

BUDR has been found a more efficient mutagen than BU (Freese, 1959; Litman and Pardee, 1960) probably because it is more easily converted into deoxynucleotide triphosphate and interfers less with the formation of uracil and cytosine. Litman and Pardee (1960) have shown that BUDR does not require some fixation process to induce mutations and does not require protein synthesis.

If we conceive of some mutations as consisting of at least two steps the first could be the incorporation of the BUDR in a position on one strand of the double helix of DNA and the second step would be the replication in the enol state from this template. There is the possibility that base analogs may enter more easily where certain combinations of other bases exist and also the analog might enter more easily under conditions of other than normal pH, temperature, and growth media. The incorporation of a base analog may result in an instability which is unique for that special spot. It is also possible that an analog in a certain spot might choose the right partner more often than the base normal to that spot and result in fewer mutations rather than more. It is probably as important for us to recognize factors leading

to stability as those causing change. There is also the possibility that an analog might be incorporated into DNA without replication by an exchange reaction. Possible types of base alterations in DNA are replacements, deletions, insertions, and inversions.

Description of Mutations

During this research with Drosophila melanogaster criteria, as described, as to what could be counted as a "Maxy" type mutant were followed quite closely. The surprising thing about the research was the number of partial mutants which could not be classified as "Maxy" type. In later broods as well as in the F3 and F4 generations many flies showed more than one partial mutation. It was impossible to try to breed all flies with aberrant traits.

In the F1 generation numerous flies with one or both wings outstretched were found; in the limited breeding tried this outstretched wing was not transmitted, so that it was impossible to know if these flies were partial odsy. They were not counted as mutations. Another type wing frequently found had a twisted look and was shorter than the normal wing. It is possible that both these characteristics were due to mutations that had spontaneously arisen and spread through the stock.

In late broods as well as in F3 and F4 generations, eye malformations were frequent. Eyes of different size

and shape were found in the same fly. Some eyes were divided into large and small parts with other tissue interposed. Eyes were often malpositioned and of odd shapes.

In Series 1 experiment an eyeless mutant was not counted as it was not of "Maxy" type and it died before it could be bred. Two very dark eyed mutant females were counted as one event because they were found in brood 2. The apricot mutation found in Exp. 5, brood 1 died. The white mutation in Exp. 7, brood 3 was transmitted and a stock started. One partial wing mutant was found; both of the wings were of normal structure, one was short.

No lethal mutations were found and 2 semi-lethals were found in the 675 individual lethal checks done. The breeding was not continued to the F3.

In the second series of experiments, the many types of abnormalities observed, especially in the later broods and in the F3 and F4 generations of the lethal checks, came to be the most interesting aspect of the research, even though they could not be tallied as mutants under the system used.

In the vat 1 experiments 3 whole body mutations were found. The apricot mutant tested garnet and the white transmitted the white mutation. In brood 2 a forked bristle mutant was found. In brood 3B 16 females developed a melanotic type abdominal tumor. The flies appeared normal for about the first day then developed a semi-oval area

which was outlined by heavier pigmentation; this area extended over the left half of the abdomen. The pigmented area filled with fluid until the area was greatly distended. The flies died within two days and none of them were fertile. We could not identify the type of tumor.

The remaining brood 3B were inbred and the population continued for over two years. No more tumors appeared. The stock was vigorous and easy to maintain. After a number of generations every type of "Maxy" mutation could be found, in addition to the eye shape and size abnormalities, which could be observed in about half the population. Flies from this stock were used in the Genetics lab at University of Houston for observation of mutant types. A number of crosses were made of flies which seemed to have similar mutations; in each case their offspring had mutations common to the entire stock rather than to the specific type of the P1.

The "Maxy" stock carries a heavy load of mutations and is always carefully controlled by selection so that there is no control group to which to compare the brood 3B results.

In the vat 1 experiment 3 partial mutants, all of the left-right type were checked. The dark eye mutation lived 12 days and was not fertile. The yellow mosaic from brood 1B was not fertile. The forked bristles were left scutellars and this fly also had the left wing of the

twisted type; this fly lived 15 days and was not fertile.

In Table 4 only the lethals and semi-lethals are given; the cases discussed are marked by asterisks. In 265 lethal checks 8 (7 shown in Table) vials with no males were found in the F2 but on breeding these to the F3, one gave a 9-12 ratio of males to females and is not shown because it was not counted a semi-lethal. Another gave a 1-10 F3 ratio and a 0-17 F4 ratio and is shown as a semi-lethal in the Table. Another F2 giving a 0-14 ratio had an F3 of 5-10 and an F4 of 1-4; one of the 4 females had vermilion eyes and a small right eye with the right side of the head flat. She was crossed to her brother and one of the resulting F5 females had very small eyes, as had the one F5 male (F5 not shown in Table 4). A ratio of 3 males to 11 females in the F2, in the F3 gave a 1-7 ratio; the one male was very small and both wings were short (not in Table). In an F2 with a 1-12 count, the F3 had 6 males to 27 females, and one of the males had a large right eye which was nearly dorsal on his head (not counted as a semi-lethal and not in Table).

In the 0-4 check talled as lethal all four females had heavy melanin deposits around the ocelli. An F2 check of 1-9 gave an F3 of 2-8, and one of the females had apricot eyes. In the semi-lethal check 1-6 of the F2, the F3 gave 0-12 with one forked bristle female. The F4 check

was 2-9 and both males were very small. In a 1-8 F2 count, the F3 was 4-10 and the F4 5-16; one of the males had short wings.

In the Vat 2 experiment 1,187 F1 females were examined for mutations. Brood 1 had to be discarded because a 3 day early hatch indicated vat contamination. Four whole body mutations were found in the Vat 2 experiments. In brood 2, two light rose-white eye-color mutations were counted as one event. These were not fertile. The white mutation shown in Table 5 was fertile and transmitted the trait. The apricot female was bred to f41 stock male and produced 1 male and 11 females, indicating a probable semi-lethal. The yellow, singed bristle mutant found in brood 3A had the left eye missing, lived 13 days and was not fertile.

A total of 291 individual lethal checks were carried out for the Vat 2 experiment. From brood 2B a 2-16 F2 gave an F3 of 3-16, and an F4 in which the 2 males were small and one of the females was vermilion, forked bristle, and with a small right eye which was almost in two parts. The second semi-lethal shown in Table 5 had two visible female mutants in the F3; one had a very small eye just to the right of the right eye--the other female had a section of white in the center of the right eye.

The third semi-lethal had the right eye divided with the red dorsal and white ventral. This female was fertile and gave a 3-25 ratio with no eye mutations. In the 1-15 semi-lethal the male had a twisted right wing.

Twelve of the lethal checks had no males in the F2 but on testing to the F3 only one checked lethal, another gave no F3. Three of these F2 checks with no males gave visible mutations in the F3. A 0-5 F2 gave an F3 of 14--one of the males had an antenna in an "up" position near the center of the head. He was mated with a sib and all females were normal while the 2 males were very small. A 0-3 F2 gave a 4-10 F3; three of the 4 males had outstretched wings and all females appeared normal.

If the lethal checks had not been carried beyond the F2, we would have found 20 lethals in 1,231 individual lethal checks; this means the lethal rate was 1.6%. This is well above spontaneous rates which have been reported by Schalet (1958) of from .34% to .40% depending on the age of the sperm and whether maternally or paternally derived chromosomes are being tested. By breeding beyond the F2 a lethal rate of .41% was found. This does not differ greatly from spontaneous rates.

The semi-lethal rate was about 1.4% which we found means that one chromosome out of 72 would bear a semi-lethal mutation. Among the F3 and F4 generations 11 eye mutations

were observed. Six of the males were very small which may mean that they were viable although under a genetic load. Other types of mutations found in the F3 and F4 were 2 forked bristle mutants, 3 males with outstretched wings and 1 male with an antenna defect.

Table 6 gives a summary of the data from all the experiments. A visible mutation rate of .19% among the F1 females derived from "Maxy" males treated with BUDR was found. This compares with a rate of .008% for spontaneous mutations observed at 13 loci on the x-chromosome of the F1 "Maxy" female (Schalet, 1958).

A per locus rate for the 14 loci observed in our research is about 1.3×10^{-4}. This can be compared with the 6×10^{-6} rate found by Schalet. Muller, Valencia, and Valencia (1949) reported a per locus spontaneous rate of 3×10^{-5} based on maternally derived chromosomes studied at 9 sex-linked loci in a stock of Drosophila melanogaster.

The Russells (1959) announced a per locus rate for paternally derived chromosomes in mice of 8.4×10^{-6}. Muller (1956) had estimated a per locus rate for man of about 10^{-5}.

In our research 8.5 of the 12.5 mutations observed in the F1 female were eye mutations. Four of these were transmitted, 3 of them at the white locus, and one apricot tested as being at the garnet loci. There is no certainty that the untransmitted mutations were whole body.

The large number of eye mutations carried on the "Maxy" chromosome might account for the number of eye mutations observed in the F1 female rather than being an indication that BUDR in some way affected genetic material more frequently at these loci.

The purpose of this research was to determine if BUDR was a mutagenic agent, using the most sensitive and unbiased technique so far devised, and one that had been used successfully with a number of other mutagenic agents. If the number of semi-lethal mutations and visible mutations seen in the F3 and F4 generations can be interpreted as events at the genetic level which were invisible in the F1 female then it could be suggested that scoring of visible F1 mutations might be an inadequate measure of the degree of mutagenicity of BUDR, or that there is a difference between the mutagenic action of BUDR and other agents, since visible mutations appearing in further testing of lethal and semi-lethal cultures are not usually observed. To know the genetic nature of these events would require a good deal of further experimentation.

The high visible mutation rate of BUDR compared to its lethal rate, as well as the high rate of visible mosaic mutations recovered, suggests that there may have been produced a number of lethal mosaic mutations which would have appeared as non-lethals in the absence of further testing to

detect them. Some of the semi-lethals in the F_2 which failed to appear lethal in the F_3 are possibly of this category.

CHAPTER V

SUMMARY

1. Young males of Muller's "Maxy" stock were administered BUDR by abdominal injection and then mated with virgin Drosophila melanogaster of the "Maxy" stock. The resulting F1 females were examined for visible mutations at 14 selected loci on the paternally derived X-chromosome.

2. Normal appearing virgin F1 females were individually mated with special male stock according to Muller's "Maxy" technique to detect lethal mutations which arose in the paternally derived X-chromosome of the F1 female.

3. Experimental methods and criteria for classifying both visible and lethal mutations were described.

4. Among 6,598 females examined 12.5 mutations arising on the X-chromosome of the BUDR treated male were scored. This would be 1 mutation per 528 chromosomes tested or a .19% rate. This may be compared with a spontaneous mutation rate of 1 mutation per 12,564 chromosomes or ,008% found by previous workers with the "Maxy" stock. Visible mutations among F1 females carrying the X-chromosome from the BUDR treated male were found to be about 24 fold the spontaneous rate.

5. Lethal mutations were found among 5 of 1,231 chromosomes tested or one lethal mutation per 246 chromosomes, a rate of .41%. In previous spontaneous lethal mutation studies in "Maxy" stock 14 lethal mutations in 4,082 paternally derived X-chromosomes were reported. This would be a .34% spontaneous rate. Among the BUDR treated paternal chromosomes 17 semi-lethal mutations per 1,231 chromosomes were found. One chromosome out of 72 would bear a semi-lethal mutation; this would be a rate of about 1.4%.

6. Many of the visible whole body mutations were untransmitted because the females bearing them did not reproduce. Some of these may actually have been fractional mutations. The high rate of semi-lethal mutations could be an indication that at least some of the mutations scored as whole body may actually have been mosaics.

7. BUDR, as used in this research, was found to be a potent mutagenic agent in Drosophila melanogaster.

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