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THE EFFECTS OF FORMALDEHYDE ON THE FREQUENCY OF REVERSION

OF THE white-ivory MUTANT OF DROSOPHILA MELANOGASTER

by

Ruth Ellen D. Wood

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Zoology

Approved:

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Logan, Utah

1970

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<div align="right">Ruth Ellen D. Wood</div>

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ABSTRACT

The Effects of Formaldehyde on the Frequency of Reversion

of the white-ivory Mutant of Drosophila melanogaster

by

Ruth Ellen D. Wood, Master of Science

Utah State University, 1970

Major Professor: Dr. James T. Bowman
Department: Zoology

This study is an analysis of the effects of formaldehyde, a chemical which is both recombinogenic and mutagenic, on white-ivory (w^i) a highly mutable allele of the white locus of Drosophila. Formaldehyde is shown to significantly increase the reversion frequency of w^i. The results of this investigation differ from earlier observations in three respects: (1) there is a positive correlation between RNA (ribonucleic acid) concentration and reversion frequency in the presence of formaldehyde, but reversion does not appear to be totally RNA dependent; (2) female germ cells are more sensitive than those of the male; and (3) a cluster of revertants has been recovered. These indicate that formaldehyde-induced w^i reversion may involve a different mechanism than that proposed for formaldehyde-induced sex-linked lethals. Different mechanisms have been proposed to account for the induction of sex-linked lethals and for the increase in recombination. Formaldehyde-induced reversion of white-ivory follows the parameters of recombination.

(41 pages)

INTRODUCTION

The study of highly mutable loci in higher organisms is concerned among other facets, with the manner in which mutational events are initiated, the nature of the changes in the genetic material involved, and the possible role of repair mechanisms. As yet, little information is available. White-ivory is one such highly mutable locus in Drosophila melanogaster. It has the properties of length, high spontaneous reversion frequency, and transposability of one of its derivatives. These properties indicate that this mutant may involve an addition of genetic material to the white locus and that removal of this material may lead to reversion from the mutant to wild-type.

The study of agents which alter the reversion frequencies of such mutable genes should help elucidate the genetic peculiarities that result in high mutability. Formaldehyde, one such agent, induces chromosome breakage, mutation, and recombination. Its effect on w^i has not, until now, been ascertained.

The following abbreviations are used frequently throughout this thesis: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; CH_2O, formaldehyde; H-bonds, hydrogen bonds; w^i, white-ivory; w, white; and w^c, white-crimson. Other, less frequently used, abbreviations are explained in the text.

REVIEW OF LITERATURE

The white array is a series of eye pigment mutants located at 1.5 on the X-chromosome. White-ivory is a mutant of this array which is characterized by a number of unique properties. It differs from alleles located to the left in the white array by showing no dosage compensation and differs from the noncompensating alleles by neither being enhanced by enhancer of white-eosin (en-w^e) nor acting as a dominant suppressor of zest (z) (Green, 1959). Length is a characteristic of w^i noted by Lewis (1959), Green (1959), and Bowman (1965). Recombination normally occurs between white-buff (w^{bf}) and white-cherry (w^{ch}) alleles but has not been observed in the presence of the w^i homologue. Crossing-over, in general, is drastically reduced within the white locus in the presence of w^i.

Lewis (1959) noted that w^i reverted at a frequency of 15 non-cross-over revertants among 290,000 w^i female gametes, or 5.2 X 10^{-5}, many times the reversion frequency of most mutants. This reversion of white-ivory to wild-type (w^+) appears to be a mutational event that is not associated with exchange of outside markers (Lewis, 1959) and is unaffected by heterozygosity for Ubx^{130} (Ultrabithorax-130), Cy (Curly), or M(1)n (Minute) cross-over enhancers (Bowman, 1965).

White-ivory females heterozygous for deficiencies in the white region show a ten-fold reduction in the frequency of spontaneous reversion. This indicates dependence of the reversion mechanism upon synapsis with a normal homologue. X-ray induced revertants, on the other hand, appear to be synapsis independent since they

occur frequently in clusters and show a dependence on the number of loci present (Bowman, 1965; Bowman and Green, 1966).

White-ivory revertants are phenotypically indistinguishable from wild-type. They have no further tendency to mutate and cause no reduction in intragenic recombination at the white locus (Lewis, 1959; Bowman, 1965; Bowman and Green, 1966). This indicates that the recombination inhibiting element present with the mutant has been removed.

Partial revertants show a varied combination of revertant and mutant characteristics. Two stable, X-ray-induced, partial revertants (w^{ip1} and w^{ip2}) phenotypically resemble apricot and do not reduce recombination within the white locus. They have been mapped within the white locus and found to be recombinationally inseparable from w^{a} (white-apricot) (Bowman and Green, 1966; Bowman, unpublished). One white-eyed revertant (w^{62k}) retains the property of length (or recombination suppression within the white locus) but fails to revert to wild-type (Bowman and Green, 1966). A recent X-ray induced partial revertant of w^{i}, w^{c}, produces an array of mutational deriva- tives with unusual properties (Figure 1). White-crimson is likewise recombinationally inseparable from w^{a} and maps specifically between w^{col} (white-colored) and w^{ch} (Green, 1969a). However, none of the other derivatives have been accurately mapped due to their frequent mutation (Green, 1967). The properties of the derivatives of w^{i} are:

1. The mutable derivatives exhibit a reversion frequency approxi- mately 10,000 times that of spontaneous w^{i} reversion (5.2×10^{-5} as compared to 2.2×10^{-1}) (Green, 1967).

2. Wild-type, white and partial revertants are produced through mutational events and not through mitotic, meiotic or sister

$\rightarrow$ = X-ray induced, $\longrightarrow$ = spontaneous event, $\square$ = stable allele, $\bigcirc$ = mutable allele, w = white, i = ivory, + = wild-type, c = crimson, d = dark, N = Notch deficiency.

Figure 1. Phenotypic mutation spectrum of $\underline{w}^i$ and its derivatives at the $\underline{w}$ locus (Bowman, 1959; Bowman, unpublished; Bowman and Green, 1966; Green, 1967).

strand recombination (Green, 1967).

3. Clustering indicates that these mutational events are premeiotic and, therefore, presumably synapsis independent (Green, 1967). Spontaneous germinal revertants of white-ivory occur singly, suggesting a meiotic origin (Bowman, 1969) and rarely occur in the absence of a structurally normal homologue (Bowman, 1965). This suggests a synapsis dependent mutational event.

4. The mutationally unstable derivatives all mutate to similar stable and mutable phenotypes.

5. Transpositions of $\underline{w}^c$ to the third chromosome have been recently substantiated (Green, 1969b). Transpositions of other derivatives have not yet been verified.

6. Deficiencies within the $\underline{w}$ locus and adjacent loci have been produced by all of the unstable genes (Green, 1969b).

Four of the above are also properties which characterize controlling elements found in maize: (1) high mutability, (2) non-recombinational origin, (3) transposition, and (4) occurrence of deficiencies (McClintock, 1965; McClintock, 1967). The controlling elements regulate gene action according to the genetic portion transposed and the attachment or integration site. These foreign genetic particles behave similarly to the integrated phage, mu-1, which induces mutation in $\underline{E}$. $\underline{coli}$ (Taylor, 1963).

Campbell proposed a recombination mechanism for the excision of such episome-like elements. An analogous intra-strand exchange model proposed by Laughnan (1955) and Peterson and Laughnan (1963) for pairing in a serial tandem duplication has been adapted to the $\underline{w}^i$ reversion event by Bowman (1965). It was proposed that a serial tandem duplication could pair intrachromosomally in a double loop formation. A single crossover event could then be responsible for chromosomal repair through excision of the interfering genetic material (Figure 2). If, as Rasmusun postulated (1962), a repeat in the $\underline{white}$ region is the explanation for the length characteristic of $\underline{w}^i$, then this proposed mechanism may indeed be responsible for mutation at the $\underline{w}^i$ locus.

The initiation of such action could be affected by mutagenic agents specific for lesions which result in breakage and affect

original ‾‾‾‾‾‾‾‾‾‾ A B C D E F B C D E F G ‾‾‾‾‾‾‾‾‾‾
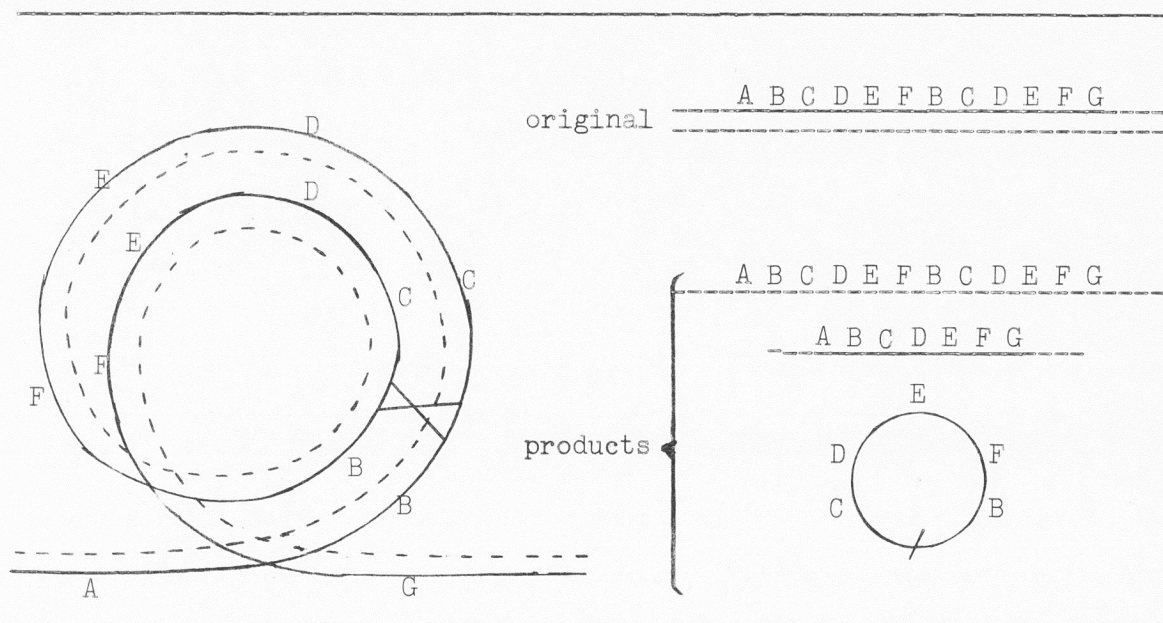
ABCDEFBCDEFG

ABCDEFG

products

Figure 2. Intrachromosomal pairing and crossing-over between segments of a serial tandem duplication to generate a wild-type sequence and a genetic element (redrawn from Bowman, 1965).

recombination. The characteristics associated with formaldehyde treatments have shown these properties and should, therefore, affect the reversion frequency of $\underline{w}^i$ and its derivatives. These charac-teristics are:

1. A high incidence of duplications (or repeats and deficiencies) occur as a result of CH_2O treatment. Repeats and reciprocal deficiencies characterize 60.3% of all induced changes brought about by formaldehyde-food (a combination of CH_2O and standard Drosophila medium) treatment as compared with 7-19% of the similar changes induced by X-ray treatments (Slizynska, 1963a; Slizynska, 1963b).

2. An increase in total recombination on the second and third chromosomes from 0.9% to approximately 10% is observed in treated Drosophila males (Sobels, Bootsma and Tates, 1959;

Whittinghill and B. Lewis, 1961).

In a study of carbonyl compounds in mutagenesis, Rapoport (1946) discovered the mutagenicity of formaldehyde and the specificity it exhibits toward male germ cells. His hypothesis of mutagenic action, the interaction of formaldehyde with the amino groups of the gene proteins, was based on a high incidence of amine attacks observed in formaldehyde reactions and his finding no evidence for the induction of chromosome breakage or rearrangements (Rapoport, 1947).

Auerbach (1949) and Auerbach and Moser (1953a) verified reports by Rapoport (1946), Kaplan (1948) and Herskowitz (1950) concerning the sensitivity of male germ tissue and suggested that the sensitive stage of development is in the late auxocyte stage or the period of growth just prior to meiosis. Auerbach's findings were not consistent with the mode of mutagenic action postulated by Rapoport in 1947. She found chromosome breakage and rearrangements as well as other anomalies known to be induced by X-rays and mustard gas treatments. The other anomalies found were: recessive and dominant lethals, large and small rearrangements, small deficiencies, mosaics, gynandromorphs, and visible mutations.

Auerbach was originally attracted to the study of formaldehyde because Rapoport's postulate of action did not seem feasible. She proposed that formaldehyde in its highly reactive free form has little chance of reaching the gene protein without first being bound to products in the media or broken down during digestion. A product of CH_2O and casein (high in contaminating RNA) was found to be mutagenic. The reaction, however, was readily reversible and extensive washings made the CH_2O-casein product non mutagenic. Therefore, Auerbach and Moser (1949) questioned the CH_2O-casein product as the cause of muta-

genesis. Minimal or starvation media was found to be mutagenically ineffective when CH_2O was introduced, suggesting an essential interaction of CH_2O with an ingredient of the normal medium. In general, when CH_2O was used in solution or in the gaseous state no mutagenic effect was observed although Auerbach (1952) did report a slight increase in sex-linked lethals which were induced in mature sperm. It was concluded that CH_2O in complete Drosophila medium (formaldehyde-food) is the most effective combination for mutagenic action (Auerbach, 1956; Auerbach and Moser, 1953b).

Alderson (1954) suggested that the effective mutagenic ingredient in Drosophila media is RNA, specifically adenylic acid or adenosine. He established this in a series of experiments using chemically defined media which contained controlled quantities of either RNA of adenylic acid (Alderson, 1960a; Alderson, 1960b). The specific requirement for adenine was demonstrated by replacing the reactive 6-amino group of adenylic acid with a stable hydroxyl group and forming inosine. When inosine was substituted for adenosine, the mutagenic activity of formaldehyde was completely removed (Alderson, 1961) (Figure 3). The mutagenic activity of CH_2O, therefore, depended on the presence of the 6-amino group of adenine.

Alderson (1964) later concluded that the monohydroxymethylation reaction at this 6-amino site was the "sufficient and necessary" condition for mutagenesis. In this study he treated adenosine with a four fold excess of formaldehyde and combined the two ingredients 18 hours before adding them to an RNA free medium. The number of sex-linked lethals produced by this product was compared to the number produced by direct addition of the same concentrations of CH_2O and
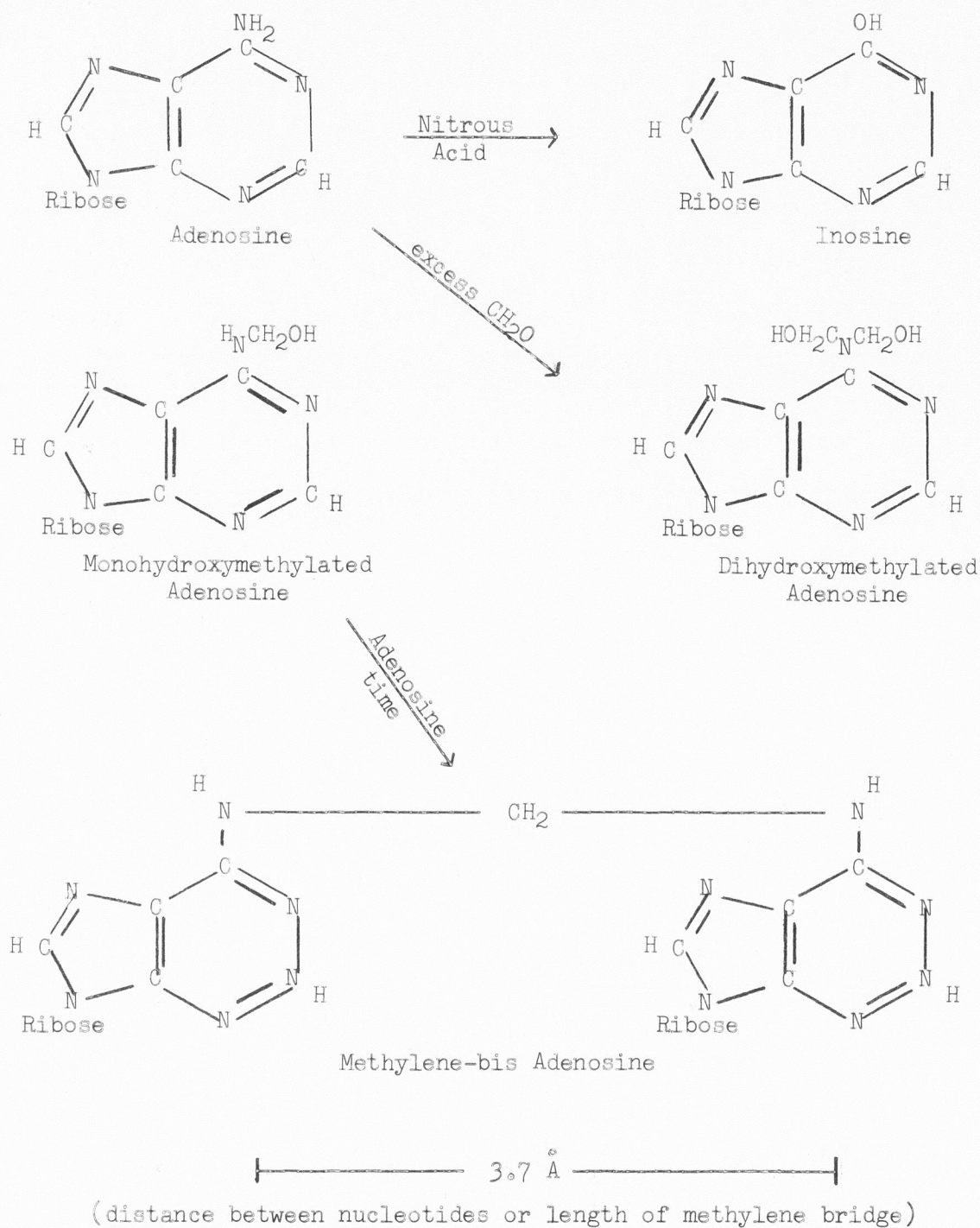
Figure 3. Reactions of adenosine with formaldehyde and nitrous acid
(Alderson, 1960b; Feldman, 1964).

adenosine to the RNA-free media. The direct addition of the two components increased the frequency of CH_2O interaction with other food constituents which would reduce the possibility of the formation of a dihydroxymethyl-adenine product. Sex-linked lethals were increased by the second treatment but no significant increase in frequency was observed after the pretreatment. It was concluded that the dihydroxymethylated product was not mutagenic (Alderson, 1964). Alderson (1960b) proposed the production of an adenine dimer as the probable mutagenic product of CH_2O-RNA interaction. His proposal was later recinded (Alderson, 1964) since he postulated that formation of such a product is improbable under physiological conditions; however, no evidence was provided for such a conclusion.

Studies on TMV (tobacco mosaic virus) carried out by Fraenkel-Conrat (1954) gave conclusive evidence against Rapoport's proetin-CH_2O interaction. His analysis of changes in ultra-violet absorption due to formaldehyde treatment indicated little interaction with the TMV protein and that formaldehyde reacts more readily with RNA than with DNA. CH_2O was bound to RNA in a slow reaction (48 hours at room temperature) which proved to be readily reversible.

Staehelin (1958) found two forms of formaldehyde binding after treating TMV-RNA: (1) a very labile form which is lost upon prolonged dialysis, and (2) a more stable form which is only slowly reversible in the cold. The greater proportion of the bound formaldehyde appeared to be in the labile form with short incubations whereas the more stable form predominated if the reaction were allowed to proceed for a number of days.

Feldman (1964) found indirect evidence that a monomethylol product (RNA-CH_2O) is the labile derivative and the stable product

is a methylene bis-derivative. Later, the products of CH_2O and adenosine 2' and 3' phosphates were chromatographically separated and found to be: P-2'-adenosine-CH_2-adenosine-2'-P, P-2'-adenosine-CH_2-adenosine-3'-P, and P-3'-adenosine-CH_2-adenosine-3'-P (Feldman, 1967). On enzymic dephosphorylation, all three isomers produced a compound identified chromatographically and spectrophotometrically as methylene bis-adenosine. Formation of the methylol derivatives was complete in two to four days whereas the complete reaction to form the methylene-bis derivatives required many days and even months.

There is no evidence that formaldehyde produces a monohydroxy-methylated (labile) form or an adenine dimer (stable) form in vivo. However, since both of these products have been identified in vitro, and the labile product has been found to be effective in increasing sex-linked lethals (Auerbach and Moser, 1949), it may be assumed that similar products are formed and are active in the formaldehyde-fed living organism.

Single stranded or denatured strands of DNA or RNA are more susceptible to reaction with formaldehyde than the double stranded forms (Berns and Thomas, 1961; Haselkorn and Doty, 1961; Tikchonenko and Dobrov, 1969). This makes two observations of significance: (1) the 6-amino group of adenine is necessary for mutagenesis (Alderson, 1961) and (2) an H-bond associated with this nucleotide in the amino form is normally at the 6-amino site. These indicate that H-bonds would have to be broken before a formaldehyde lesion could occur. Consequently, CH_2O interaction would be most favorable at the time the strands separate for replication. This would be in full agreement with the observation of Auerbach and Moser (1953a)

that the sensitive period for CH_2O mutagenesis is in the growth stage just prior to meiosis or the late auxocyte stage of Drosophila development. The sensitivity of this stage indicates that CH_2O mutagenesis is due to a direct chromosomal lesion which effects the 6-amino group (Alderson, 1961) of the replicating or transcribing strand. How such a lesion may induce the observed anomalies is not known.

Mutagenic agents may also affect the cellular environment. This would alter normal activities in the cell (Auerbach and Ramsay, 1968). Such a secondary effect may be the result of delayed nucleic acid synthesis such as observed after treatments with metabolic inhibitors. It has been proposed that mitomycin C-induced recombination is due to inhibition of DNA synthesis. This produces a meiotic environment in a normal mitotic cell encouraging synapsis of homologous chromosomes (Holliday, 1961; Holliday, 1964). Gofmekler, Pushkina, and Klevtsova (1968) and Pushkina, Gofmekler, and Klevtsova (1968) have observed a similar inhibition of nucleic acid synthesis through formaldehyde treatments on rat tissue. Synapsis caused by this inhibition could affect intra-strand recombination, particularly in a tandem duplication. Duplicated regions would be more readily synapsed than normal chromosomal homologues due to easier accessibility. This would provide a ready means for intra-strand recombination.

There are other similarities between mutagenic treatments with CH_2O and mitomycin C:

1. Mitomycin C is an inter-strand cross-link former (Iyer and Szybalski, 1964; Szybalski and Iyer, 1964) whereas CH_2O is

an intra-strand (Freifelder and Davison, 1963) and may be
an inter-strand cross-link former (Tikchonenko and Dobrov,
1969; Axelrod et al., 1969).

2.   Both mutagens produce episome-like fragments (Bradley, 1967;
      Slizynska, 1963b).

3.   Recombination in <u>Drosophila</u> <u>melanogaster</u> is enhanced by both
      mutagens (Suzuki, 1965; Alderson, 1967).

These similarities are of significance since mitomycin C induces
somatic reversion of $\underline{w}^i$ (Bowman, personal communication).

MATERIALS AND METHODS

Stocks

The white locus is located at 1.5 on the X-chromosome with outside markers y (yellow body) and sc (scute bristles) located 1.5 map units to the left, and spl (split bristles) and ec (echinus eye) 1.5 and 4 map units to the right, respectively (Figure 4). Df(1)3C2 is a deficiency of the white region and is described with the above mutants by Lindsley and Grell (1967). White-ivory-quintuplication (Qn(1)w^i) was developed by Dr. E. B. Lewis. All white stocks were derived from the same strain and are on a Canton-S background.

Figure 4. Left segment of the X-chromosome.

Media

In all control experiments the flies were grown at 25 C in sterile, half pint milk bottles containing approximately 50 ml of Drosophila media consisting of:

1 l H_2O

15 gr agar (Nutritional Biochemical Corporation)

70 gr cornmeal in 500 ml cold H_2O

60 gr sugar in 60 ml H_2O

15 gr brewer's yeast (Nutritional Biochemical Corporation)

8 ml propionic acid added when the media cooled to 60 C

In the formaldehyde-food used in mutagenic treatments, RNA-Na (Sodium Nucleate, Schwarz Bioresearch Inc.) and formaldehyde were added to give the concentrations indicated in the Results. Percentage concentrations of both CH_2O and RNA-Na are approximations based on weight of substance added to the total weight of the medium at 50 C. Variations in the percentage might occur as a result of differing environmental conditions influencing evaporation of H_2O or CH_2O during the cooling and growth periods.

Control RNA determination

It was necessary to modify the standard medium for this test by omitting the agar. The modified control media and added 0.5% RNA-Na media were compared to determine the natural level of RNA present. The RNA was extracted through a modification of the Schmidt and Thannhouser technique (1945). The RNA-phosphorous of the control media and the media with RNA added was measured by the Fiske and SubbaRow techniques (1925).

Method of treatment for inducing somatic revertants

Adults of the specific genotypes mentioned in the Results were placed on treatment or control media and allowed to lay eggs for seven days. The adults were then removed and the progeny allowed to continue their development to eclosion. Fourteen media modifications were tested for their effects on variations in somatic

reversion frequency. The modifications were in stepwise increments of CH_2O while RNA-Na was held at a constant level and in stepwise increments of RNA-Na while CH_2O was held constant.

Scoring. Flies emerging during the first ten days of the eclosion period, which had been treated for entire larval life, were scored for mosaics (red pigmented facets in the normal ivory-colored eye). A dissecting microscope at 45 X magnification and a high intensity light source were used.

Statistical analysis. A regression analysis and t-test limits on the Y-intercepts of the regression slopes were based on a method discussed by Ostle (1963).

Method of treatment for inducing germinal revertants

Homozygous $y^2 sc\ w^i$ females and $y^2 sc\ w^i$ males as well as $y(w^i)_5 ec$ males were fed for full larval life on 0.9% CH_2O and 0.05% RNA, 0.9% CH_2O and 0.15% RNA, or 0.9% CH_2O and 0.30% RNA media. They were scored for somatic revertants 0 to 4 hours after eclosion. Both revertant and normal, young virgin females were then maintained in an adult feeding chamber (Figure 5) for 24 hours before mating to ensure thorough germ cell treatment. The adult feeding chamber was adopted from Yousef (personal communication) and requires the following materials:
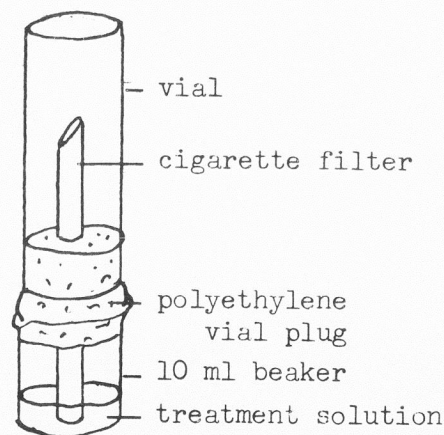

- vial
- cigarette filter
- polyethylene vial plug
- 10 ml beaker
- treatment solution

Figure 5. Adult feeding chamber.

10 ml beaker, polyethylene vial plug, the standard _Drosophila_ vial,
and full length cigarette filter (Phillip Morris menthol filters).
The 10 ml beaker contained 4 ml of a 3% glucose solution and the
percentages of CH_2O and RNA-Na which corresponded with the concen-
trations used in the larval treatment media. The filters were kept
saturated during the treatment.

The treated virgin, $y^2sc\ w^i/y^2sc\ w^i$ females were then mated
to $y^2sc\ w^{bf}w^{ch}spl$ males. $y(w^i)_5ec$ males were treated, scored for
somatics, and mated to $y\ f$=: females. All parents were transferred
at 4-day intervals to new media to produce 3 broods, and were then
discarded.

Scoring for germinals. Germinal revertants are phenotypically
wild-type and are easily noticed among the w^i progeny. After scoring
the w^i flies are placed in a detergent solution and counted by an
electronic fly counter modified from an original model of Keighly
and Lewis (1950) by J. T. Bowman. Since flies were collected from
ten bottles at a time for scoring, if more than one wild-type fly
occurred from the same ten bottles they were considered a cluster
and scored as a single mutational event.

Statistical analysis for germinals. Fiducial limits were placed
on the frequencies of complete revertants according to the method of
Stevens (1942).

RESULTS

Control RNA level

Determination of the level of RNA present in the control medium was necessary in order to determine if RNA is essential for formaldehyde mutagenesis as suggested by Auerbach and Moser (1953a, 1953b) and Alderson (1954, 1960a, 1960b). A RNA-free control was impossible since RNA must be present for development of Canton-S flies (Geer, 1963). The Fiske and SubbaRow technique (1925) used in this determination established the RNA-phosphate level in the control as approximately one-tenth that found in a comparative medium containing 0.5% additional RNA-Na (0.076 uM RNA-P/ml to 0.86 uM RNA-P/ml). This indicated a minimal level of approximately 0.05% RNA in all media. This 0.05% correction was added to all references to RNA concentrations in experimental media.

Somatic studies

Two studies involving somatic reversion were undertaken with the formaldehyde-food treatment described in Materials and Methods. The first was used as a pilot study to determine the influence of CH_2O on the reversion frequency per $\underline{w}^i$ locus present (Table 1). The series of six experiments indicated an increase in the reversion frequency directly proportional to the number of $\underline{w}^i$ loci present per fly as predicted from X-ray treatments (Bowman, 1965). The fourth experiment provided a means of treating one and two $\underline{w}^i$ loci per female in the same formaldehyde-food culture. The cross $\underline{y}^2\underline{sc}\ \underline{w}^i/\underline{Df(1)3C2}$

Table 1.   The effect of formaldehyde on the somatic reversion
frequency per number of w^i loci present per fly with
no RNA added.

Exp.	Treatment	Genotype	Revertants Total Flies	Number of Loci	Percentage
#1	control	$w^i/w^i=$; w^i/Y	4/1028 3/1503	2 1	0.4 0.2
#2	.09% CH_2O	$w^i/w^i=$; w^i/Y	29/627 15/745	2 1	4.6 2.0
#3	.09% CH_2O	$y^2sc\ w^i/Df(1)3C2$ $y^2sc\ w^i/Y$	53/1411 75/1880	1 1	3.8 4.0
#4	.09% CH_2O	$y^2sc\ w^i/y^2sc\ w^i$ $y^2sc\ w^i/Df(1)3C2$ $y^2sc\ w^i/Y$	50/1009 8/327 30/900	2 1 1	5.0 2.5 3.3
#5	.045% CH_2O	$y(w^i)_5ec/Y$	29/277	5	10.5
#6	.045% CH_2O	$y^2sc\ w^i/y^2sc\ w^i$ $y^2sc\ w^i/Y$	32/1014 28/1036	2 1	3.2 2.7

X $y^2sc\ w^i/Y$ gives female progeny with genotypes $y^2sc\ w^i/Df(1)3C2$

and $y^2sc\ w^i/y^2sc\ w^i$.   In the first genotype .025 mosaics were

scored per fly (or 2.5%) and .050 mosaics were scored per fly in

the second genotype (Table 1).   Quintuplication white-ivory (a five

fold repeat of the w^i locus) shows a pronounced increase in reversion

and a clear dependence on the number of loci present.   Differences

between experiments on the same genotypes are presumably due to

variations in experimental conditions.

The second somatic study (Table 2) was to determine the influence

of various concentrations of RNA and CH_2O on the somatic reversion

Table 2. The effect of formaldehyde and RNA on the somatic reversion frequency

Percent CH_2O	Percent RNA	Revertants per No. of Flies Scored			Reversion Frequency per Locus
		$\frac{y^2sc\ w^i}{Y}$	$\frac{y^2sc\ w^i}{y^2sc\ w^i}$	$\frac{y(w^i)_{5ec}}{Y}$	
0.00	0.05	3/1503	2/508	2/208	0.00190
0.00	0.15	-	-		
0.00	0.30	1/495	0/462	1/138	0.00095
0.00	0.55	3/1259	6/1324	5/1500	0.00122
0.09	0.05	9/316	29/529	17/260	0.02058
0.09	0.15	25/565	38/569	29/247	0.03360
0.09	0.30	34/779	81/964	45/439	0.03250
0.09	0.55	5/207	23/342	47/182	0.05000
0.1125	0.05	0/8	6/40	1/13	0.04570
0.1125	0.15	40/582	97/1083	85/301	0.05220
0.1125	0.30	15/312	87/634	71/168	0.07140
0.1125	0.55	17/168	26/143	81/187	0.08920
0.135	0.05	-	-	-	-
0.135	0.15	10/284	40/248	19/82	0.04450
0.135	0.30	7/139	38/280	30/69	0.07170
0.135	0.55	10/74	29/158	21/40	0.10160

frequency of w^i. Four levels of RNA and four levels of CH_2O were used in 16 treatments. It was found that RNA alone had no significant effect on the reversion frequency of w^i. However, an increase in RNA at a constant level of formaldehyde did bring about a corresponding increase in the somatic reversion frequency of w^i. The probability that the increase in RNA is responsible for this observed variance is significant at the .01 level ($F=19.24$). The method of least squares gives Y axis intercepts for lines B, C, and D at .0241, .0312, and .0253 respectively (Figure 6). T-test probability limits from the data indicate with 90% accuracy that these lines will intercept
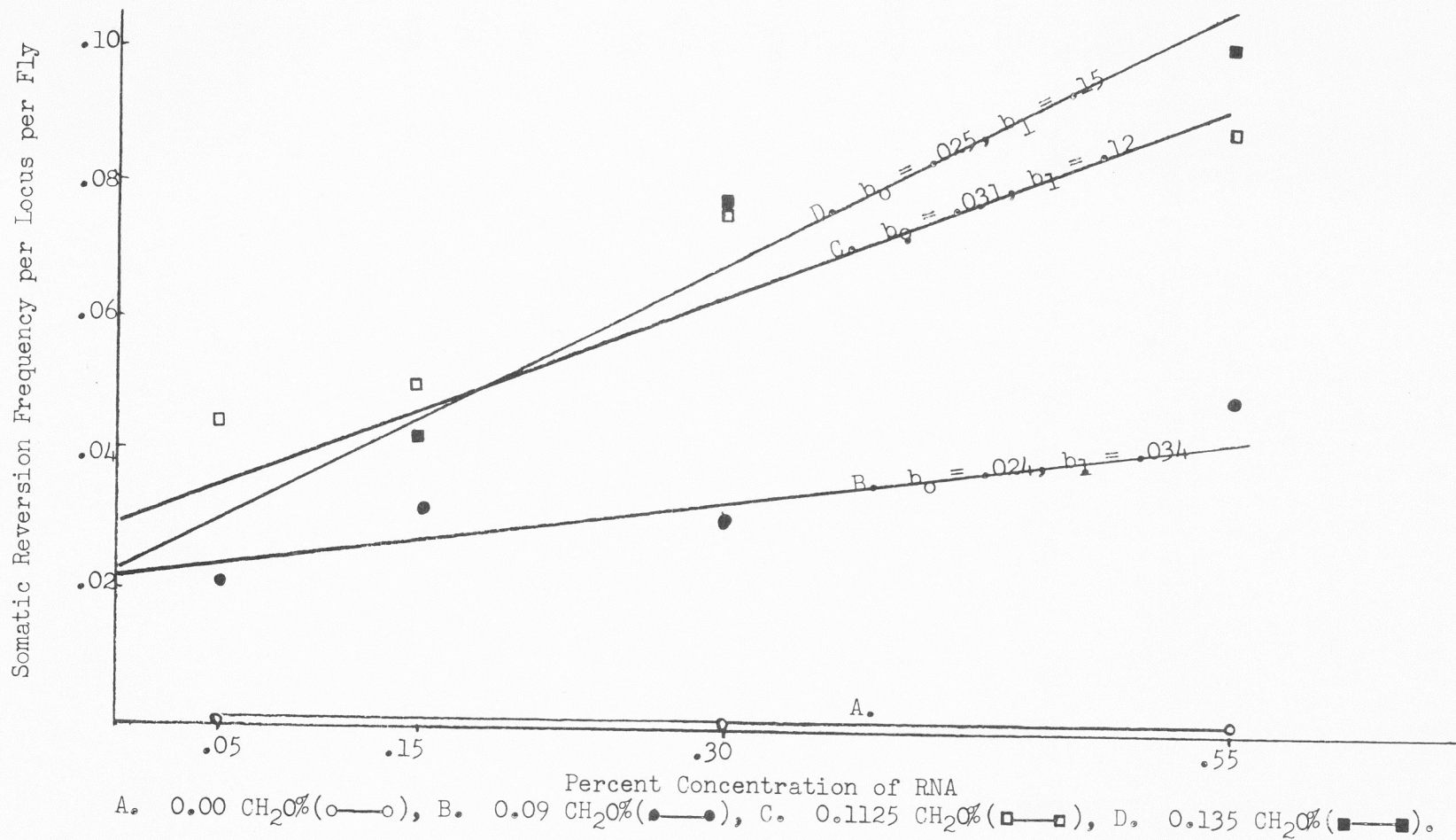
Figure 6. The somatic reversion frequency as affected by varying increments of RNA at four formaldehyde levels.

significantly above the level for spontaneous reversion on the Y

axis (B = .014 to .034, C = .016 to .046, and D = .007 to .042).

This indicates that if the experimentation could have been done in

the absence of RNA, CH_2O alone could have been responsible for at

least a portion of the observed mutagenic effect. This questions

the strict dependence of this mutagenic response on the presence

of adenine or RNA in the media as proposed in the production of

sex-linked lethals (Auerbach and Moser, 1953b; Alderson, 1960a;

Alderson, 1960b) and may indicate an increase in recombination which

is CH_2O dependent (Alderson, 1967). The interaction between CH_2O

and RNA, however, is also significant (P = .01, F = 4.89), as 86%

of the total variability can be explained by RNA, CH_2O, and the

interaction between the two reactants.

Figure 7 is a graphic representation of values in Table 2

showing the effect of increasing quantities of CH_2O at constant

levels of RNA

Formaldehyde induced germinal reversion

A small germinal study was made in order to determine the

activity of CH_2O in germinal reversion. At a constant level of

CH_2O the increase in RNA brings about an apparent increase in

germinal reversion though the correlation is not significant (Table

3). The corrected fiducial limits (Stevens, 1942) on the spontaneous

germinal frequency are 5.1×10^{-5} and 8.1×10^{-5} (Bowman, 1965). The

germinal reversion frequency for the total progeny of treated males

and females was not significantly above the spontaneous level. Taken

alone, the progeny of treated females showed a germinal reversion

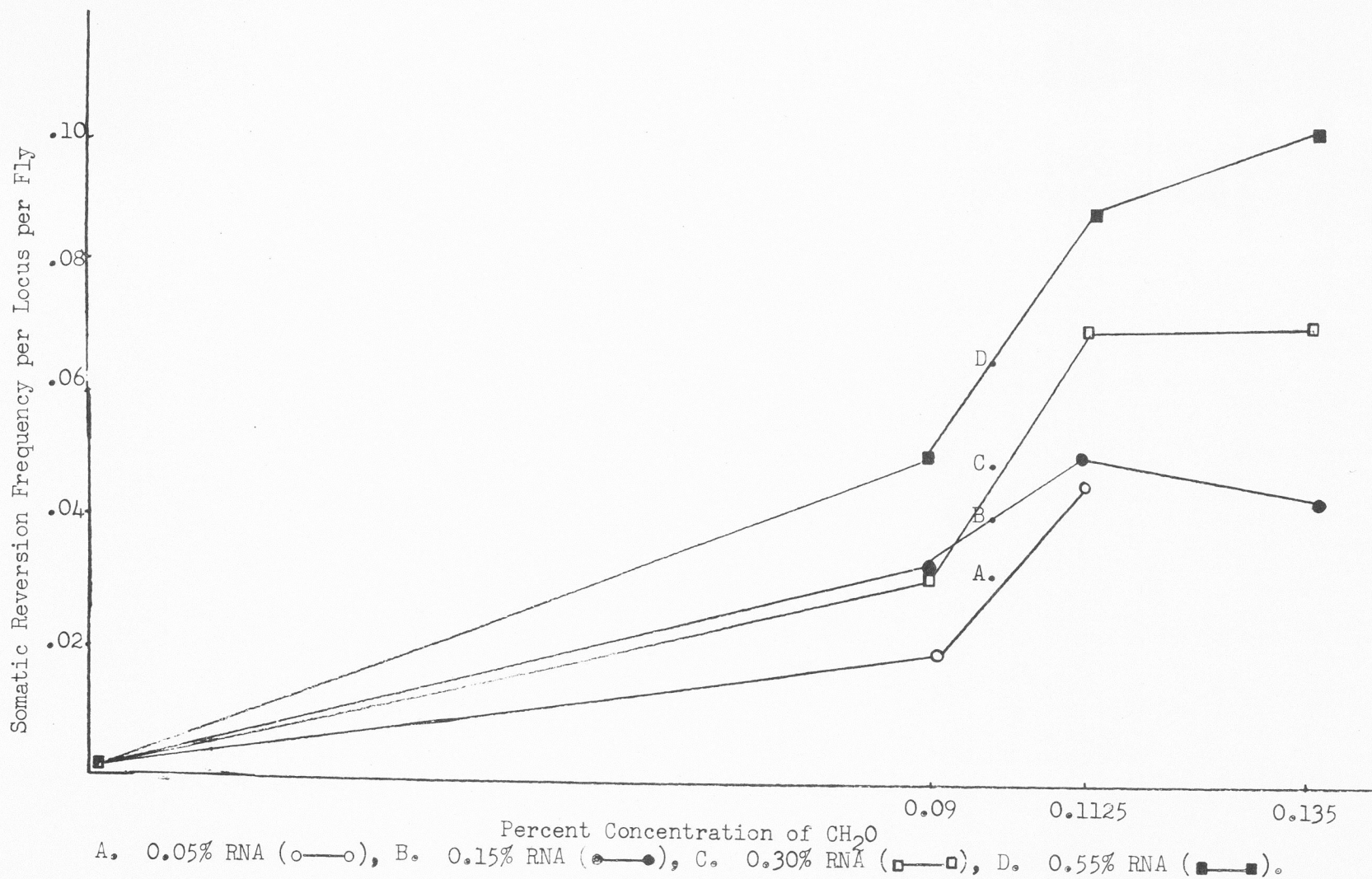frequency significantly above the spontaneous value (95% fiducial

Figure 7. The somatic reversion frequency as affected by varying increments of CH_2O at four levels of RNA.

A. 0.05% RNA (o——o), B. 0.15% RNA (●——●), C. 0.30% RNA (□——□), D. 0.55% RNA (■——■).

Table 3. The effect of formaldehyde and RNA on the germinal reversion
frequency of <u>white-ivory</u>

Percent CH_2O	Percent RNA	Revertants per No. of Flies Scored		Reversion Frequency $X\ 10^{-5}$	Fiducial Limits $X\ 10^{-5}$
		$\dfrac{y^2 sc\ w^i}{Y}$	$\dfrac{y^2 sc\ w^i}{y^2 sc\ w^{bf} w^{ch} spl}$		
0.09	0.05	0/6,719	4*/19,560	7.6	.4 to 35.3
0.09	0.15	2/16,941	0 /13,459	6.6	.4 to 30.4
0.09	0.30	1/26,058	9 /46,369	13.8	5.1 to 29.5
Total Females			11 /79,388	16.3	8.7 to 28.1**
Total Males		3/49,718		6.0	1.2 to 17.4
Total Flies		14/129,106		12.4	6.7 to 19.9

      * cluster of 3 flies
      ** significant at .05 level

limits at $8.7\ X\ 10^{-5}$ and $28.1\ X\ 10^{-5}$). Formaldehyde, therefore,

appears to be inducing a germinal event in females.

DISCUSSION

From information previously available it is known that formaldehyde has two effects: (1) it is mutagenic as is indicated by the induction of sex-linked lethals, and (2) it increases crossing-over (see Review of Literature).

The following conditions characterize the production of sex-linked lethals:

1. The production of sex-linked lethals requires both formaldehyde and adenosine (Alderson, 1960a; Alderson, 1960b). Free CH_2O injected into larvae at different stages of development (Sobels, 1954) and inosine plus CH_2O (Alderson, 1961) were found to be ineffective in increasing the frequency of sex-linked lethals. (Inosine is identical to adenine except for a 6-hydroxyl group in the position of the 6-amino group.)

2. Formaldehyde-food induces sex-linked lethals in males only.

3. The post spermatogonial stage of Drosophila sperm development is by far the most sensitive stage to CH_2O treatment. The spermatogonial stage may also be affected, but this is improbable since clusters of identical lethals are rarely recovered after treatment, and the frequency of sex-linked lethals induced during this period of development is not significantly increased (Auerbach and Moser, 1953a).

The second observed genetic effect of formaldehyde treatment, an increase in crossing-over, was observed by Sobels and van Steenis (1957), Sobels, Bootsma, and Tates (1959), and later by Whittinghill

and B. Lewis (1961). In all three experiments, formaldehyde-food
was used. An increase in recombination from 0.9% to 10% was obser-
ved only in males. Alderson (1967) found the increase in recombination
to be the same when either inosine or adenine was used. The parameters
of the increased crossing-over effect differ from the effect measured
by sex-linked lethals since: (1) clusters of identical recombinants
are recovered after treatment indicating an effect on the spermato-
gonial cells (Sobels, Bootsma, and Tates, 1959) and (2) adenine is
not essential for the induction of recombination (Alderson, 1967).

The present research has established the following parameters
for the action of formaldehyde on $\underline{w}^i$:

1. CH_2O significantly increases the reversion frequency of $\underline{w}^i$.

2. CH_2O-induced reversions are similar to X-ray induced reversion
   (Bowman, 1965) since both are locus dependent and both differ
   from spontaneous reversion by virtue of their being independent
   of synapsis.

3. There is a positive correlation between RNA concentration and
   the increase in reversion frequency in the presence of CH_2O.

4. CH_2O-induced reversion, however, appears not to be strictly
   RNA dependent.

5. The frequency of germinal reversion in treated females is signi-
   ficantly higher than in males. This contrasts with the lack of
   induced sex-linked lethals in females observed by Rapoport (1946),
   Auerbach and Moser (1953a), and Sobels and Simmons (1956).

6. Whereas recovery of 1 cluster is not proof, it does suggest
   that spermatogonial cells are affected by CH_2O treatment.

The data reported here are consistant with parameters established for the second genetic effect of formaldehyde. Both Formaldehyde alone and formaldehyde in the presence of RNA (formaldehyde-food) are effective reverting agents. The lack of RNA dependence as shown by the regression curve and statistical limits on the Y-intercept of Figure 6 suggests that CH_2O alone can increase the frequency of $\underline{w}^i$ reversion in somatic cells. The influence of RNA concentration indicates that both formaldehyde $\underline{and}$ a RNA-formaldehyde product may be effective in increasing reversion at the $\underline{w}^i$ locus. The action of formaldehyde alone might reflect hydroxymethylation of adenine which is incorporated in DNA.

A recombination mechanism is also in keeping with the observation that mitomycin C is a recombinogenic agent as well as an inductor of $\underline{w}^i$ somatic reversion.

The one cluster observed in the germinal reversion study indicates that spermatogonial cells are responsive to CH_2O treatment. More extensive research is desirable to confirm this observation. It may be argued, however, that the mutagenic action of CH_2O on $\underline{w}^i$ is not limited to post spermatogonial cells. To the extent that this is true, the germinal reversion of $\underline{w}^i$ parallels the observation of clusters of CH_2O-induced recombinants by Sobels, Bootsma, and Tates (1959). The observation that germinal revertants are recovered from CH_2O treated females is of particular interest. As discussed earlier, sex-linked lethals have been recovered only from treated males. The increase in sex-linked lethals is limited to males, whereas increased recombination is observed in females as well as males.

The fact that the observations of this thesis are comparable

to those noted for CH_2O-mediated recombination indicates that a cross-over-like mechanism (breakage and rejoining), such as proposed by Bowman (1965), is most likely involved in $\underline{w}^i$ reversion. The observation of induction of phage-like particles after mitomycin C treatments and the knowledge of the effectiveness of this mutagen on the somatic reversion frequency of $\underline{w}^i$ give added support for a recombination event similar to the proposed mechanism for the induction of episomes (Campbell, 1962). Since Green (1969b) has established that $\underline{w}^c$ involves a transposable factor and $\underline{w}^c$ was derived from $\underline{w}^i$, it is reasonable to propose that $\underline{w}^i$ is also a transposable element with properties similar to those of controlling elements in maize (Green, 1967; Green, 1969b; McClintock, 1965; McClintock, 1967).

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