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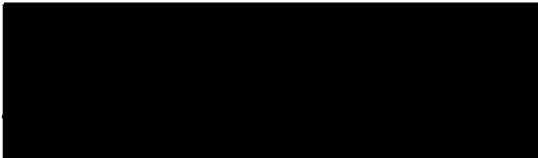

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EFFECT OF EXOGENOUS PYRIMIDINE NUCLEOSIDES AND THEIR HALOGENATED ANALOGUES ON THE RADIATION-INDUCED GENETIC DAMAGE IN *DROSOPHILA MELANOGASTER* MALE GERM CELLS

by

RENGASWAMI RAJARAMAN

A thesis submitted in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY
in Biology

DALHOUSIE UNIVERSITY
Halifax, Nova Scotia, Canada

March, 1972.

Approved by

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ABSTRACT

The mutagenic and the radiosensitizing properties of the halogenated pyrimidine nucleoside analogues BUdR, IUdR, BCdR, and ICdR, etc., are well established in the phages, the bacteria and the mammalian cells but not in the higher organisms. Using *D. melanogaster* as genetic test system, the mutagenic and radiosensitizing effect of the nucleoside analogues BUdR and BCdR, with TdR and CdR as controls, were studied in the different stages of the spermatogenous cells by standard brood technique. Dominant lethality, non-disjunction, chromosome loss, autosomal and sex-chromosomal translocations, and sex-linked recessive lethals were taken as the genetic parameters.

The nucleosides and the analogues were not mutagenic by themselves. The pre-meiotic cells, that synthesized DNA after the injection of the nucleosides or the analogues showed an increase in the genetic radiation damage only with BUdR and BCdR but not with TdR or CdR, probably due to the analogue incorporation in the DNA. The post-meiotic cells, that synthasized DNA before the injection of the nucleosides showed a reduction in the genetic damage. The different possible mechanisms of the radio-protective action of the nucleosides were discussed. Since the protection was afforded by the DNA precursors and the genetic repair would be expected to take place at the DNA level, the probable involvement of DNA repair synthesis was suggested, though repair by structural protein synthesis was not ruled out.

ABBREVIATIONS AND SYMBOLS

A	Autosome
AdR	Deoxyadenosine
ATP	Adenosine triphosphate
B	Bar eye*
BC	5-Bromocytosine
BCdR	5-Bromodeoxycytidine
br; brs	Brood; broods
BU	5-Bromouracil
BUdR	5-bromodeoxyuridine
bw	Brown eye*
CdR	Deoxycytidine
ClU	5-Chlorouracil
ClUdR	5-Chlorodeoxyuridine
D; d	Day
DNA	Deoxyribonucleic acid
F_1	First filial generation
F_2	Second filial generation
FU	5-Fluorouracil
FUdR	5-Fluorodeoxyuridine
GA	Gamma irradiation before brood A
GE	Gamma irradiation before brood E
GdR	Deoxyguanidine
IC	5-Iodocytosine
ICdR	5-Iododeoxycytidine
In	Inversion*
IU	5-Iodouracil
IUdR	5-Iododeoxyuridine
kr	Kilo rad
L	Sex-linked recessive lethal mutation
MF	Modification Factor (% lethals in nucleoside or analogue + radiation / % lethals in S + radiation)

mRNA	Messenger ribonucleic acid
p^p	Pink peach eye*
S; saline	Physiological saline (0.7% NaCl w/v in distilled water)
RDL	Relative dominant lethal frequency
RNA	Ribonucleic acid
sc	Scute*
st	Scarlet eye*
TdR	Thymidine
Te	Tested
Tr; Trans.	Translocation
UdR	Deoxyuridine
y	Yellow body*
♂	Male
♀	Female
$>$	Not significant
$<$	Significant
$\ll$	Highly significant

* See Lindsley and Grell (1967) for further information.

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INTRODUCTION

The halogenated pyrimidine analogues are a series of compounds in which a halogen atom has been substituted for the hydrogen atom at the 5-carbon position of uracil or cytosine. The resultant pyrimidines, 5-fluorouracil (FU), 5-chlorouracil (ClU), 5-bromouracil (BU), 5-iodouracil (IU), 5-bromocytosine (BC), and 5-iodocytosine (IC) and their corresponding deoxyribosides 5-fluorodeoxyuridine (FUdR), 5-chlorodeoxyuridine (ClUdR), 5-bromodeoxyuridine (BUdR), 5-iododeoxyuridine (IUdR), 5-bromodeoxycytidine (BCdR), and 5-iododeoxycytidine (ICdR) have been useful as molecular probes in the study of the mutation process (Freeze, 1959), the semi-conservative replication of DNA and the detection of non-semi-conservative 'repair' synthesis of DNA (Kaplan, 1970). In addition, they comprise a new class of radio-sensitizing agents which played an important role in molecular radiation biology by enhancing the direct radiation damage to DNA (Djordjevic and Szybalski, 1960; Erikson and Szybalski, 1961).

The metabolic properties of these various halogenated pyrimidine analogues depend on the radii of the van derWaal's forces that surround the halogen atoms (Szybalski, 1962). Thus, FU behaves like uracil and is incorporated into RNA in the place of uridylate after enzymatic conversion into

the riboside triphosphate. After phosphorylation, FUdR selectively inhibits thymidylate synthetase, blocking the conversion of uridylate to thymidylate and thus inhibiting the DNA synthesis (Cohen _et al_., 1958). The analogues substituted with heavier halogen atoms (BU and IU; BUdR and IUdR) behave as thymine or thymidine analogues, their triphosphates being selectively incorporated into DNA (Zamenhof and Griboff, 1954; Dunn and Smith, 1957). The halogenated deoxycytidines (BCdR and ICdR) may be deaminated intracellularly to BUdR and IUdR, respectively, and incorporated into DNA (Frisch, _et al_., 1960; Cramer _et al_., 1962; Prusoff, 1963) or may be incorporated in the place of deoxycytidine (CdR) (Herskowitz, 1967). The selectivity and specificity of the incorporation of these analogues into DNA or RNA makes their radiosensitizing properties directly interpretable in terms of effects on the corresponding types of nucleic acid.

Although the mechanism of the radiosensitization by the halogenated pyrimidine analogues has not been fully elucidated, there is a consensus of agreement that the process is intimately associated with radiation damage at the DNA level. The explanations that have been proposed fall into two main groups:

a. The presence of BU in one strand of the DNA sensitizes it to the lethal effect of ^{32}P disintegration in the opposite strand (Ragni and Szybalski, 1962), suggesting that

the presence of BU in some way labilizes the phosphodiester back bone of the DNA, thus increasing the radiochemical lesions within the DNA (Szybalski, 1962).

b. The primary sensitization effect may be due to the interference by the halogenated analogues with the DNA repair system which operate in the irradiated cells (Lett *et al*., 1964).

Recently, it has been demonstrated by Humphrey and Dewey (1965) in mammalian cell lines and by Kaplan (1967) in *Escherichia coli* that radiosensitization by BUdR is caused by an increased yield of DNA strand breaks, which are non-reparable.

While the induction of mutation and radiosensitization with 5-halopyrimidines have been clearly demonstrated in microorganisms and mammalian cell lines, comparatively little information is available on these phenomena in higher organisms. These analogues have a variety of physiological effects on the main characteristics of the life cycle in higher organisms. Apart from replacing thymidine (TdR) in the cell nuclei in the root meristem of *Vicia faba* (Kihlman, 1963) and in the gametophytic tissue of *Arabidopsis thaliana* (Brown and Smith, 1964), BUdR could induce chromosome breaks in the treated cells (Hsu and Somers, 1961) and increase the radiosensitivity (Humphrey and Dewey, 1965). In *A. thaliana*, BUdR had other effects such as (a) reduction of fresh

and dry weight, (b) altered morphological characters, and (c) delayed flowering (Jacobs, 1969) in the treated (P) generation. Similar alterations in the morphological characters (somatic mutation) have been observed in the imagos of *Drosophila melanogaster*, when the larvae were treated with BUdR (Rizki and Rizki, 1968). However, the information on mutation induction and radiosensitization of genetic damage by these analogues in higher organisms is scanty and contradictory.

In *A. thaliana*, Brown and Smith (1964) demonstrated with autoradiography that ^{125}I-labelled IUdR was incorporated in the nucleus of the gametophytic cells. They obtained some lethal and viable laggards that segregated in the successive generations but the evidence for the induced mutation was inconclusive. Immersion of seeds in BUdR solution did not induce mutation. Röbbelen (1964) and Müller (1965) also failed to induce mutations with base analogues in *A. thaliana*. When the analogue was supplied in the medium, Hirono and Smith (1969) obtained segregating Mendelian gene mutations in *A. thaliana*, while using the same method Jacobs (1969) obtained only heritable "laggards" of cytoplasmic origin with no Mendelian segregation.

The mutagenic action of BUdR has been first studied in *D. melanogaster* by Kaufman *et al.*, (1961). When BUdR was injected into the adult males intra-abdominally, lethal

mutations were induced in the spermatozoa and in the spermatids but no chromosomal rearrangements, suggesting secondary effects rather than a direct involvement of the DNA (Kaufman and Gay, 1970). Herskowitz and Bakula (1965) tentatively claimed that BUdR induced lethal mutations in D. melanogaster, when grown in defined sterile culture medium containing BUdR. However, negative evidence for the mutagenic action of BUdR in the injected males of D. melanogaster was obtained by Sobels (1965), Fahmy et al., (1966) and Sharma (1969). Similarly, larval or adult feeding on BUdR solution did not induce any lethal mutation (Khan and Alderson, 1968). Sobels (1965) observed an enhancement of the radiation-induced genetic damage as shown by an increase in the lethal mutations, while Sharma (1969) failed to observe the radiosensitizing effect.

Since the phenomena of the mutagenicity and the sensitization of radiation-induced genetic damage with the halogenated pyrimidine analogues in higher organisms was inconclusive, it was undertaken to investigate these phenomena with BUdR (a TdR analogue) and BCdR (a Cdr as well as TdR analogue) in the different stages of male germ cells of D. melanogaster, using the standard techniques of genetic analysis.

MATERIALS AND METHODS

1. Genetic Stocks
2. Mating Scheme and Brood Pattern
3. Culture Conditions
4. Administration of Chemicals
5. Irradiation
6. Treatment Conditions
6.1. 1-Day Gamma A series
6.2. 1-Day Gamma E series
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8.4. Extraction of Sex-linked Recessive Lethal Mutations

1. Genetic Stocks:

Male *Drosophila melanogaster* flies having the genetic constitution $\underline{X^{c2}y\ B}$ / $\underline{y+}$ $\underline{Y}$ were used in conjunction with $\underline{y}$ $\underline{sc^{S1}}$ $\underline{In.49}$ $\underline{sc^{8}}$; $\underline{bw}$; $\underline{st}$ $\underline{p^{p}}$ virgin females in all the experiments.

The males have a closed ring X-chromosome with a recessive visible marker *y* (yellow body; location in a rod X-chromosome 1-0.0) and a dominant marker *B* (bar shaped eye; location in a rod X-chromosome 1-57.0). The Y-chromosome has the tip of $\underline{In}$ $\underline{(1)}$ $\underline{sc^{8}}$ including the *y+* locus of an X-chromosome transferred to the tip of the long arm ($\underline{Y^{L}}$); (*y+* *Y* is a synonym of *sc.8* *Y*) (Lindsley and Grell, 1967). Phenotypically these males are bar eyed, with the normal body colour.

Since the base analogues are known to produce point mutations by replacing the normal bases, males carrying a ring shaped X-chromosome were preferred so as to restrict the recovered recessive lethals mainly to point mutations (Sobels et al., 1967). Translocations involving a ring chromosome will, by the formation of dicentrics, lead to inviable gametes, restricting the recovered lethals mainly to point mutations and small deficiencies (Oster, 1958). This would eliminate picking up lethals due to chromosomal rearrangements.

The females have markers in three chromosomes. The X-chromosome is marked with y (yellow body), while bw (brown eye; location 2-104.5) is the second chromosome marker. The third chromosome is marked by two closely linked genes (4 map units) st (scarlet eyes; location 3-44.0) and p^p (pink-peach eye; location 3-48.0). All the genes are described by Lindsley and Grell (1967). The locus bw blocks the synthesis of the red pigments, resulting in brownish wine eye colour on emergence, darkening to garnet. The locus st produces light vermillion eyes by blocking the synthesis of brown pigments. When a fly is homozygous for bw and st, the eyes are colourless or white. When the locus p^p is present along with st, the eye colour is a bright orange and the classification is easier.

2. Mating Scheme and Brood Pattern:

Treated X^{c2} y B / y+ Y males were crossed with y sc^{S1} In.49 sc^{8}; bw; st p^{p} virgin females (Fig. 1). The age of the males was either one day or seven days at the time of the initiation of crossing, depending on the experimental protocol (detailed information given below). The age of the virgin females at the time of the initiation of the cross was always 3-4 days after eclosion.

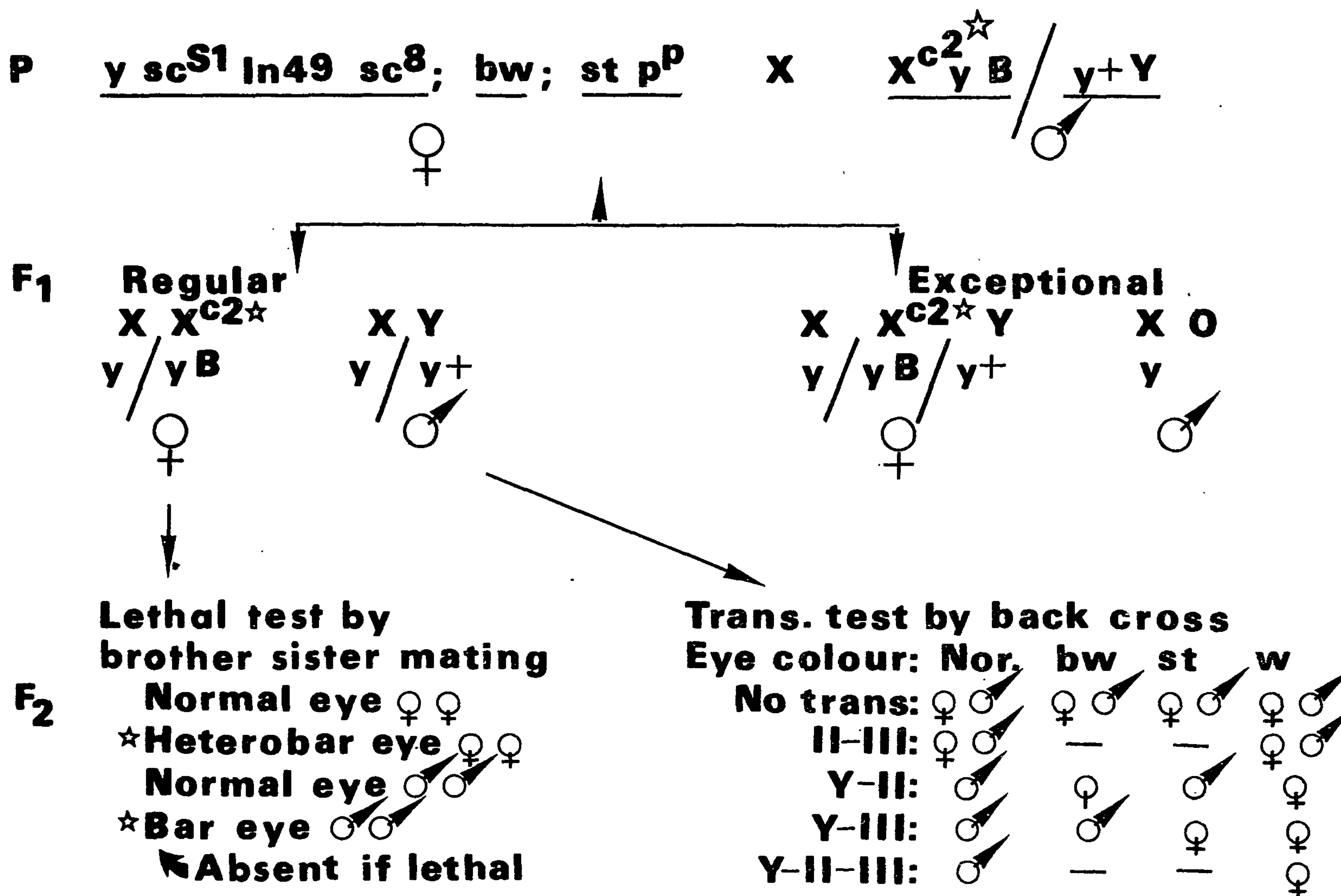
Since the object of the studies was to investigate the mutagenicity of the halogenated pyrimidines (BUdR and BCdR) and their radiosensitizing properties, when incorporated in the DNA of the nucleus, the time of DNA synthesis in the spermatogonial cells was taken into consideration. Accordingly, the treatment and the mating schedule was designed so as to separate the germ cells that synthesized DNA before and after the injection of the nucleosides or their analogues.

The testes of the adult male D. melanogaster have an array of progressive stages of maturing spermatogenous cells in the following order: Sstem cells, early and late spermatogonial cells, spermatocytes, meiotic cells, spermatids, immature spermatozoa (in bundles) and mature spermatozoa (free swimming). The major episode of DNA synthesis during the development of the male gametes occurs after the premeiotic mitosis of the spermatocytes resulting in tetraploid

Fig. 1. Standard mating scheme to extract sex-linked recessive lethals and II, III and / or Y chromosome translocations induced in the treated males. The chromosomal basis of scoring lethals and translocations are shown in Figs. 5 and 6.

☆ Treated X-chromosome.

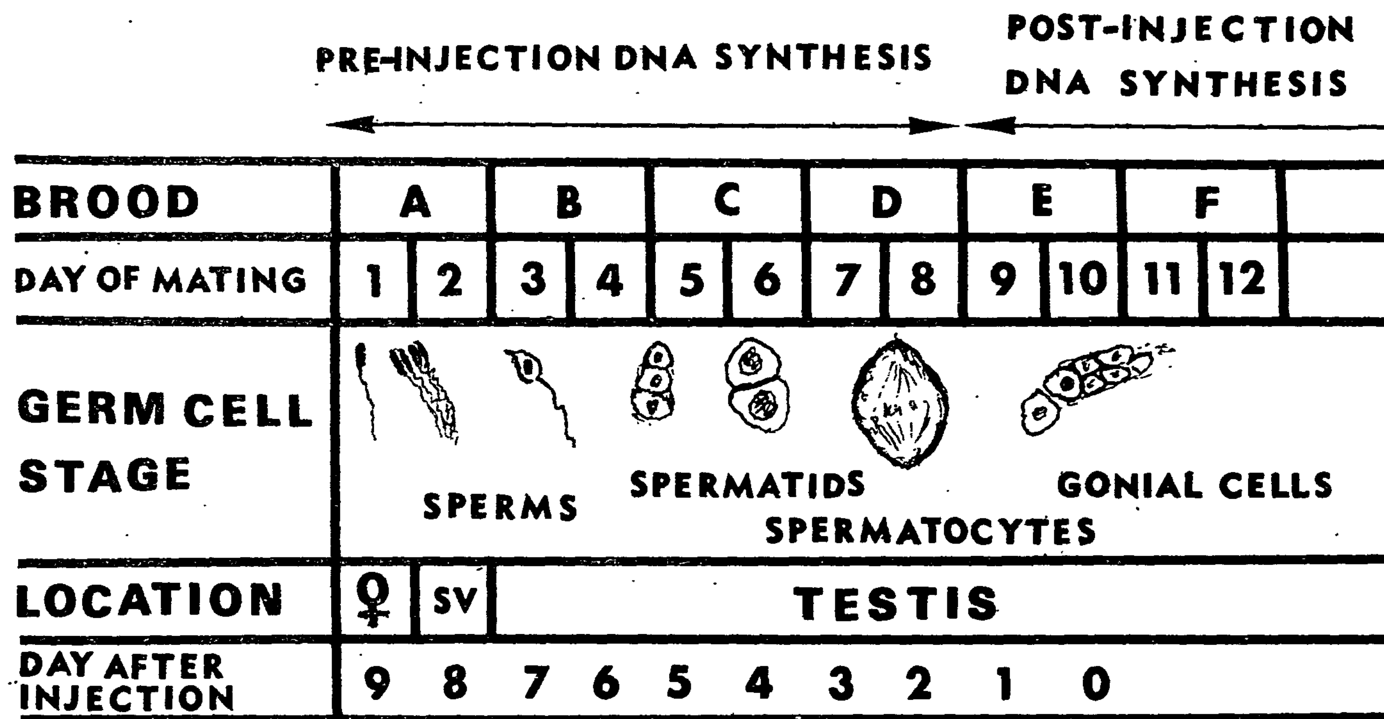
MATING SCHEME



(4C) quantity of DNA (Swift, 1950). The time required from the 4C stage of the spermatocyte to the formation of mature spermatozoa in D. melanogaster is 10 days ± few hours. This time table has been well documented by different authors (Chandley and Bateman, 1962; Olivieri and Olivieri, 1965; Kaplan and Sisken, 1960) by the use of tritiated TdR and autoradiography. Taking this into consideration, the standard 2-day brood pattern widely used in D. melanogaster genetic studies (Sobels, 1966) was employed to separate the F_1 offspring fractions derived from the different germ cell stages at the time of treatment (Fig. 2).

Each treated male (one day old at the time of the initiation of mating unless otherwise stated) was numbered individually and allowed to mate with six virgin females for 48 hours. At the end of 48 ± 1 hr, the males were transferred by etherization to fresh vials with a fresh batch of six 3-day old virgin females per male for the next 48 hrs. Each mating period of 48 hrs comprises one brood. The males were carried through six such broods (A through F) for a total period of 12 days in order to sample the post-meiotic and pre-meiotic spermatogenous cells. The F_1 offsprings of the successive broods raised in this way would sample mainly the mature sperms in brood A, the immature sperms in brood B, the spermatids in brood C, the meiotic cells in brood D, the spermatocytes in brood E and the late spermatogonial cells

Fig. 2. Brooding Schedule observed to isolate the progenies derived from the different germ line sectors based on the time table of DNA synthesis and the spermatogenesis in male *D. melanogaster* at 25°C (Chandley and Bateman, 1962 and Sobels, 1966).


PRE-INJECTION DNA SYNTHESIS
POST-INJECTION DNA SYNTHESIS
BROOD
A
B
C
D
E
F
DAY OF MATING
1
2
3
4
5
6
7
8
9
10
11
12
GERM CELL STAGE
SPERMS
SPERMATIDS
SPERMATOCYTES
GONIAL CELLS
LOCATION
♀
SV
TESTIS
DAY AFTER INJECTION
9
8
7
6
5
4
3
2
1
0

in brood F (Fig. 2)(Sobels, 1966). However, it should be mentioned that a strict separation of the cells in different stages cannot be obtained by any brood pattern.

After each mating period, the group of six inseminated females was transferred to vials with fresh medium and was left for another four days before being killed. This routine subculture avoids overcrowding and assures maximal recovery of the F_1 progeny (Ives, 1963).

3. Culture Conditions:

All cultures were raised in a temperature controlled room (25°C ± 1), on standard cream of wheat-yeast-sugar medium (Demerec and Kaufman, 1961) with slight modifications.

The following was the proportion of the different constituents of the medium: water 79 cc; agar 0.3 gm; dry yeast 6.0 gm; methyl-P-hydroxy benzoate (fungicide, 10% solution in 95% ethanol) 0.7 cc. Yeast and sugar were dissolved in about 15 cc of water and allowed to ferment for about 10-15 minutes. The cream of wheat was soaked in about 20 cc of water. Agar was dissolved separately in boiling water. Fermenting yeast-sugar solution was boiled for 30 minutes to kill the yeast. Soaked cream of wheat was added to the cooked yeast-sugar solution. Cooking was continued for another 20 minutes. When the yeast-cream of wheat mixture was cooked properly, dissolved agar and the fungi-

cide solutions were added and thoroughly mixed. Constant stirring of the medium at all stages of cooking was necessary to avoid burning the medium at the bottom of the pot. Approximately 7-8 cc of the medium was poured into heat sterilized (350°F for 1 hr) standard shell vials (9 cm high x 2.5 cm diameter). Fermenting the yeast and sugar before cooking eliminated the seeding of individual vials with yeast before starting a culture, since the medium was already having the fermented yeast smell. The addition of a small quantity of agar in the medium helped stop the medium falling down when the bottles or vials were inverted to empty the flies, which is a nuisance in the regular cream of wheat medium (Demerec and Kaufman, 1961).

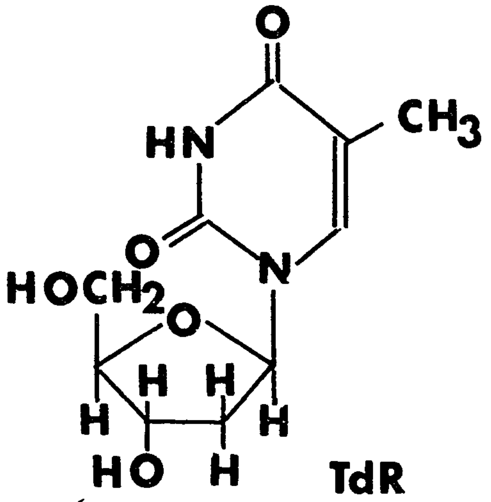
4. Administration of Chemicals:

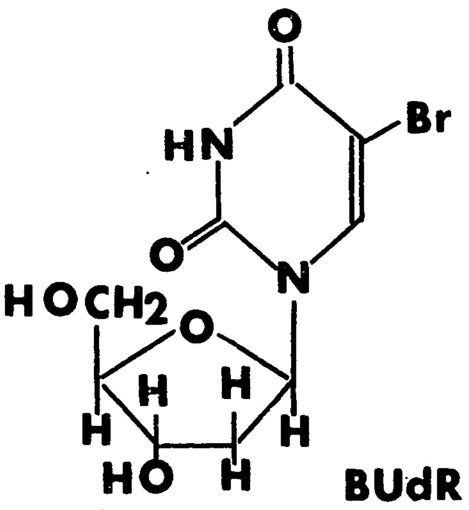
The nucleosides and their brominated analogues used in the present study are as follow:

1. Thymidine (TdR)
2. 2-Deoxycytidine (CdR)
3. 2-Deoxyguanosine (GdR)
4. 2-Deoxyadenosine (AdR)
5. 5-bromodeoxyuridine (BUdR)
6. 5-Bromodeoxycytidine (BCdR)

The structural formulae of the above nucleosides and their analogues are shown in Fig. 3. TdR, GdR, BUdR, and BCdR were obtained from Nutritional Biochemicals, Cleveland, Ohio. AdR and CdR (as the hydrochloride) were obtained from

Fig. 3. Structural formulae of the nucleosides and the brominated pyrimidine nucleoside analogues used in this study: TdR = thymidine; CdR = 2-deoxycytidine; GdR = 2-deoxyguanosine; AdR = 2-deoxyadenosine; BUdR = 5-bromodeoxyuridine; BCdR = 5-bromodeoxycytidine.


O
CH$_3$
HN
O
N
HOCH$_2$
O
H
HO
TdR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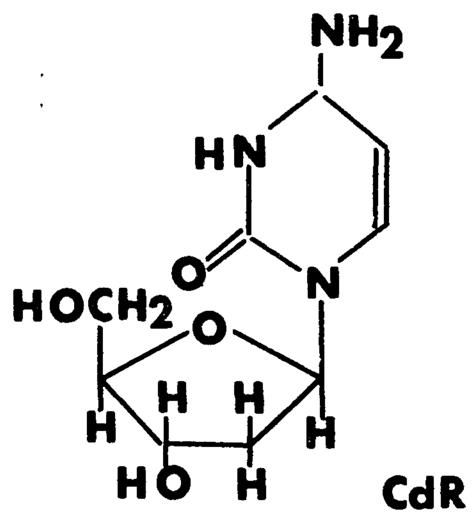
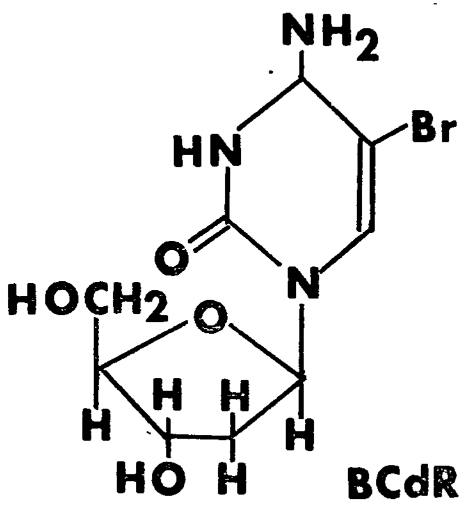
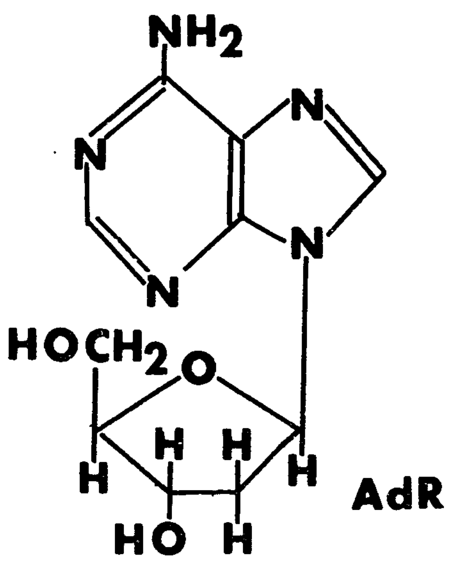
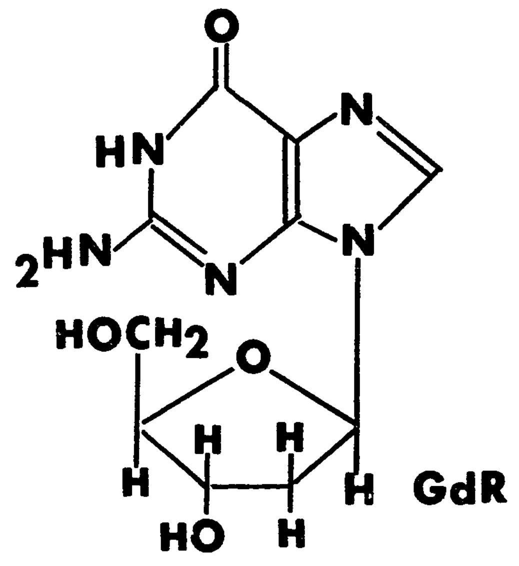
Br
HN
O
N
HOCH$_2$
O
H
HO
BUdR


NH$_2$
HN
O
N
HOCH$_2$
O
H
HO
CdR


NH$_2$
Br
HN
O
N
HOCH$_2$
O
H
HO
BCdR


NH$_2$
N
HOCH$_2$
O
H
HO
AdR


O
HN
N
$_2$HN
HOCH$_2$
O
H
HO
GdR

Sigma Chemical Company, St. Louis, MO., U.S.A. The nucleosides or the analogues were dissolved at the required concentration in isotonic or physiological saline (saline or S) (0.7% w/v NaCl in distilled water) and administered by microinjection into the abdominal cavity of the adult $\underline{X^{c2}}$ $\underline{y}$ $\underline{B}$/ $\underline{y+}$ $\underline{Y}$ males of D. melanogaster. Etherized males were injected with saline of 0.1% nucleoside or analogue solution in saline with the aid of a glass microneedle attached to a Gilmont micrometer syringe (Cole-Parmer Instrument and Equipment Company, Chicago, Ill., U.S.A.) through a plastic leuer end catheter. The tip of the needle was gently inserted ventro-laterally into the anterior side of the abdomen and the solutions injected until the dorsal tergites distended slightly. The approximate quantity of the solution injected was 0.2µl / fly; however, strict quantitation of injected solution is not reliable since a varying amount of the solution may ooze out from the fly (Carlson and Oster, 1962).

5. Irradiation:

In all the experiments gamma rays were delivered from "Gamma Beam" ^{137}Cs source (Atomic Energy of Canada Ltd., Ottawa, Canada). Total doses of 1,200, 2,400 or 3,600 Rads were delivered at a rate of 4.1 to 4.2 Rads/sec at room temperature. The dose rate was measured with the aid of a Baldwin-Farmer Secondary Standard Dosemeter Mark 2 (Baldwin Instrument Co., Ltd., Dartford, England), calibrated by the

National Physical Laboratory, England.

Perforated size 00 gelatin capsules were loaded with 25-30 males at a time and the capsule was placed in a tes-tube during irradiation. Etherized males in the capsules were allowed to recover completely from anaesthesia before irradiation in order to avoid any possible anoxic effects on the mutation frequencies (Mukherjee, 1965).

6. Treatment Conditions:

Three series of experiments were conducted in this study.

6.1. 1-Day Gamma A series:

In this series of experiments, the post-injection DNA synthesis cells (spermatocytes and late spermatogonial cells sampled in brs E and F--see Fig. 2) were irradiated in the pre-meiotic stage.

The males that emerged during a period of 1 hr were used in this series of experiments. The males were 12±0.5 hrs old at the time of injection of the solutions (as outlined below) and 24±0.5 hrs old at the time of irradiation. An interval of 12 hrs between the injection and the irradiation was allowed to give sufficient time for the nucleosides or analogues to be incorporated in the DNA (Olivieri and Olivieri, 1965). Immediately following the irradiation (within 10-15 minutes) the males were individually crossed with

six virgin females through six successive 2-day broods A through F.

The following test conditions were obtained:

1. Saline-unirradiated control (S)
2. S + 1.2 kr gamma rays before br A (S + 1.2 GA)
3. 0.1% TdR-unirradiated control (TdR)
4. 0.1% TdR + 1.2 kr gamma rays before br A (TdR + 1.2 GA)
5. 0.1% BUdR-unirradiated control (BUdR)
6. 0.1% BUdR + 1.2 kr gamma rays before br A (BUdR + 1.2 GA)
7. 0.1% CdR-unirradiated control (CdR)
8. 0.1% CdR + 1.2 kr gamma rays before br A (CdR + 1.2 GA)
9. 0.1% BCdR-unirradiated control (BCdR)
10. 0.1% BCdR + 1.2 kr gamma rays before br A (BCdR + 1.2 GA)
11. 0.1% equimolar solution of TdR, CdR, AdR and GdR-unirradiated control (TdR + CdR + AdR + GdR)
12. 0.1% equimolar solution of TdR, CdR, AdR and GdR + 1.2 kr gamma rays before br A (TdR + CdR + AdR + GdR + 1.2 GA)

In the treatment conditions no. 11 and 12, the offspring derived from the mature sperms alone (br A: first two days of mating--see Fig. 2) were studied with reference to lethals.

For all other treatment conditions, the mortality of the treated males, the temporal pattern of dominant lethals, the fertility, the incidence of non-disjunction, chromosome loss and lethal mutations were studied through the six successive broods, while the incidence of translocations were studied only through the first five broods (brs A through E). The translocation tests were not carried out in the late spermatogonial cells (br F), since most of the translocation bearing gonial cells are selectively eliminated during meiosis from being able to form viable gametes (Puro, 1966).

6.2. 1-Day Gamma E series:

In this series of experiments, the post-injection DNA synthesis cells (the spermatocytes and the late spermatogonial cells sampled in brs E and F--see Fig. 2) were irradiated in the post-meiotic (spermatozoa stage) stage; to achieve this, irradiation was postponed until after br D.

As in the previous set of experiments, the males that ecloded during a period of 1 hr were used in these experiments also. The virgin males were injected when 12 ± 0.5 hrs old. When they were 24 ± 0.5 hrs old, they were crossed individually with six virgin females, through four 2-day broods (brs A, B, C and D). Before the fifth br E was initiated (that samples the post-injection DNA synthesis cells), the males were exposed to 1.2 kr gamma rays. The F_1 progenies of brs E and F were studied for translocations and lethals,

while those of brs A through D were rejected. The following test conditions were obtained in this set of experiments:

1. S + 1.2 kr gamma rays before br E (S + 1.2 GE)
2. 0.1% TdR + 1.2 kr gamma rays before br E (TdR + 1.2 GE)
3. 0.1% BUdR + 1.2 kr gamma rays before br E (BUdR + 1.2 GE)
4. 0.1% CdR + 1.2 kr gamma rays before br E (CdR + 1.2 GE)
5. 0.1% BCdR + 1.2 kr gamma rays before br E (BCdR + 1.2 GE)

At the time of irradiation on the 8th day (before br E) the mature sperms in the testes would have DNA synthesized after the injection of the nucleosides or analogues (Fig. 2). By irradiating the base analogue incorporated sperms, it was expected to bypass the meiotic elimination of cells with chromosomal rearrangements that would likely have happened in the previous series of experiments and thus increasing the chances of picking up more lethals and/or translocations induced as a result of the radiosensitizing effect of the base analogues in the DNA.

6.3. <u>7-Day Gamma A series</u>:

In this set of experiments, the pre-injection DNA synthesis cells were irradiated in the fully mature spermatozoan stage in the presence of the exogenous nucleosides or their

analogues.

The male flies that emerged during a period of 3 hrs were used in this set of experiments. The males were virgins and 7 days old (168±1.5 hrs) at the time of irradiation. Nucleoside or analogue solutions were injected 12±0.5 hrs before irradiation. Each treated male was individually mated with three virgin females with two 1-day broods (first two days only) and was rejected. Using the inseminated females, a second sub-culture was raised to recover more F_1 flies. The females were rejected on the 7th day after the initiation of the culture. The F_1 progeny was screened only for lethal mutations with the following test conditions:

1. S + 2.4 kr gamma rays (7-Day S + 2.4 GA)
2. 0.1% TdR + 2.4 kr gamma rays (7-Day TdR + 2.4 GA)
3. 0.1% BUdR + 2.4 kr gamma rays (7-Day BUdR + 2.4 GA)
4. 0.1% CdR + 2.4 kr gamma rays (7-Day CdR + 2.4 GA)
5. 0.1% BCdR + 2.4 kr gamma rays (7-Day BCdR + 2.4 GA)
6. 0.1% Equimolar solution of TdR, CdR, GdR and AdR + 2.4 kr gamma rays (7-Day TdR + CdR + GdR + AdR + 2.4 GA)
7. S + 3.6 kr gamma rays (7-Day S + 3.6 GA)
8. 0.1% Equimolar solution of TdR, CdR, AdR and GdR + 3.6 kr gamma rays (TdR + CdR + AdR + GdR + 3.6 GA).

The males were aged for 7 days without being allowed to mate. This would ensure accumulation of mature sperms in the

testes, as the maturation of the spermatogonial cells is a continuous process (Lefevre and Jonsson, 1964 a). This set of experiments was conducted to study the effect of the exogenous nucleosides and analogues on the radiation-induced genetic damage in the mature spermatozoa.

Two different higher radiation doses (2.4 kr and 3.6 kr) were employed in order to confirm the protective effect observed with 1-day old males exposed to 1.2 kr gamma radiation with exogenous nucleosides or their analogues.

7. <u>Toxicity Assays</u>:

After a preliminary trial of different concentrations of nucleoside and analogue solutions in saline, 0.1% solution of the nucleosides or analogues in saline was preferred since this concentration has been successfully used in <u>D. melanogaster</u> by other workers (Herskowitz,and Bakula, 1965; Sharma, 1969). This would approximately give a dose of 0.34μg / fly around the testes (Fahmy <u>et al</u>., 1966). However, due to lack of biodata for these chemicals on <u>D. melanogaster</u>, the rate of mortality of the treated males as well as their temporal pattern of fertility through the successive broods were taken as toxicity assays (Mukherjee, 1965; Fahmy and Fahmy, 1970). Higher concentrations (0.5-1.0%) were found toxic to the treated males (Fahmy <u>et al</u>., 1966).

7.1. <u>Rate of Mortality in Treated Males</u>:

The number of dead males were recorded at intervals of

2 days till the 10th day. The percent dead males in relation to the previous count of live males gave the rate of mortality of the treated males.

7.2. Fertility of Treated Males:

The fertility of treated males involved the counting of all the F_1 offspring of each male per each brood.

The rate of mortality among the treated males and their fertility were studied only in the 1-Day Gamma A series.

8. Mutation Assays:

Using the F_1 progeny of the cross: $y\ sc^{S1}\ In.49\ sc^{8}$ females x $X^{C2}\ y\ B\ /\ y+\ Y$ males, it is possible to study the frequencies of dominant lethals, non-disjunction, chromosome loss, translocations between II, III and / or Y chromosomes and sex-linked recessive lethal mutations (lethals).

8.1. Dominant Lethals:

Dominant or embryonic lethality may result due to several causes, but mainly due to gross chromosomal rearrangements that hinder cleavage (Auerbach, 1962; LaChance, 1964). Hence, this is also commonly referred to as % unhatchability of the egg (Shankaranarayanan, 1967). The method used by Abrahamson and Herskowitz (1957) for the study of dominant lethals was used with minor modifications. Each egg collecting unit was hexagonal in shape and was made from a set of seven cylindrical plastic tubes (8 cm length x 1 cm diameter) glued to-

gether laterally with both ends open. At one end of the unit a nylon netting was held taut by cementing. Leaving the central tube empty, one inseminated female was placed in each tube and the tubes were sealed with cotton plugs. The unit was then placed gauze side downwards in a petriplate containing the fly medium. The medium was dyed with methylene blue (Brink, 1969) for optical clarity (0.5 mg/100 cc of cooked medium).

One unit was used per batch of six females that mated with one male. About 90-120 inseminated females (15-20 males) per brood per treatment were used to collect sufficient number of eggs. Each female was numbered to keep an accurate record of the eggs laid by the individual females. The females were allowed to lay for three successive 12 hr periods after each brood period (Fig. 2). The eggs were counted at the end of each sitting and after 25-28 hrs of incubation at $25 \pm 1^{o}C$ in a fly proof incubator, the unhatched eggs were counted. The eggs laid by unfertilized females (when none of this female's eggs hatched in all the three sittings) were excluded from the calculations to avoid spurious increase in the dominant lethal frequency (Sankaranarayanan, 1967).

8.2. Non-disjunction and Chromosome Loss:

The F_1 progeny of the cross: $\underline{y}$ $\underline{sc^{S1}}$ $\underline{In.49}$ $\underline{sc^{8}}$; $\underline{bw}$; $\underline{st}$ $\underline{p^{p}}$ females x $\underline{X^{c2}}$ $\underline{y}$ $\underline{B}$ / $\underline{y+}$ $\underline{Y}$ males consists of yellow body, heterobar females and wild type females. In the event of chro-

mosome loss (Y) or non-disjunction (X and Y entering the same nucleus during meiosis) exceptional zygotes resulting in XO males (yellow body) and $XX^{c2}Y$ females (wild type, heterobar eye) are produced. (Fig. 1 and 4). Thus, the F_1 progeny count per treated male (data on the fertility of treated males) also gave the data on the frequencies of non-disjunction and chromosome loss. The frequency of non-disjunction ($XX^{c2}Y$ females) females was computed in relation to XX^{c2} females, while that of chromosome loss (XO males) in relation to XY males (Browning, 1969). Since the exceptional zygotes produced due to non-disjunction in the female gametogenesis could not have been influenced by the treatment given to the males, these females were ignored (Fig.4) (Leigh, 1969).

8.3. Extraction of Translocations:

The F_1 males of the cross between y sc^{S1} In.49 sc^8 females and X^{c2} y B / y+ Y males could be used to detect the translocation events between IInd, IIIrd and/or Y chromosomes induced in the treated male germ cells. In order to extract the different translocations, the F_1 males were individually back-crossed with two or three y sc^{S1} In.49 sc^8; bw; st p^p virgin females. In the absence of any translocation event involving II, III and/or Y chromosomes, the back-cross progeny would yield four eye colour phenotypes, i.e., normal or wild (red), brown, scarlet and white eyed males and females

Fig. 4. The different genotypes of the gametes produced by the male and the female in a cross between *y* sc^{S1} *In.49* sc^{8} female and X^{c2} *y* *B* / *y+* *Y* male and the genotypes and the phenotypes of the F_1 flies (See Fig. 1 also) (Leigh, 1969).

FEMALE GAMETES	MALE GAMETES			
	Regular		Exceptional	
	$X^{c2}yB$	y^+Y	O	$X^{c2}yB/y^+Y$
Regular Xy	X/X^{c2} y/yB ♀	X/Y y/y+ ♂	X/O y ♂	$X/X^{c2}/Y$ y/yB/y+ ♀
Exceptional O	O/X^{c2} Lethal	O/Y Lethal	O/O Lethal	$O/X^{c2}/Y$ yB/y+ ♂
Xy/Xy	$X/X/X^{c2}$ Lethal	X/X/Y y/y/y+ ♀	X/X/O y/y ♀	$X/X/X^{c2}$ Lethal

(Figs. 1 and 5.1.). The presence of a translocation between II and III chromosomes in the treated male parent would be indicated by the occurrence of only normal and white eyed males and females in the back-cross progeny (Figs. 1 and 5.2.) The occurrence of the males with normal eyes and scarlet eyes and the females with brown eyes and white eyes would indicate the translocation between II and Y chromosomes (Figs. 1 and 5.3.). The presence of III-Y translocation would be evidenced by the back-cross progeny consisting of normal and brown eyed males and scarlet and white eyed females (Figs. 1 and 5.4.). The induction of a Y-II-III complex translocation would give a back-cross progeny of normal eye males and white eye females only (Figs. 1 and 5.5.).

Whenever the back-cross progeny was not sufficient (at-least 20 flies) to score the translocation event, a retest was conducted by back-crossing the wild type male of that culture.

8.4. Extraction of Sex-linked Recessive Lethal Mutations (Lethals):

In the absence of loss or gain of sex chromosomes in the sperms during the spermatogenesis in the treated males, wild phenotype males and yellow body, heterobar eye females are produced (Figs. 1, 4 and 6). After brother sister mass mating, the inseminated F_1 females were placed individually in culture vials and their F_2 progenies were tested for the

Fig. 5.1. Translocation test: Chromosomal basis of the different F_2 phenotypes of a back-cross of F_1 male obtained from a cross between $\underline{y}$ $\underline{sc^{S1}}$ $\underline{In.49}$ $\underline{sc^{8}}$; $\underline{bw}$; $\underline{st}$ $\underline{p^{p}}$ female and $\underline{X^{c2}}$ $\underline{y}$ $\underline{B}$ / $\underline{y+}$ $\underline{Y}$ male, when no translocation involving II, III and/or Y chromosome was induced in the treated male parent.

Sperm Genotypes of F_1 ♂ with no trans.	Back cross Progeny Phenotypes: Eye colour	Sex
III $st^+ p^{p+}$; II bw^+; X	+	♀
III $st^+ p^{p+}$; II bw^+; Y	+	♂
III $st^+ p^{p+}$; II bw; X	bw	♀
III $st^+ p^{p+}$; II bw; Y	bw	♂
III $st\ p^p$; II bw^+; X	st	♀
III $st\ p^p$; II bw^+; Y	st	♂
III $st\ p^p$; II bw; X	w	♀
III $st\ p^p$; II bw; Y	w	♂

Fig. 5.2. Translocation test: Chromosomal basis of the different F_2 phenotypes of a back-cross of F_1 male obtained in the cross between $\underline{y}$ $\underline{sc^{S1}}$ $\underline{In.49}$ $\underline{sc^{8}}$; $\underline{bw}$; $\underline{st}$ $\underline{p^{p}}$ female and $\underline{X^{c2}}$ $\underline{y}$ $\underline{B}$ / $\underline{y+}$ $\underline{Y}$ male, when translocation between II and III chromosomes was induced in the treated male parent.

Sperm Genotypes of F_1 ♂ with II–III trans.	Back cross Progeny Phenotypes: Eye colour	Sex
III $st^+ \; p^{p+}$ / II bw^+ / X	+	♀
III $st^+ \; p^{p+}$ / II bw^+ / Y	+	♂
III $st \; p^p$ / II bw^+ / X	Lethal	
III $st \; p^p$ / II bw^+ / Y	Lethal	
III $st^+ \; p^{p+}$ / II bw / X	Lethal	
III $st^+ \; p^{p+}$ / II bw / Y	Lethal	
III $st \; p^p$ / II bw / X	w	♀
III $st \; p^p$ / II bw / Y	w	♂

Fig. 5.3. Translocation test: Chromosomal basis of the different F_2 phenotypes of a back-cross of F_1 male obtained in the cross between $\underline{y}\ \underline{sc}^{S1}\ \underline{In.49}\ \underline{sc}^{8}$; $\underline{bw}$; $\underline{st}\ \underline{p}^{p}$ female and $\underline{X}^{c2}\ \underline{y}\ \underline{B}$ / $\underline{y+}\ \underline{Y}$ male, when translocation between Y and II chromosomes was induced in the treated male parent.

Sperm Genotypes of F_1 ♂ with Y–II trans.	Back cross Progeny Phenotypes Eye colour	Sex
III st pp II X	Lethal	
III st pp II Y bw+	st	♂
III st pp II bw X	w	♀
III st pp II bw Y bw+	Lethal	
III st+ pp+ II X	Lethal	
III st+ pp+ II Y bw+	+	♂
III st+ pp+ II bw X	bw	♀
III st+ pp+ II bw Y bw+	Lethal	

Fig. 5.4. Translocation test: Chromosomal basis of the different F_2 phenotypes of a back-cross of F_1 male obtained in the cross between $\underline{y}$ $\underline{sc^{S1}}$ $\underline{In.49}$ $\underline{sc^{8}}$; $\underline{bw}$; $\underline{st}$ $\underline{p^{p}}$ females and $\underline{X^{c2}}$ $\underline{y}$ $\underline{B}$ / $\underline{y+}$ $\underline{Y}$ male, when translocation between Y and III chromosomes was induced in the treated male parent.

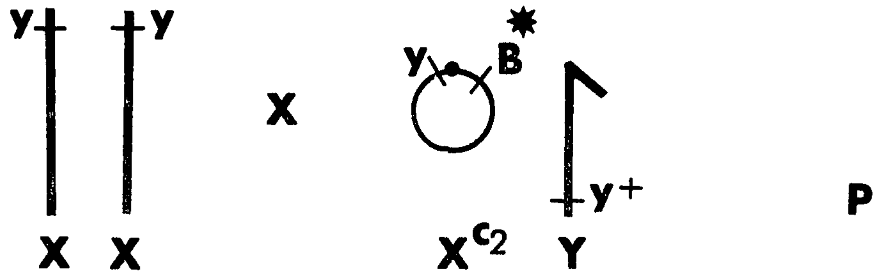
Sperm Genotypes of F_1 ♂ with Y–III trans.	Back cross Progeny Phenotypes: Eye colour	Sex
II bw; III; X	Lethal	
II bw; III; Y st+ pp+	bw	♂
II bw; III st pp; X	w	♀
II bw; III st pp; Y st+ pp+	Lethal	
II bw+; III; X	Lethal	
II bw+; III; Y st+ pp+	+	♂
II bw+; III st pp; X	st	♀
II bw+; III st pp; Y st+ pp+	Lethal	

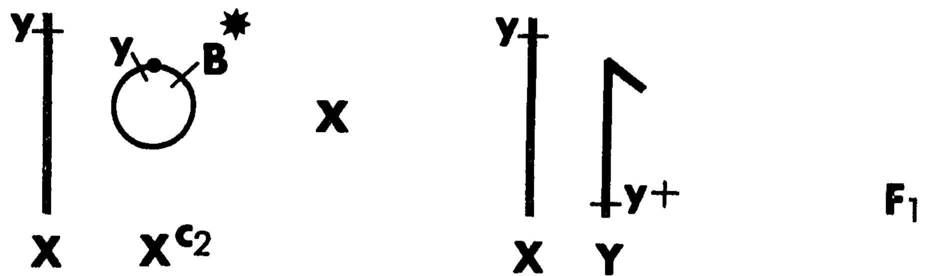
Fig. 5.5. Translocation test: Chromosomal basis of the different F_2 phenotypes of a back-cross of F_1 male obtained in the cross between $\underline{y}\ \underline{sc}^{S1}\ \underline{In.49}\ \underline{sc}^{8}$; $\underline{bw}$; $\underline{st}\ \underline{p}^{p}$ female and $\underline{X}^{c2}\ \underline{y}\ \underline{B}\ /\ \underline{y+}\ \underline{Y}$ male, when a complex translocation between Y, II and III chromosomes was induced in the treated male parent.

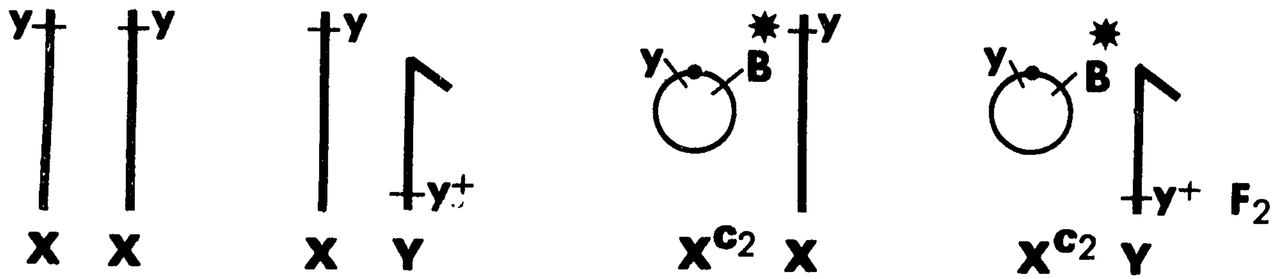
Sperm Genotypes of F_1 ♂ with Y–II–III trans.			Back cross Progeny Phenotypes	
III	II	Sex chromosome	Eye colour	Sex
st pP	bw+	Y	Lethal	
st pP	bw+	X	Lethal	
st pP	bw	Y	Lethal	
st pP	bw	X	w	♀
st+ pP+	bw+	Y	+	♂
st+ pP+	bw+	X	Lethal	
st+ pP+	bw	Y	Lethal	
st+ pP+	bw	X	Lethal	

Fig. 6. Lethal test: Chromosomal basis of extracting sex-linked recessive lethal mutations induced in the treated $\underline{X^{c2}}$ $\underline{y}$ $\underline{B}$ / $\underline{y+}$ $\underline{Y}$ males when crossed with $\underline{y}$ $\underline{sc^{S1}}$ $\underline{In.49}$ $\underline{sc^{8}}$ females.

✱ Treated $\underline{X^{c2}}$ chromosome.







Absent in lethal bearing culture

presence or absence of bar eye males. The absence of bar eye males in the F_2 progeny of a single F_1 female indicates that this F_1 female received from her father an $\underline{X^{c2}}$ $\underline{y}$ $\underline{B}$ chromosome with an induced lethal mutation (Figs. 1 and 6). When there was insufficient number of males (less than 10) in the F_2 culture, a retest for lethality was conducted using the heterobar females of the F_2 progeny.

RESULTS

1. 1-Day Gamma A series
1.1. Toxicity Assays
1.2. Dominant Lethals
1.3. Non-disjunction and Chromosome Loss
1.4. Translocations
1.5. Lethals
2. 1-Day Gamma E series
2.1. Translocations
2.2. Lethals
3. 7-Day Gamma A series
3.1. Lethals

1. 1-Day Gamma A series:

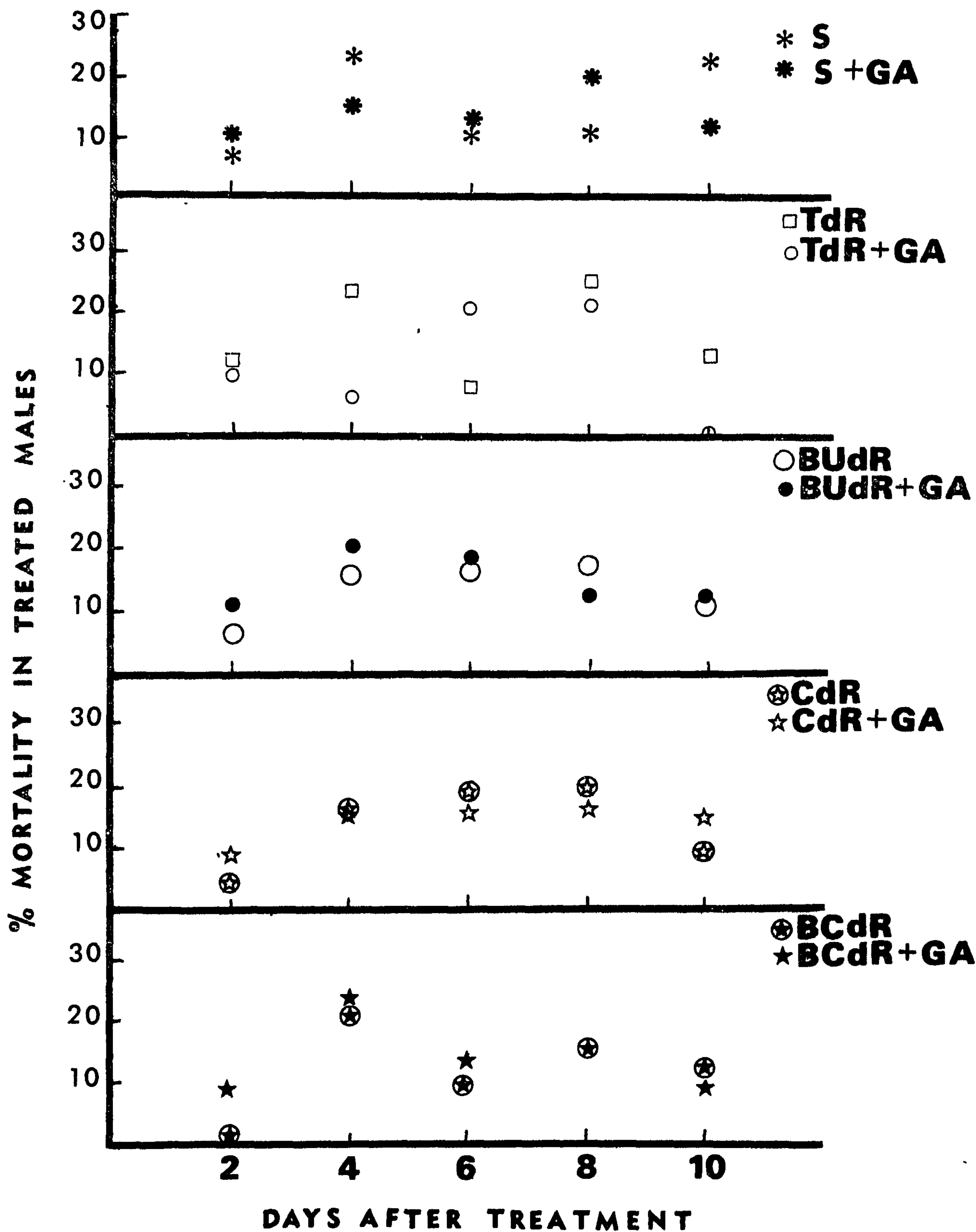
In this series of experiments, the males were injected with different nucleosides or analogues when they were 12 $\pm$ 0.5 hrs old and were irradiated with 1.2 kr gamma radiation when 24 $\pm$ 0.5 hrs old, and were allowed to mate with the females, along with an unirradiated control batch of flies (vide methods and materials above). Toxicity as well as mutation assays were carried out for most of the treatments.

1.1 Toxicity Assays:

The data on the toxicity assays of the different treatment conditions are shown in Tables 1, 2 and 3 (all tables are included in the appendix) and graphically represented in Figs. 7, 8 and 9.

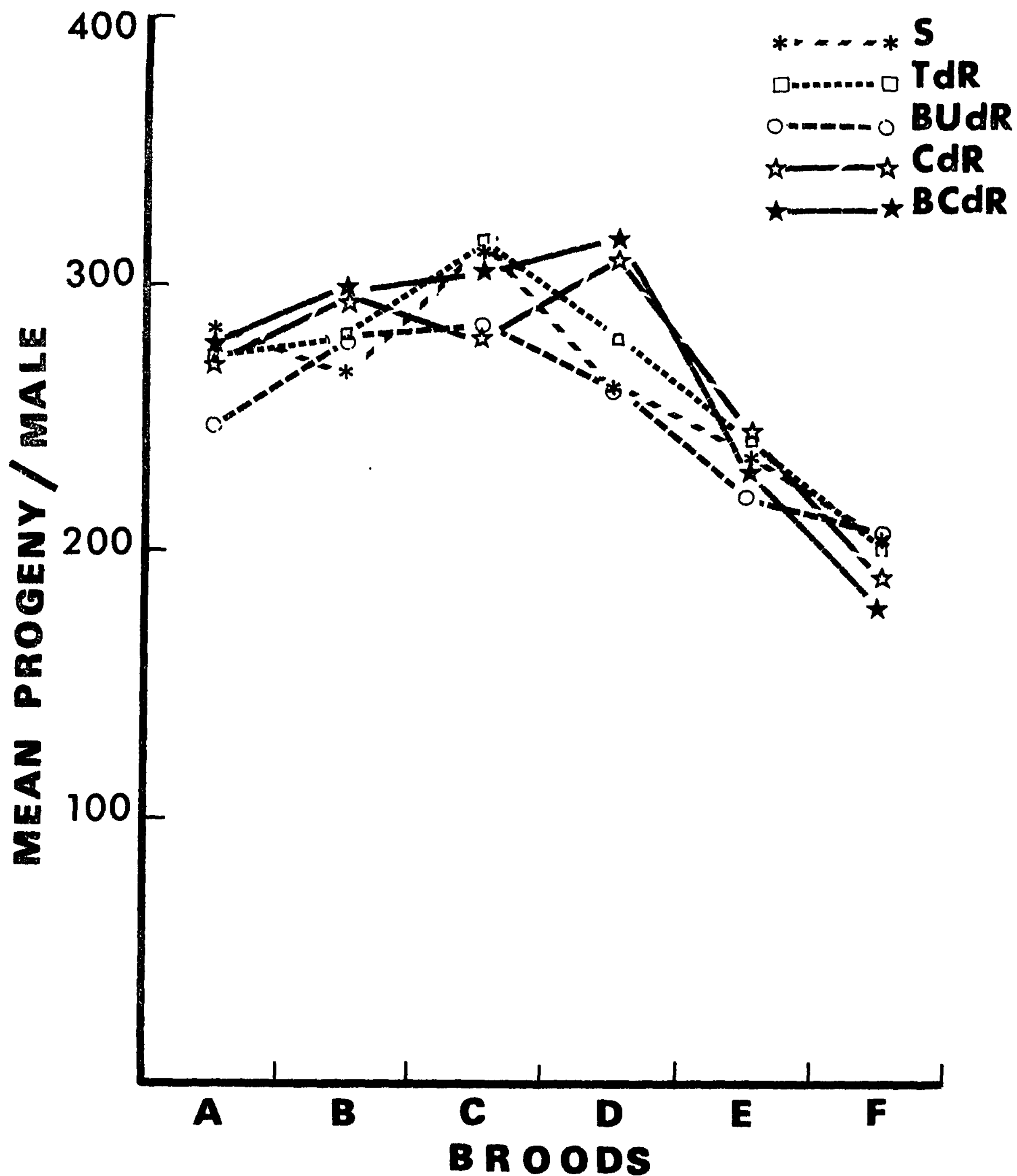
The rate of mortality among the treated males was random (Table 1; Fig. 1) and did not show any difference due to

Fig. 7. Rate of mortality among the treated males through the successive broods in 1-day gamma A series.



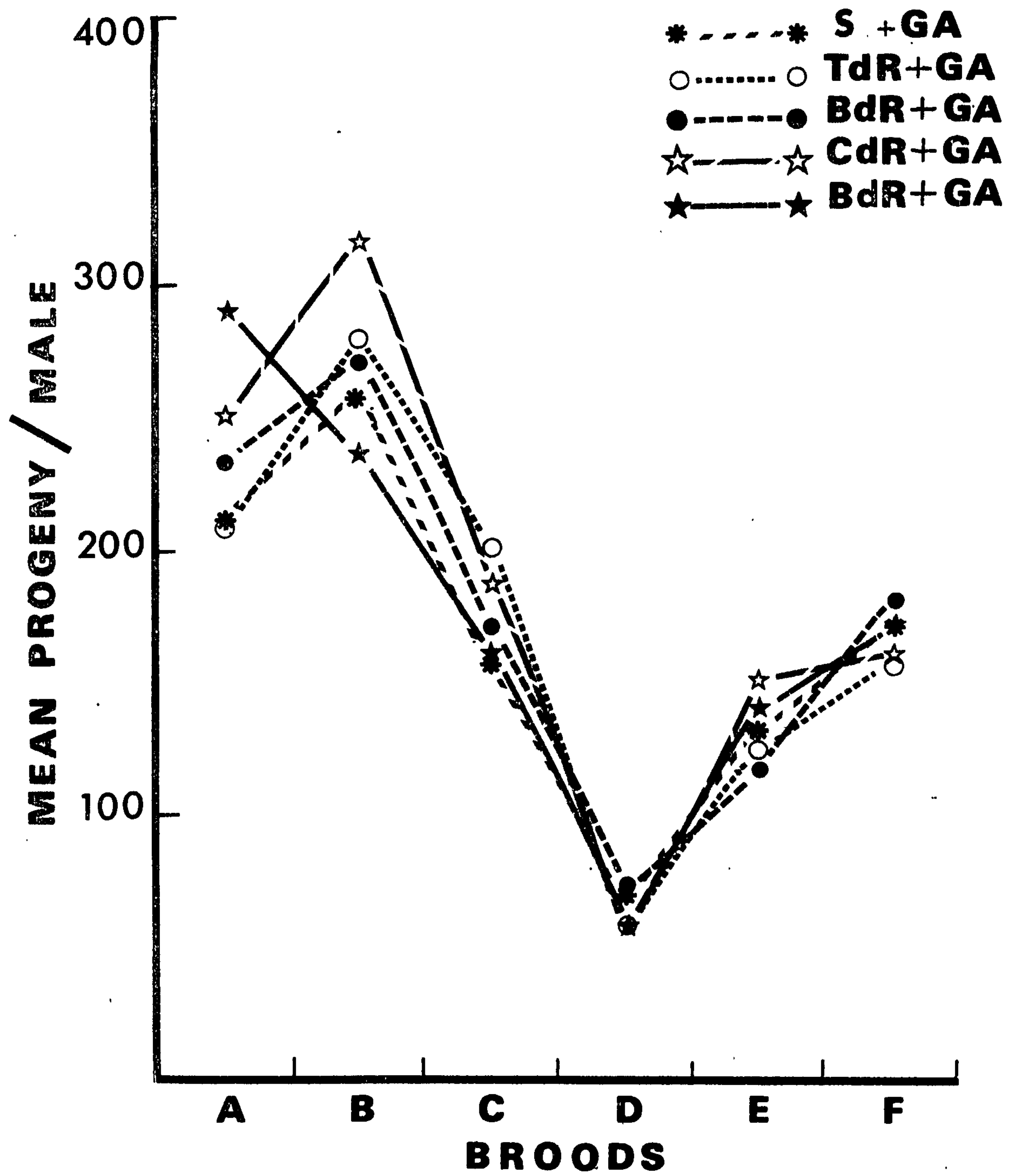
% MORTALITY IN TREATED MALES
DAYS AFTER TREATMENT
S
S +GA
TdR
TdR+GA
BUdR
BUdR+GA
CdR
CdR+GA
BCdR
BCdR+GA
30
20
10
2
4
6
8
10

Fig. 8. Mean F_1 progeny per male in the nucleoside or analogue treated unirradiated control experiments during the course of 12 days sampled through six 2-day broods A through F.



400
300
200
100
MEAN PROGENY / MALE
S
TdR
BUdR
CdR
BCdR
A
B
C
D
E
F
BROODS

Fig. 9. Mean F_1 progeny per male in the nucleoside or analogue treated and irradiated (1.2 kr) experiments during the course of 12 days sampled through six 2-day broods A through F.


S +GA
TdR+GA
BdR+GA
CdR+GA
BdR+GA
400
300
200
100
MEAN PROGENY / MALE
A
B
C
D
E
F
BROODS

either the chemicals or irradiation. The death of the treated males through different broods could have been caused by the probable strain due to the injection or due to repeated etherization during the brood changes.

The fertility (F_1 progeny) of the treated males in the different broods (A through F) are shown in Table 2 and Fig. 8 for the unirradiated control series (S, TdR, BUdR, CdR or BCdR) and for the irradiated series (S, TdR, BUdR, CdR or BCdR + 1.2 kr gamma radiation) in Table 3 and Fig. 9. The mean number of offspring per treated male did not differ within the control series or within the irradiated series in all the six broods. In the control series, the mean number of F_1 flies per male per brood was more or less equal in the post-meiotic and meiotic cells (brs A through D) but in the spermatocytes and the late gonial cells (brs E and F) there was a decrease in the F_1 progeny consistently in all the treatments. In the irradiated series, there was a decrease in the mean progeny per male in the spermatids (br C), the meiotic cells (br D) and the spermatocytes (br E), when compared with the unirradiated control series. Sterility was highest in the meiotic cells (br D) in all the treatments with irradiation irrespective of the nucleoside or analogue pre-treatment.

The studies on the rate of mortality and fertility of the treated males have shown that the concentration of the nucleosides and analogues used was non-toxic to the adult

males and the germ cells.

1.2. Dominant Lethals:

The results on the studies of dominant lethals (% unhatchability) are summarised in Table 4. The Relative Dominant Lethal Frequency (RDL) was calculated from the following equation:

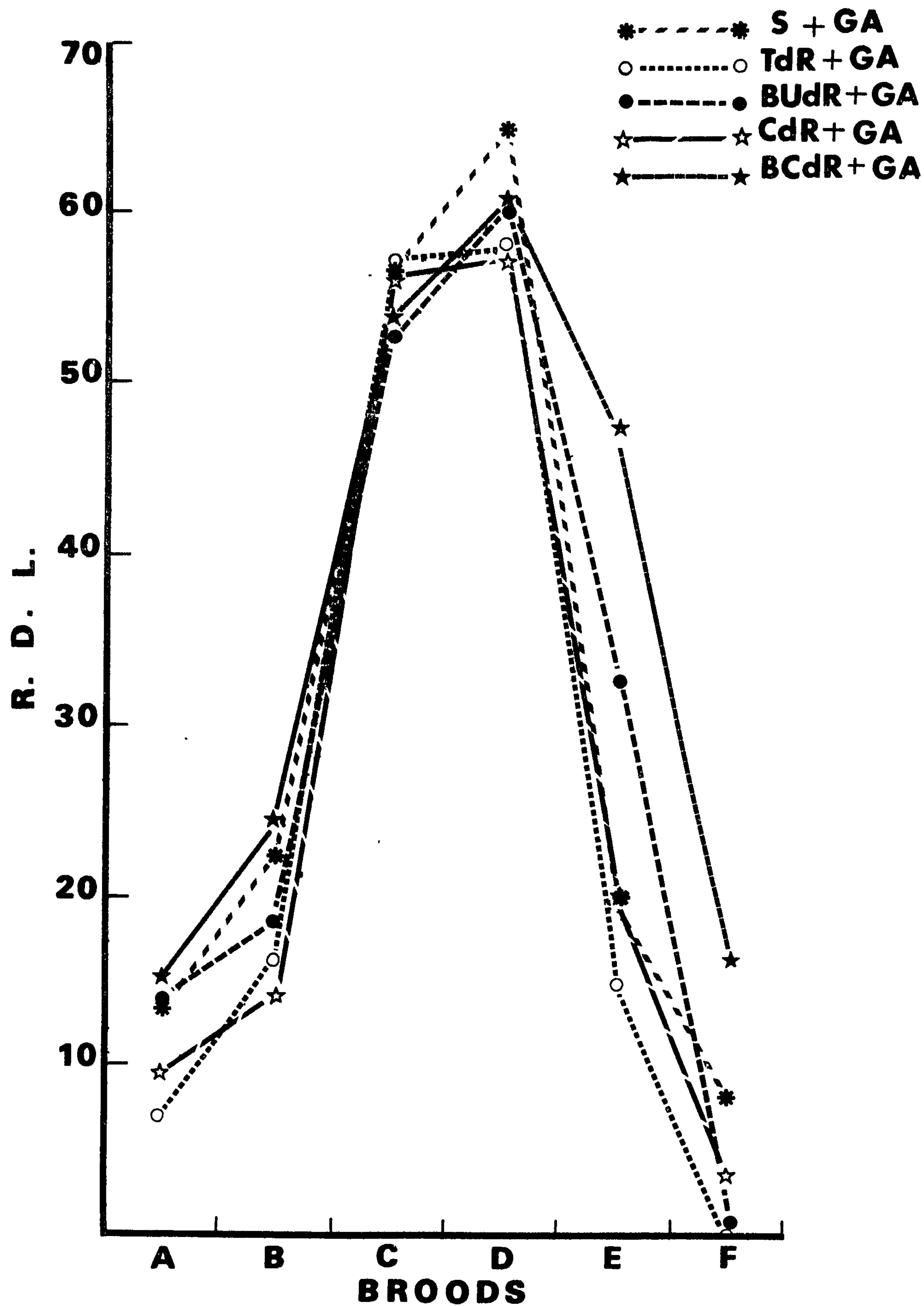
$$RDL = 100-(ht \times 100/hc)$$

where ht = % hatchability for the treated and

h_c = % hatchability for the respective control series (Ratnayake, 1968).

The significance of difference in RDL between any two pairs was estimated by converting the RDL frequency to absolute numbers and using X^2 test with 2 x 2 contingency tables. The graphic presentation of the RDL frequency for the different treatment pairs is shown in Fig. 10.

There was a significant reduction in RDL in TdR or CdR + 1.2 kr GA in the mature sperms (br A) and the immature sperms (br B) when compared with S + 1.2 kr GA. With the halogenated nucleoside analogues BUdR or BCdR + 1.2 kr GA, there was no such reduction in RDL; on the other hand, BUdR or BCdR + 1.2 kr gamma radiation showed a significantly higher RDL in the mature sperms (br A) and partly in the immature sperms (br B) than the TdR or CdR + 1.2 kr gamma radiation treatments. There was no significant difference in RDL in


S + GA
TdR + GA
BUdR + GA
CdR + GA
BCdR + GA
70
60
50
40
30
20
10
R. D. L.
A
B
C
D
E
F
BROODS

in the spermatids (br C) and the meiotic cells (br D) between any of the treatments, but the RDL frequencies were high for all the treatments since the spermatids and the meiotic cells are very sensitive to radiation damage (Sobels, 1966). In the spermatocytes (br E: post-injection DNA synthesis cells), BUdR or BCdR + 1.2 kr gamma radiation treatments resulted in a highly significant increase in RDL, when compared with the rest of the treatments. In the late gonial cells (br F), the RDL frequency tailed out in all treatments except in BCdR pre-treatment, where the RDL frequency was 18.2%, significantly higher than in all other treatment conditions (Table 4; Fig.10).

In general, TdR or CdR pre-treatment reduced the radiation induced RDL frequency in the spermatozoa (brs A and B), while BUdR and BCdR pre-treatments sensitized the post-injection DNA synthesis cells (spermatocytes) with a significant increase in the radiation-induced RDL frequency; the spermatids and the meiotic cells (brs C and D) were highly sensitive to radiation and did not show any appreciable difference in the RDL frequency.

1.3. Non-disjunction and Chromosome loss:

The F_1 progeny counts (fertility) for the different treatment conditions (Tables 2 and 3) also yielded data on the frequencies of non-disjunction ($XX^{c2}Y$) females and chromosome loss (XO males) (Fig. 4), computed on the basis of

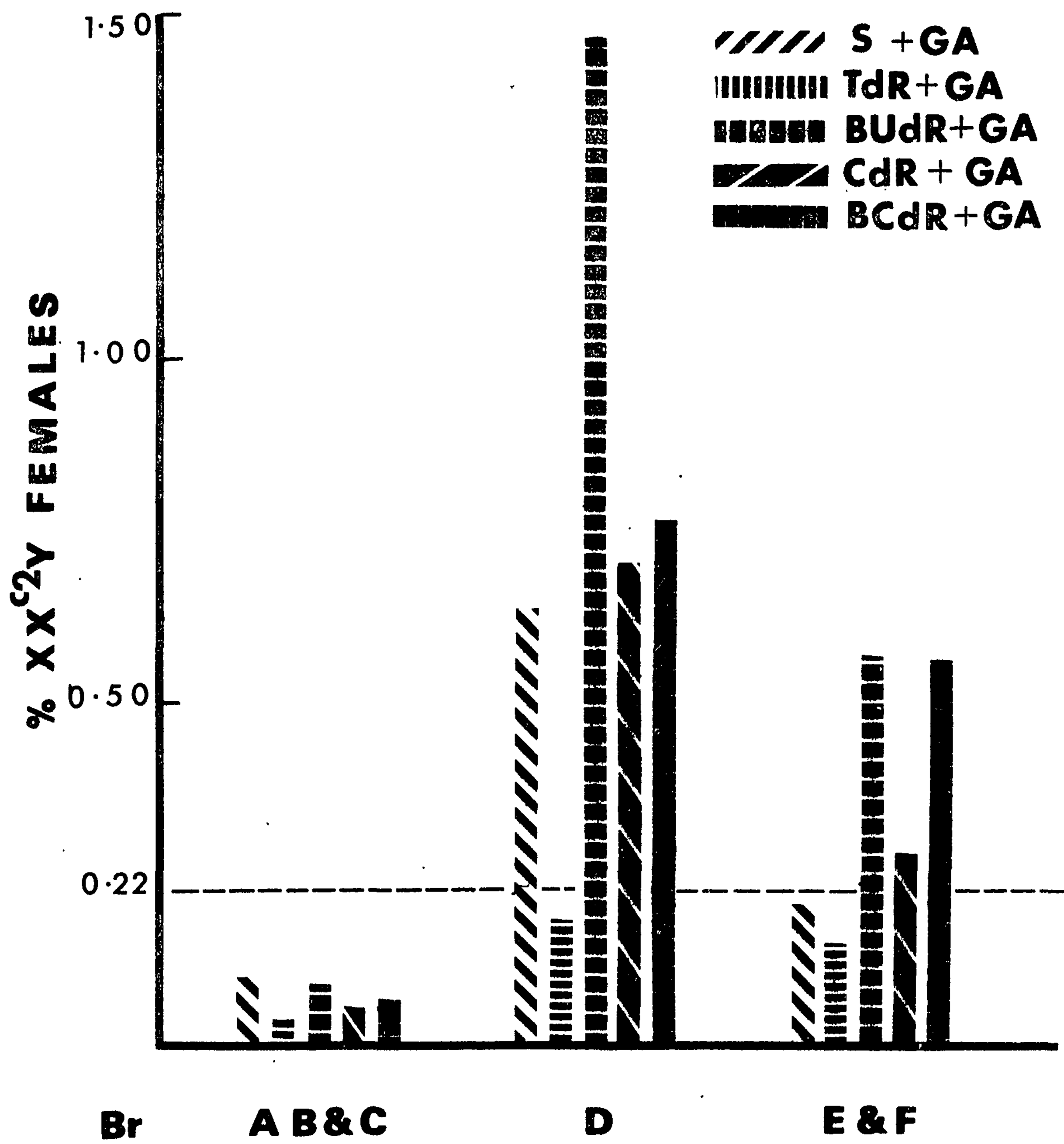
the total number of regular XX^{c2} females and XY males respectively.

In all the unirradiated control treatments (S, TdR, BUdR, CdR or BCdR), the frequencies of non-disjunction and chromosome loss in the different broods were within the range of the spontaneous frequencies for the ring X^{c2} stocks reported in the literature (0.28% $XX^{c2}Y$ females and 0.38% XO males--Clark and Clark, 1968; 0.14% $XX^{c2}Y$ females and 0.47% XO males--Mittler _et al._, 1967). Since the pyrimidine nucleosides TdR and CdR or their brominated analogues BUdR and BCdR, by themselves, did not increase the frequencies of non-disjunction or chromosome loss (Table 2), the mean frequencies of non-disjunction (0.22%) and chromosome loss (0.33%) in the unirradiated control series are shown as dotted base lines in Figs. 11 and 12 respectively.

The data on the frequencies of $XX^{c2}Y$ females and XO males (Table 3) in the irradiated series (S, TdR, BUdR, CdR or BCdR + 1.2 kr gamma radiation) in the different broods (brs A to F) were pooled into three groups on the basis of post-meiotic, meiotic or pre-meiotic stages of the male germ cells at the time of irradiation as shown in Tables 5 and 6 and in Figs. 11 and 12.

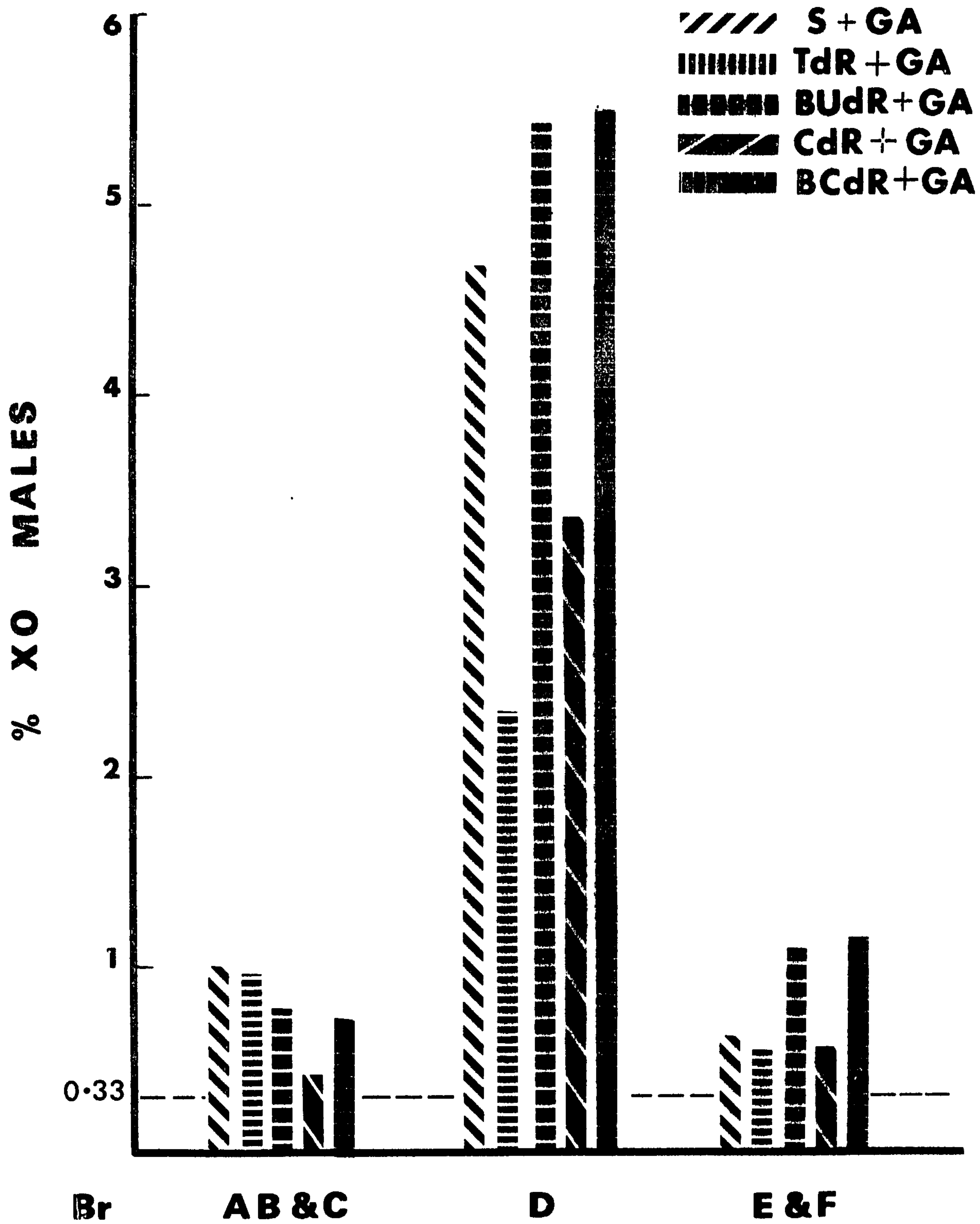
In the post-meiotic germ cells (brs A, B and C), the radiation-induced frequency of non-disjunction was not influenced by the pre-treatment with TdR and CdR or their analogues

Fig. 11. The effect of pre-treatment with pyrimidine nucleosides TdR or CdR and their brominated analogues BUdR or BCdR on the radiation-induced (1.2 kr gamma radiation) frequency of non-disjunction ($XX^{c}2Y$ females) in the post-meiotic (brs A, B and C), meiotic (br D) and pre-meiotic (br E and F) stages of male germ cells in *D. melanogaster*. The dotted base line shows the frequency of non-disjunction females in the unirradiated control series.



S +GA
TdR+GA
BUdR+GA
CdR + GA
BCdR+GA
1·50
1·00
0·50
0·22
% XX^{c2}Y FEMALES
Br
A B&C
D
E & F

Fig. 12. The effect of pre-treatment with pyrimidine nucleosides TdR or CdR and their brominated analogues BUdR or BCdR on the radiation-induced (1.2 kr gamma radiation) frequency of chromosome loss (XO males) in the post-meiotic (brs A, B and C), meiotic (br D) and pre-meiotic (brs E and F) stages of male germ cells in *D. melanogaster*. The dotted base line shows the frequency of XO males in the unirradiated control series.



S + GA
TdR + GA
BUdR + GA
CdR + GA
BCdR + GA
% XO MALES
6
5
4
3
2
1
0·33
Br
AB &C
D
E &F

BUdR or BCdR and was within the spontaneous range. There was an increase in the frequency of non-disjunction in the meiotic cells (br D) in all the irradiated treatments (S, BUdR, CdR or BCdR + 1.2 kr gamma radiation) except in TdR + 1.2 kr gamma radiation treatment, which did not show any increase beyond the spontaneous level in all the broods. With the exception of TdR and BCdR pre-treatments, the peak incidence of non-disjunction females occurred in the meiotic cells (br D). The frequencies of non-disjunction in the meiotic cells did not differ significantly between treatments except in one case (TdR + 1.2 kr gamma radiation vs BUdR + 1.2 kr gamma radiation). In the pre-meiotic cells (brs E and F) (post-injection DNA synthesis cells), BUdR and BCdR pre-treatments showed significant increase in the frequency of non-disjunction, except in the case of CdR vs BCdR pre-treatments (Table 5; Fig. 11).

The post-meiotic cells were not affected by the nucleoside or analogue pre-treatment in terms of non-disjunction, while the meiotic cells were maximally affected, in general, without significant difference between the various treatments. The radiation-induced non-disjunction frequency in the pre-meiotic cells was preferentially sensitized by BUdR and BCdR pre-treatments.

The frequencies of XO males (chromosome loss) in the post-meiotic cells (brs A, B and C) of the irradiated series

were higher than the spontaneous frequency unlike in the case of non-disjunction. TdR, CdR, BUdR or BCdR pre-treatments followed by 1.2 kr gamma radiation showed a lower frequency of chromosome loss in the post-meiotic cells, when compared with S + 1.2 kr gamma radiation, the difference between S + 1.2 kr and CdR + 1.2 kr treatments being significant (1.00% and 0.42% respectively with $X^2_{1\ DF} = 19.28$ and P = .001) (Table 6; Fig. 12). In the meiotic brood D, the frequency of XO males was high in BUdR (5.42%) and BCdR (5.48 %) treated cells, low in TdR (2.33%) and CdR (3.36%) treated cells and intermediate in S treated cells (4.68%). The response of the pre-meiotic cells (brs E and F) in terms of the frequency of chromosome loss was similar to that of the meiotic cells (br D), with BUdR and BCdR pre-treatments inducing high frequency of chromosome loss (1.09% and 1.15% respectively), the latter being significantly high (Table 6 and Fig. 12).

The radiation-induced frequency of chromosome loss in the post-meiotic germ cells (brs A, B and C) was higher in the S pre-treatment than in the nucleoside or analogue treated cells; but in the meiotic (br D) and pre-meiotic (brs E and F) germ cells, BUdR and BCdR pre-treatments gave higher frequencies, while the pre-treatments with TdR and CdR gave lower frequencies of chromosome loss, as compared with the S pre-treatment.

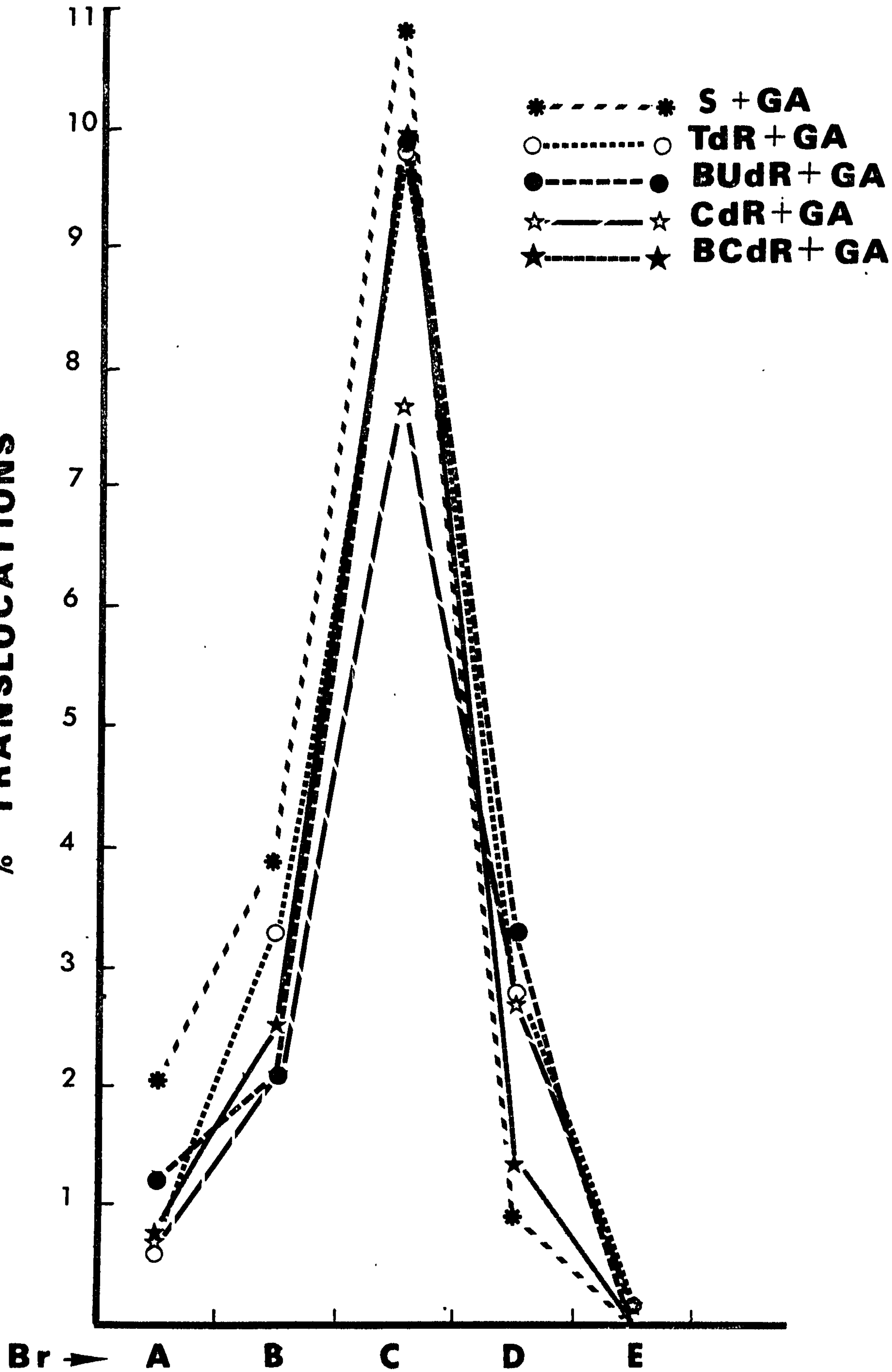
1.4. Translocations:

Translocations involving II, III and/or Y chromosomes were conspicuous by their absence in all the unirradiated control treatments (S, TdR, BUdR, CdR or BCdR) (Table 7).

Different types of translocation events involving II, III and/or Y chromosomes (Fig. 5.1.-5.5.) were recovered in the irradiated series of experiments (S, TdR, BUdR, CdR or BCdR + 1.2 kr gamma radiation) in the post-meiotic and meiotic stages (brs A, B, C and D) of the spermatogenous cells but not in the spermatocytes (br E), where the translocation events were virtually absent. (Late gonial cells (br F) were not screened for translocation.) The different types of translocations (Fig. 5.1.-5.5.) were summed up to compute the translocation frequency in each brood. Translocation studies were repeated twice or thrice for each treatment in order to screen a reasonable number of gametes (atleast 400 gametes per brood per treatment). After homogeneity test using X^2 analysis, the results were pooled as shown in Table 8. The significance of differences between the different treatments was analyzed by 2 x 2 contingency tables (with Yate's correction) using the pooled data; the $X^2_{1\ DF}$ and P values for each brood are also given in Table 8. Percent translocation events in the different treatments through br E are graphically shown in Fig. 13.

The pyrimidine nucleoside or analogue pre-treatments

Fig. 13. Effect of pre-treatment with pyrimidine nucleosides and their brominated analogues (TdR, BUdR, CdR or BCdR) on the radiation-induced (1.2 kr gamma radiation) translocation frequencies (II, III and/or Y chromosomes) in the different stages of male germ cells in _D. melanogaster_.



% TRANSLOCATIONS
11
10
9
8
7
6
5
4
3
2
1
Br
A
B
C
D
E
S + GA
TdR + GA
BUdR + GA
CdR + GA
BCdR + GA

(TdR, BUdR, CdR or BCdR + 1.2 kr gamma radiation) induced lower translocation frequencies in the post-meiotic cells (brs A, B and C) than in the S + 1.2 kr gamma radiation treatment, with the peak frequency in the spermatids (br C) for all the treatments. However, the differences were not statistically significant. In the meiotic cells (br D) the reverse was true, with TdR and BUdR pre-treatments showing significant increase in the translocation frequency (2.8% and 3.3% respectively) than S pre-treatment (0.9%). Virtually no translocations were recovered in the spermatocytes (br E) (pre-meiotic) as would be expected since the translocation bearing cells are usually eliminated during meiosis. Only two translocations (one in TdR pre-treatment and one in CdR pre-treatment) were recovered in a total of 2,841 gametes tested in all the treatments in the spermatocytes (br E) (Table 8; Fig. 13).

1.5. Lethals:

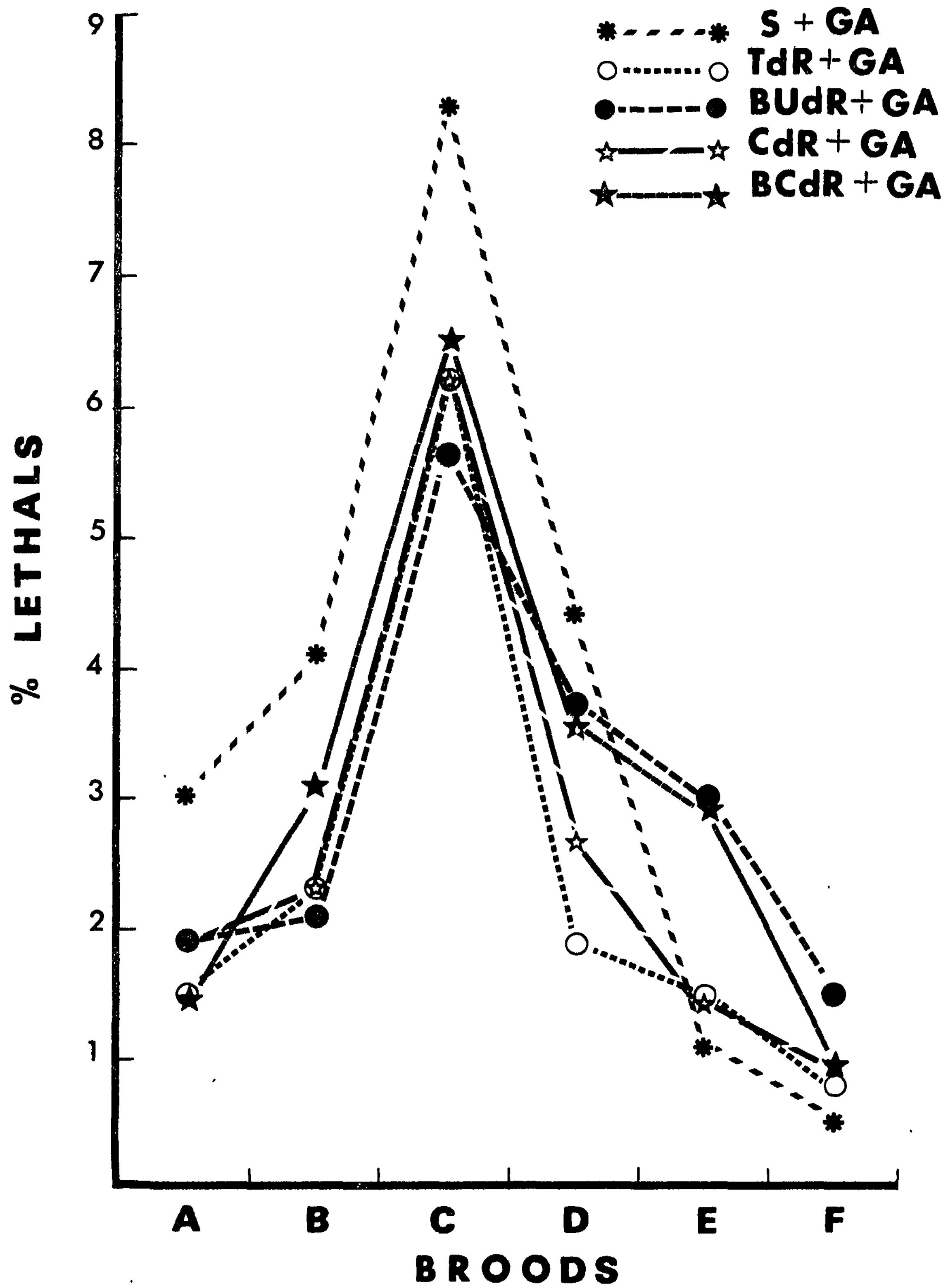
In the unirradiated control experiments (S, TdR, BUdR, CdR or BCdR) the lethal mutation frequencies were not above the spontaneous level (0.2-0.3%--Oftedal, 1964). The results of the control treatments are shown in Table 7, where the last column represents the total number of chromosomes tested in all the broods in each treatment. The analogues BUdR and BCdR, by themselves, did not induce lethal mutations.

In the irradiated treatments (S, TdR, BUdR, CdR or BCdR

+ 1.2 kr gamma radiation), lethals were recovered in all the broods. Studies on the lethal mutation frequency were repeated in order to screen at least 500 chromosomes per brood per treatment. After homogeneity test using X^2 analysis, the results of the repeat experiments were pooled (Table 9); and the temporal distribution of the frequencies of lethal mutations in the different treatments are plotted in Fig. 14. The significance of differences in the lethal frequencies between the treatments was analysed by the 2 x 2 contingency tables (with yate's correction) using the pooled data, the $X^2_{1\ DF}$ and the P values also being shown in Table 9.

There was a reduction in the lethal frequencies in TdR, BUdR, CdR or BCdR pre-treated experiments in all the broods that sampled the germ cells with pre-injection DNA synthesis (brs A, B, C and D), when compared with S pre-treatment. The reduction was significant with TdR and BCdR pre-treatments in the mature sperms (br A) (1.5% and 1.4%), with BUdR pre-treatment in the immature sperms (br B) (2.1%) and in TdR pre-treatment in the meiotic cells (br D) (1.9%) in relation to S pre-treatment in the mature sperms, immature sperms and meiotic cells (brs A, B and D) (3.0%, 4.1% and 4.4% respectively). Though there was a reduction in the frequency of lethals in the nucleoside and analogue pre-treated cells in the spermatids (br C), the difference, however, was not significant. As in the case of translocations, the spermatids (br C) showed maximal radiosensitivity to induction of lethal

Fig. 14. The effect of pre-treatment with pyrimidine nucleosides and their brominated analogues (TdR, BUdR, CdR or BCdR) on the radiation-induced (1.2 kr gamma radiation) lethal mutations in the different stages of male germ cells of *D. melanogaster*.

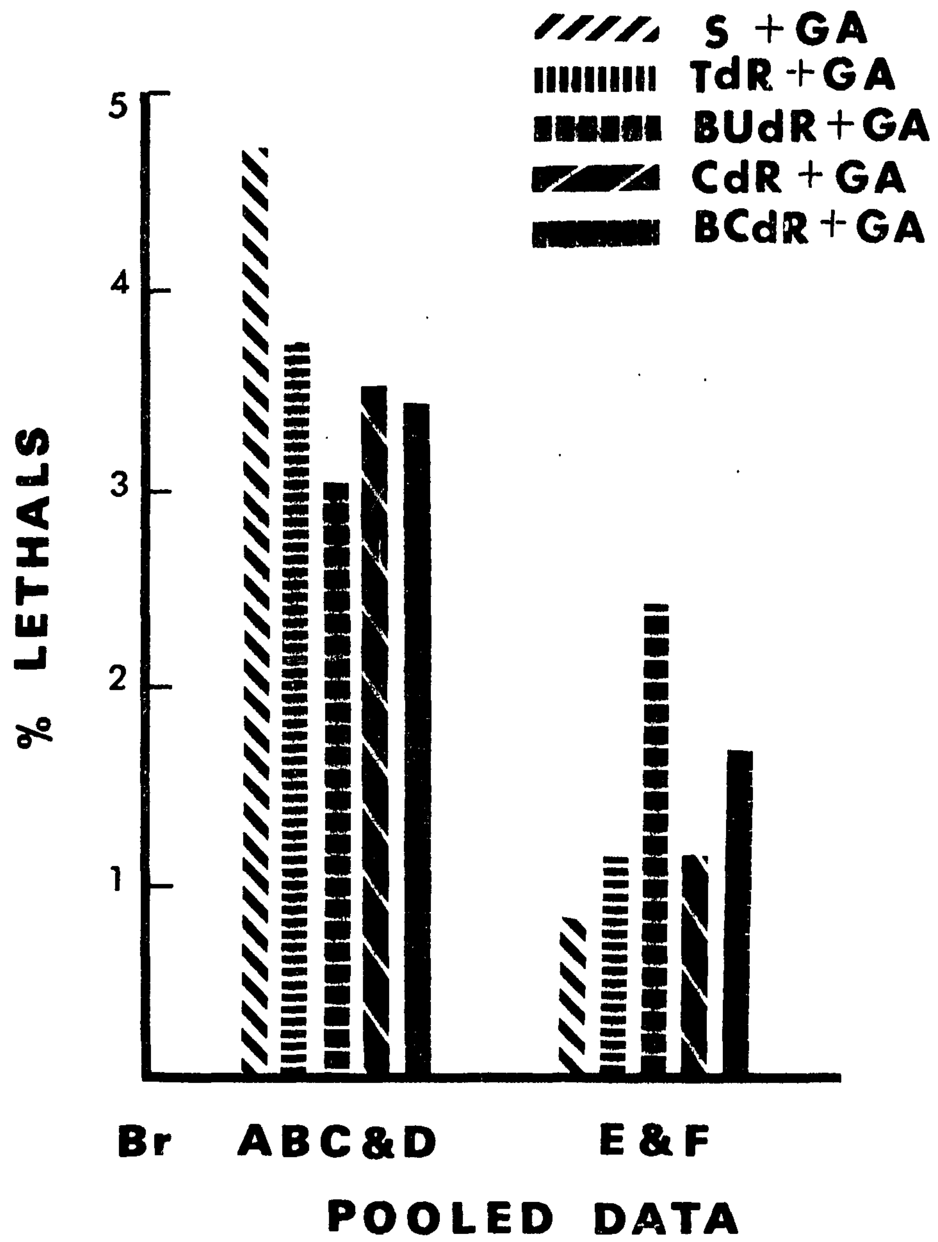


S + GA
TdR + GA
BUdR + GA
CdR + GA
BCdR + GA
9
8
7
6
5
4
3
2
1
% LETHALS
A
B
C
D
E
F
BROODS

mutations also. BUdR and BCdR pre-treatments showed a significant increase in the lethal frequency in the spermatocytes (br E: post-injection DNA synthesis cells) with 3.0% and 2.9% as opposed to 1.1% in S pre-treatment ($X^2_{1\ DF} = 8.00$, P = 0.01 and $X^2_{1\ DF} = 5.32$, P = 0.05); but this tendency tailed out in the late gonial cells (br F) with no significant difference between treatments (Table 9; Fig. 14).

Since the brood pattern was designed to separate the germ cells with pre-injection DNA synthesis (mature, immature sperms, spermatids and meiotic cells sampled in brs A, B, C and D) and the post-injection DNA synthesis (the spermatocytes and the late gonial cells sampled in brs E and F) (Fig. 2), the results on lethal tests were accordingly pooled into two groups (Table 10; Fig. 15). In the cells with pre-injection DNA synthesis, the nucleoside or analogue treated cells showed significant reduction in the lethal frequency when compared with S pre-treatment, with the modification factor (MF = % lethals in nucleoside or analogue + 1.2 kr / % lethals in S + 1.2 kr) ranging from 0.78-0.68 (Table 10). The cells with post-injection DNA synthesis had a significant increase with BUdR and BCdR pre-treatments over the S pre-treatment. The modification factor for BUdR and BCdR pre-treatments were 3.0 and 2.25 (Table 10). The normal nucleosides TdR and CdR did not show significant increase in the radiation-induced lethal frequency in the spermato-

Fig. 15. Effect of pre-treatment with pyrimidine nucleosides and their brominated analogues (TdR, BUdR, CdR or BCdR) on the radiation-induced (1.2 kr gamma radiation) lethal mutation frequency in the post- and pre-meiotic stages of male germ cells of *D. melanogaster*.


S + GA
TdR + GA
BUdR + GA
CdR + GA
BCdR + GA
% LETHALS
5
4
3
2
1
Br
ABC&D
E&F
POOLED DATA

cytes and the late gonial cells (brs E and F) individually, or in the pooled data of post-injection DNA synthesis cells (Tables 9 and 10).

2. 1-Day Gamma E series:

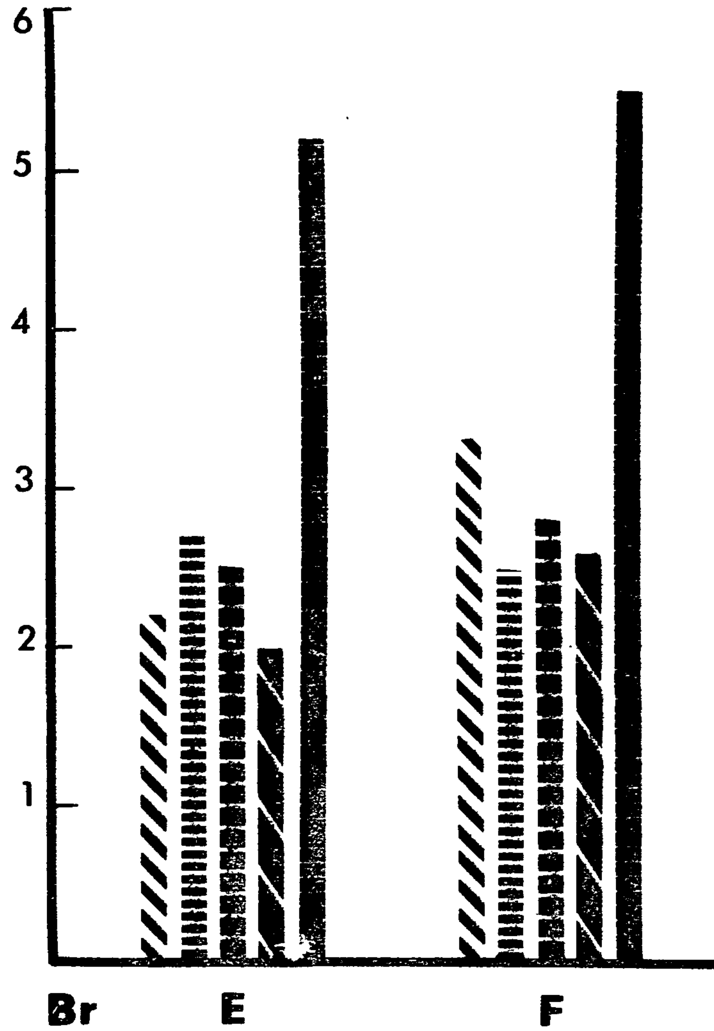
In this set of experiments, the nucleoside or analogue injected 1-day old males were allowed to mate without irradiation through brood D and were irradiated before the initiation of brood E. By doing so, it was able to allow the post-injection DNA synthesis cells (the spermatocytes and the late gonial cells sampled in brs E and F) advance to the spermatozoan stage at the time of irradiation, instead of irradiating them in the pre-meiotic stage as in the previous set of experiments (1-Day Gamma A series). It was hoped to realise higher frequencies of translocations and lethals in the BUdR and BCdR incorporated cells, which might have been otherwise eliminated during meiosis in the 1-day gamma A series.

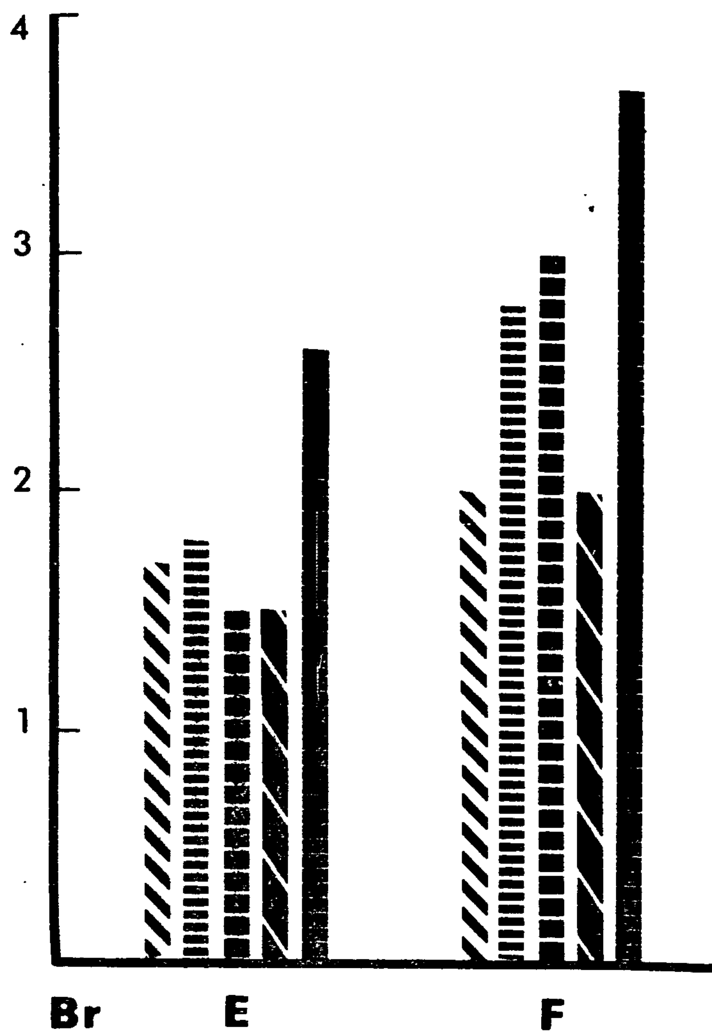
About 500 gametes in broods E and F were tested for translocations and lethals. The results of the five test conditions (S, TdR, BUdR, CdR or BCdR followed by 1.2 kr gamma radiation before brood E) and the significance of the differences are shown in Table 11 and the histogram plotted in Fig. 16.

2.1. Translocations:

There was no significant difference in the frequency of

Fig. 16. Effect of treatment with nucleosides or their brominated analogues (TdR, BUdR, CdR or BCdR) followed by 1.2 kr gamma radiation at brood E on the translocation frequency and the lethal frequency. The germ cells that synthesized DNA after the injection of the nucleosides or analogues were irradiated in their post-meiotic (spermatozoa) stage.


S +GA
TdR+GA
BUdR+GA
CdR+GA
BCdR+GA
% LETHALS
1
2
3
4
5
6
Br
E
F


% TRANSLOCATIONS
1
2
3
4
Br
E
F

translocations between the different treatments in the spermatocytes and the late gonial cells (post-injection DNA synthesis cells) when irradiated in the spermatozoan stage, (Table 11), eventhough BCdR pre-treatment + 1.2 kr GE resulted in a higher yield of translocations in the spermatocytes and the late gonial cells (2.6% and 3.7%) than in S + 1.2 kr GE (1.7% in the spermatocytes and 2.0% in the late gonial cells).

2.2. Lethals:

The yields of lethal mutations were not significantly different in the spermatocytes (br E) and in the late gonial cells (br F) between S, TdR, BUdR and CdR pre-treatments followed by 1.2 kr gamma radiation before brood E (Table 11). However, with BCdR pre-treatment, the yield of lethals was 5.2% in the spermatocytes (br E), significantly higher than 2.2% lethals in the S pre-treatment ($X^2_{1\ DF}$ = 6.26 with P = 0.02) and 2.0% lethals in CdR pre-treatment ($X^2_{1\ DF}$ = 6.96 with P = 0.01). In the brood sampling the late gonial cells (br F), BCdR + 1.2 kr GE gave 5.5% lethals, which was significantly higher than 2.1% lethals in CdR + 1.2 kr GE ($X^2_{1\ DF}$ = 9.03 with P = 0.01).

3. 7-Day Gamma A series:

In the 1-day gamma A series, the nucleoside or analogue pre-treatment significantly reduced the radiation induced lethal frequency generally in all the pre-injection DNA syn-

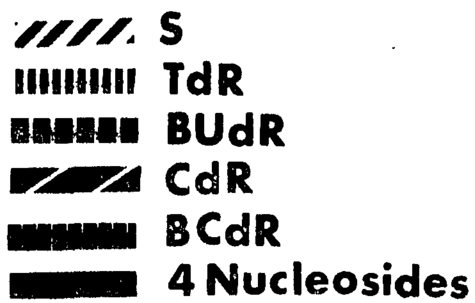
thesis broods (A through D) (Tables 9 and 10). However, this effect was conspicuous only in brood A, which sampled mainly the mature spermatozoa with a possible mixture of immature spermatozoa (first column in Tables 9 and 12). A confirmation of the 'protective' effect of the nucleosides or analogues on the radiation-induced lethal frequency in the mature sperms was considered necessary. The newly emerged males were allowed to accumulate mature sperms by keeping them without mating for 7 days, before being subjected to irradiation.

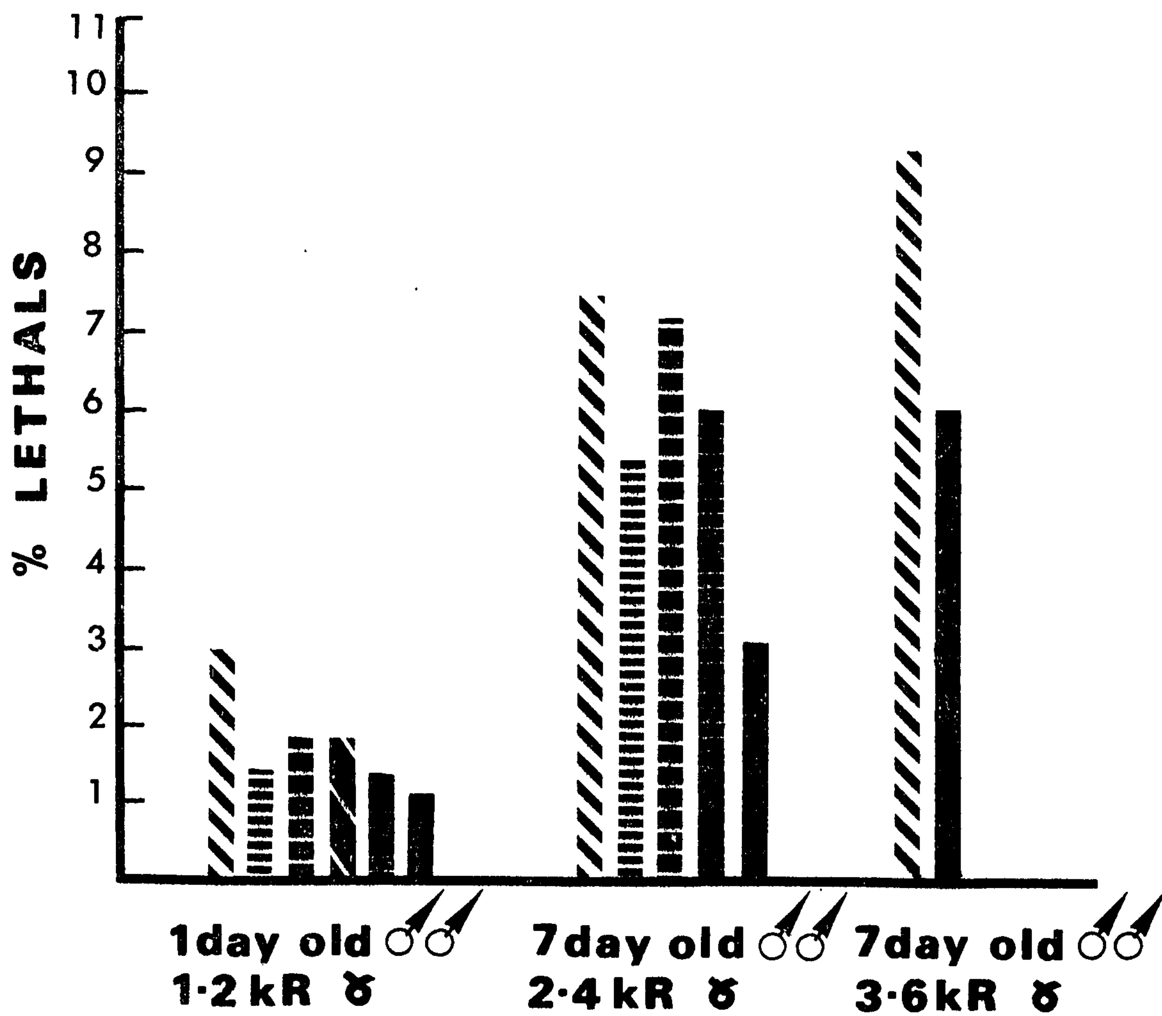
Apart from the nucleoside or analogue pre-treatments as in the previous set of experiments, an additional pre-treatment condition - injection of 0.1% equimolar solution of all the four normal nucleosides of the DNA (TdR, CdR, AdR and GdR) - was also included since such equimolar solution of the nucleosides is known to increase survival in irradiated L cells (Petrovic and Nias, 1966). Two higher doses of radiation (2.4 kr and 3.6 kr) were employed to confirm the 'protective' effect of the nucleosides with different doses of radiation.

The different test conditions using 1-day old males (as in column 1 of Table 9) and 7-day old males and the results on the modification of the lethal mutation frequencies in the mature spermatozoa are shown in Table 12 and the respective histogram plotted in Fig. 17.

The frequency of radiation-induced lethal mutations in

Fig. 17. Effect of pre-treatment with nucleosides and analogues on the radiation-induced recessive lethal frequency in the mature sperms of 1-day and 7-day old *D. melanogaster*.


S
TdR
BUdR
CdR
BCdR
4 Nucleosides


% LETHALS
11
10
9
8
7
6
5
4
3
2
1
1day old ♂♂
1·2 kR γ
7day old ♂♂
2·4 kR γ
7day old ♂♂
3·6 kR γ

mature sperms was always lower in the nucleoside or analogue pre-treatments than in the S treatment. Pre-treatment with TdR, BCdR and the equimolar solution of TdR, CdR, AdR and GdR significantly reduced the radiation-induced recessive lethals (1.2 kr) in the mature sperms (possible mixture of immature sperms) of one day old males (1.5%, 1.4% and 1.2%) when compared with the S pre-treatment (3%); the modification factor was 0.50, 0.46 and 0.40 respectively. With 7 day old males and 2.4 kr gamma radiation, pre-treatment with the equimolar solution of the four nucleosides yielded a highly significant reduction in lethal frequency (3.1%) against S pre-treatment (7.5%) ($X^2_{1\ DF}$ = 15.900; P = 0.001). Exogenous TdR, BUdR and CdR, when singly administered did not show significant reduction in the yield of lethals in the mature spermatozoa of 7-day old males exposed to 2.4 kr gamma radiation. Hence, the test with a higher dose of radiation (3.6 kr) using 7-day old males was extended only with two treatment conditions: (a) S pre-treatment and (b) pre-treatment with equimolar solution of TdR, CdR, AdR and GdR in saline. Exogenous solution of the four deoxynucleosides reduced the lethal frequency with a modification factor of 0.64 (6.0% against 9.3% in S pre-treatment); the reduction in percent lethals was highly significant with $X^2_{1\ DF}$ = 12.66; and P = 0.001.

DISCUSSION

1. Toxicity Assays
2. Mutagenicity of the Nucleoside Analogues
3. Effect of Nucleoside or Analogue Pre-treatment on the Radiation-induced Genetic Damage in the Post-injection DNA Synthesis Cells
4. Effect of Nucleoside or Analogue Pre-treatment on the Radiation-induced Genetic Damage in the Pre-injection DNA Synthesis Cells

1. Toxicity Assays:

The data on the rate of mortality among treated males (Table 1; Fig. 7) and their fertility through the different broods (Tables 2 and 3; Fig. 8 and 9) revealed that the treatment conditions were not toxic. The decrease in fertility of the irradiated males, with a maximum sterility in the meiotic cells (br D) was due to irradiation and not due to the exogenous chemicals, because this temporary loss of fertility was also found in the absence of the nucleosides or analogues (Table 3; Fig. 9), but not in the unirradiated series (Table 2; Fig. 8). Similar temporary loss of fertility in irradiated _D. melanogaster_ males with a peak sterility in the meiotic cells have been observed by all the previous workers (Bateman, 1968; Puro, 1966). Apart from being non-toxic, the present treatment conditions did not grossly alter the rate of sperm utilization or spermatogenesis. Several aspects of the results like (a) the maximal

sterility (Fig. 9), dominant lethality (Fig. 10), non-disjunction (Fig. 11) and chromosome loss (Fig. 12) occurring in the meiotic cells (br D) (Bateman, 1968; Puro, 1966; Luning, 1952 and Sävghan, 1963); (b) peak of translocation events (Fig. 13) and lethal mutations (Fig. 14) in the spermatids (br C) (Chandley, 1962 and Sobels, 1966); (c) absence of translocation events in the pre-meiotic cells (br E) (Fig. 13) (Puro, 1966) being common for all the irradiated treatments (with or without nucleosides or analogues) effectively rule out any serious delay in the process of spermatogenesis in the males due to different treatments. Thus the genetic data obtained for the various treatment conditions are highly comparable on the basis of the different stages of male germ cells sampled by the brood technique.

2. Mutagenicity of the Nucleoside Analogues:

The results presented above indicate that the halogenated pyrimidine analogues BUdR and BCdR were not mutagenic in *D. melanogaster* male germ cells in that they did not induce dominant lethals (Table 4), non-disjunction and chromosome loss (Table 2), translocation events and lethal mutations (Table 7). BUdR, BCdR or IUdR, when administered through injection or larval or adult feeding have been found inactive as regards point mutations (sex-linked recessive lethals as well as visibles) by different workers (Sobels, 1965; Fahmy *et al.*, 1966; Khan and Anderson, 1968 and Sharma,

1969). By feeding BUdR through defined culture medium, Herskowitz and Bakula, (1965) tentatively claimed that BUdR induced lethal mutations in D. melanogaster male germ cells; but this observation was not confirmed. Kaufman et al., (1961) and Kaufman and Gay (1970) observed induction of lethal mutations in the spermatozoa and the spermatids (pre-injection DNA synthesis cells) by injection of BUdR into the adult males, indicating an indirect effect. Such events were not observed in this study. The absence of translocations in the BUdR and BCdR treated unirradiated series indicates that these analogues probably do not induce gross chromosomal aberrations in D. melanogaster male germ cells (Table 7). This observation is in agreement with the results of Kaufman et al., (1961) in D. melanogaster and of Kihlman (1963) in Vicia faba root meristem cells; however, BUdR is known to induce chromosomal aberrations in some mammalian cell lines (Hsu and Somers, 1961). Fahmy et al.(1966) observed that BUdR, IUdR as well as FUdR induced small chromosomal deletions (in the IV thr chromosome) resulting in Minute phenotype often preferentially in the spermatocytes and the gonial cells of D. melanogaster. The results on dominant lethals and translocations (Tables 4 and 7) obtained in this study indicate that BUdR and BCdR are not capable of inducing gross chromosome breaks in D. melanogaster male germ cells or if broken, the chromosomes were not able to form viable rearrangements (Fahmy et al., 1966).

3. Effect of Nucleosides or Analogues on the Radiation-induced Genetic Damage in the Post-injection DNA Synthesis Cells:

The male germ cells that synthesized DNA after the injection of the nucleosides or their analogues (the spermatocytes and the late gonial cells sampled in brs E and F: 1-Day Gamma A series) were radiosensitized with BUdR or BCdR pre-treatment, while the pre-treatment with TdR or CdR had no such effect. The tendency of radiosensitizing effect of BUdR and BCdR on cells that synthesized DNA after the injection was revealed by the following: (a) the relative dominant lethal frequency (Table 4 and Fig. 10); (b) the frequencies of non-disjunction and chromosomal loss (Tables 5 and 6; Figs. 11 and 12); and (c) the lethal mutation frequency (Tables 9 and 10; Figs. 14 and 15). Virtually no translocation events were observed in the pre-meiotic broods E and F (Puro, 1966), since a translocation bearing cell would undergo only an abortive meiosis and hence be eliminated from forming viable gametes (Auerbach, 1962).

(a) Relative Dominant Lethal Frequency: In the post-injection DNA synthesis brood sampling the spermatocytes (br E), BUdR or BCdR followed by 1.2 kr gamma radiation (1-Day Gamma A series) showed a highly significant increase in the RDL than in S, TdR or CdR pre-treatments. In the late gonial cells (br F) the sensitizing effect tailed out showing

a significant increase only in BCdR pre-treatment and not in others (Table 4; Fig. 10). Dominant lethality or unhatchability of the egg could be due to aspermia or gross chromosomal abnormalities leading to developmental abnormalities (Auerbach, 1962 and LaChance, 1967). The significantly high RDL in the spermatocytes (br E) with BUdR and BCdR pre-treatments is probably mainly due to enhanced chromosomal aberrations caused by increased radiosensitivity of analogue incorporation in DNA (Kihlman, 1963 and Humphrey and Dewey, 1965), leading to embryonic lethality rather than due to aspermia; for aspermia by cell death due to irradiation of immature germ cells (Auerbach, 1962) usually results in maximal dominant lethality as observed in the meiotic cells (br D) and is accompanied by maximal sterility as is the case in all the irradiated treatments (Table 3; Fig. 9). Cytological investigation of the unhatched eggs to infer the proportions of dominant lethality due to aspermia and embryonic lethality due to incomplete cleavage (Auerbach, 1962 and LaChance and Rieman, 1964) was not attempted on account of the tedious nature of this process in large comparative experimentations like this. The probable reason for the tailing out of RDL in the gonial cells (br F) could be attributed to quick depletion of the sensitising agents, usually within 6-8 hrs (Olivieri and Olivieri, 1965) resulting in a quick recovery of normal radiosensitivity by the gonial cells as observed in the case of some chemosterilants

(LaChance _et_ _al_., 1969). The analogue incorporated gonial cells may also be selectively eliminated during the pre-meiotic mitosis or the proportion of the analogue in the DNA may also be diluted by a subsequent DNA synthesis after the metabolic depletion of the analogues from the system.

(b) Non-disjunction and Chromosome Loss: The non-disjunction of X and Y chromosome could take place only during meiosis resulting in AXY (A = haploid set of autosomes) and AO (O = no sex chromosome) bearing sperms. So, if there were any sensitizing effect due to analogue pre-treatment, this could be expected only in the meiotic (br D) and pre-meiotic (brs E and F) cells. Indeed, this is what was observed in all the irradiated treatments (Table 5 and Fig.11). In the post-meiotic broods (brs A, B and C), the incidence of $XX^{c2}Y$ females was within the range of spontaneous frequency. The maximal incidence of $XX^{c2}Y$ females was in br D since this brood samples cells undergoing meiosis at the time of irradiation (Fig. 2). Meiotic cells are very sensitive to radiation damage and this is usually reflected in cell death causing sterility (Bateman, 1969) as well as chromosomal aberrations (Table 3; Fig. 9) (Sävghan, 1963). The pre-meiotic cells that synthesized DNA after the injection of BUdR and BCdR showed a significant increase in the frequency of $XX^{c2}Y$ females (Table 5; Fig. 11). The high incidence of $XX^{c2}Y$ females in the pre-meiotic cells may be

caused by two possible factors: (1) an adverse effect of the presence of the analogue in the system, and (2) the incorporation of the analogue in the DNA and a possible stickiness of chromosomes. The former seems improbable because the data on fertility of the treated males do not show any adverse effect specific for BUdR and BCdR pretreatments (with or without radiation) during the corresponding brood period (Tables 2 and 3; Fig. 8 and 9). Since the exogenous nucleoside pool is rapidly depleted in the system within 6-8 hrs (Olivieri and Olivieri, 1965), it seems unlikely that the presence of BUdR or BCdR in the system and not their incorporation in the DNA be responsible for the high frequency of $XX^{c2}Y$ females during the spread of four days. On the other hand, stickiness due to incorporated BUdR in mammalian meiotic cells _in vivo_ has been observed (Mukherjee, 1968). Unlike BUdR, which is specific for DNA, BCdR may be deaminated and incorporated as TdR, and may be atleast partly incorporated as an analogue of CdR (Herskowitz, 1967) and hence is not strictly specific to DNA. Its possible incorporation in the RNA (RNA synthesis occurs during spermatogenesis: Olivieri and Olivieri, 1965) is also likely to cause the same effect by possibly producing structurally altered proteins necessary for the meiotic division. However, since BUdR is specific for DNA, the high incidence of $XX^{c2}Y$ females could be very well caused by its incorporation in the DNA and hence a radiosensitizing

effect.

Though the origin of AXY and AO sperms could happen as a result of the single event non-disjunction during meiosis, AO sperms could be produced in the post-meiotic cells by radiation-induced breakage or fragmentation of the Y chromosome (Traut, 1968; Brink, 1969). Thus, the production of AO sperm resulting in XO males is commonly considered as chromosome loss (Leigh, 1969). Therefore, radiation-induced increase in the chromosome loss could be expected in all the broods irrespective of the meiotic event. Accordingly, the frequency of chromosome loss higher than the spontaneous rate (0.33%) was observed in all the broods in the irradiated series (Tables 3 and 6; Fig. 12). As in the case of non-disjunction, chromosome loss was also maximal in the meiotic brood D due to the sensitivity of the meiotic cells. However, in the pre-meiotic broods E and F, BUdR (1.09%) and BCdR (1.15%) pre-treatments showed higher frequency of chromosome loss than S (0.62%), TdR (0.54%) and CdR (0.56%) pre-treatments. As in the case of non-disjunction, the increase of chromosome loss in BUdR and BCdR pre-treatments in the spermatocytes and the late gonial cells (brs E and F) was probably due to radiosensitization by the analogue incorporation in the DNA.

(c) Lethal Mutations: In the 1-Day Gamma A series (irradiation of the post-injection DNA synthesis cells in the

pre-meiotic stage), the sensitizing effect of BUdR and BCdR pre-treatments to radiation-induced lethal mutations was evident in the spermatocytes (br E) showing 3.0% and 2.9% lethals, significantly higher than the lethal frequency in S pre-treatment (1.1%); but not significantly higher than TdR (1.5%) and CdR (1.4%) pre-treatments. In the late gonial cells (br F) even this difference has tailed out (Table 9; Fig. 14). When the lethal data on the spermatocytes and the late gonial cells (br E and F) were pooled (Table 10 and Fig. 15), BUdR pre-treatment had a mean of 2.4% lethals, significantly high as compared with the S (0.8%) or TdR (1.1%) pre-treatments; but BCdR pre-treatment showed 1.8% lethal significantly higher than S pre-treatment (0.8%) and not significantly higher than CdR pre-treatment (1.1%). Thus, BUdR and BCdR pre-treatments exhibited a strong tendency of radiosensitizing the spermatocytes and the late gonial cells, while TdR and CdR pre-treatments had no such effect. Since these cells have most probably synthesized DNA atleast once after the injection of the analogues (Fig. 2), the increase in the lethal frequency is probably due to the incorporation of the halogenated analogues in the DNA before irradiation. As in the cases of RDL, non-disjunction and chromosome loss, the sensitizing effect was not pronounced in the late gonial cells (br F) with regard to the lethal mutations also, very likely due to meiotic elimination or

dilution of the proportion of the analogue in the DNA by a second cycle of DNA synthesis following the metabolic depletion of the exogenous analogues. These results on the lethal frequency agree very well with those of Sobels (1965). Apart from the present studies, Sobel's work is the only other work, in which the DNA synthesis time was controlled by adhering to the standard brood pattern. Sharma (1969) conducted similar experiments with negative results; however, he arbitrarily observed three 4-day broods, which would have very likely altered the mating intensity of the male, giving a possible error in picking up the post-injection DNA synthesis cells without serious admixture of other cells (Lefevre and Jonsson, 1964 b). Moreover, in Sharma's study (1969) there was no information available on the interval between injection of BUdR and irradiation. An interval of 12 $\pm$ 0.5 hrs between injection and irradiation probably gave ample time for the exogenous analogues to get incorporated in the DNA.

The post-injection DNA synthesis cells were irradiated in the post-meiotic stage (1-Day Gamma E series) also in order to circumvent the probable meiotic elimination of the genetically (chromosomal) damaged cells (Auerbach, 1962) and were tested for lethals as well as translocations. Under these test conditions, BUdR or BCdR did not show significant difference in the frequency of translocations in the sperma-

tocytes and the late gonial cells, when compared with the controls (Table 11 and Fig. 16). The frequency of lethal mutations, however, was significantly high with BCdR pre-treatment (5.2%) over S (2.2%) and CdR (2.0%) pre-treatments in the spermatocytes (br E), while the lethal frequency in the late gonial cells (br F) (3.7%) was significantly high against CdR (2.0%) pre-treatment. BUdR pre-treatment did not show sensitizing effect under these conditions (Table 11 and Fig. 16), even though sensitization was apparent, when the post-injection DNA synthesis cells were irradiated in the pre-meiotic stage (1-Day Gamma A series) (Table 9;Fig.14). It could be that either BUdR was not at all incorporated in the DNA (the sensitization observed in the 1-Day Gamma A series due to a deleterious indirect effect) or BUdR was incorporated in the DNA, but during spermatogenesis through eight days the base was dehalogenated, depriving of the sensitizing property at the time of irradiation. The first speculation is unlikely since it is well established that radiosensitization by the halogenated analogues is specifically attributable to their incorporation in the DNA (Kihlman, 1963; Kaplan et al., 1964) and not due to their presence in the system (Erikson and Szybalski, 1963). The second explanation for the lack of sensitizing effect due to BUdR is more likely because dehalogenation of the incorporated BUdR has been reported in mammalian liver cells (Szybalski, 1969) and the dehalogenated BUdR (now UdR) would behave

like TdR in the DNA (Kaplan *et al*., 1964). Radiosensitization by BCdR has been consistent in 1-Day Gamma A series (post-injection DNA synthesis cells irradiated in the premeiotic stage) and in 1-Day Gamma E series (post-injection DNA synthesis cells irradiated in the spermatozoan stage). Since BCdR is not strictly specific for DNA, the possibility of damage to BCdR incorporated RNA being enhanced is there. However, to the author's knowledge, there is no report to this effect in the literature. On the other hand, BCdR is mainly incorporated in the DNA as BUdR after deamination (Frisch *et al*., 1960). While BUdR could possibly be dehalogenated into UdR in the DNA (Kaplan, *et al*., 1964), it is likely that BCdR could also be subjected to the same fate. Due to absence of data on this phenomenon in the literature, it would be interesting to study this aspect with the use of autoradiography. Similar genetic studies may also be repeated under conditions favouring a higher degree of BUdR incorporation in the DNA, i.e., use of higher concentrations of the analogue (up to 1.0%--Fahmy *et al*., 1966) or BUdR injection preceded by FUdR injection to prevent *de novo* synthesis of TdR inducing temporary TdR starvation (Cohen *et al*., 1958).

4. *Effect of Nucleosides or Analogues on the Radiation-induced Genetic Damage in the Pre-injection DNA Synthesis Cells*:

The reduction of radiation-induced genetic damage in the

post-meiotic cells due to nucleoside pre-treatment was evident in (a) relative dominant lethality (TdR and CdR pre-treatments: Table 4; Fig. 10), (b) the frequency of XO males (Table 6 and Fig. 12), yeilds of (c) translocations (Table 8; Fig. 13) and (d) lethal mutations (Table 10 and 12; Fig. 15 and 17). All these criteria have the chromosomes as the target of radiation damage.

Pre-treatment with the normal pyrimidine nucleosides TdR and CdR significantly reduced the erelative dominant lethality in the mature and immature sperms sampled in brs A and B (Table 4; Fig. 10). The frequency of chromosome loss (XO males) was also lower in all the nucleoside or analogue pre-treatments than in the S pre-treatment; but this reduction was significant only with CdR and BCdR. TdR, CdR, BUdR and BCdR pre-treatments gave lower translocation frequencies (the difference was not significant) than S pre-treatment in the sperms and in the spermatids (Table 8 and Fig. 13). While there was a moderate to significant reduction in the radiation-induced gross chromosomal abnormalities (dominant lethals, chromosome loss and translocations) after nucleoside pre-treatment, the reduction in the sex-linked lethals, which are essentially point mutations (Fahmy <u>et al</u>., 1966; Sobels, <u>et al</u>., 1967), was highly significant in the post-meiotic germ cells when compared with S pre-treatment. Since the administration of equimolar solution of the four nucleosides TdR, CdR, AdR and GdR was known to increase the survi-

val of irradiated mammalian cells in culture (Petrovic and Nias, 1966), a similar experiment was conducted to study the effect on the lethal mutation frequency in the sperms (br A only) using 1-day old males. Pre-treatment with 0.1% equimolar solution of the four normal nucleosides gave the maximum reduction in the lethal frequency (MF = 0.40--Table 12; Fig. 17). The 'protective' effect of the nucleoside pre-treatment was clearly evident in the spermatozoa (Table 9; Fig. 14). Since this observation was contrary to the belief that the spermatozoa are not capable of repair (Muller, 1940; 1965; Russell, 1965; Kofman-Alfaro and Chandley, 1971) probably due to their low metabolic activity, it was considered of interest to see if this reduction of the lethal frequency could be observed in the fully mature sperms at different radiation doses with different nucleosides and if so, to what extent. Using 7-day old virgin males (accumulate unused mature spermatozoa--Lefevre and Jonsson, 1964 a), a series of experiment was carried out with 2.4 kr and 3.6 kr gamma irradiation following nucleoside(s) pre-treatment (Table 12; Fig. 17). With 2.4 kr, TdR, BUdR and BCdR, when administered singly had no significant 'protective' effect on the lethal yield and hence, these treatments were discontinued in the experiment with 3.6 kr gamma radiation. Pre-treatment with equimolar solutions of the four nucleosides (TdR, CdR, AdR and GdR) had a modification factor of 0.41 and 0.64 for the radiation doses of 2.4

kr and 3.6 kr gamma radiation respectively, showing a dose-effect relationship, an indication of repair; the degree of 'protection' decreased with the increase in the radiation dose.

The reduction in the frequency of lethals in the sperms could be brought about by a possible selective elimination of the lethal bearing sperms or by a process other than one involving elimination ("protection" or repair). If a selective elimination of the lethal bearing sperms were to take place, one would expect an increase in dominant lethality in the respective brood (br A) (Sobels, _et al._, 1967) with the nucleoside pre-treatments. However, with TdR and CdR pre-treatments, there was a significant decrease in dominant lethality (Table 4; Fig. 10). So a possible involvement of a protective or repair process in the reduction of the lethal frequency is strongly indicated. The dose-effect relationship (Table 12) also indicates repair or protection (Watson, 1967).

The primary step in radiation mutagenesis is the registration of a molecular alteration--pre-mutational lesion (including the potential chromosome breaks)--in the genetic material. Radiation-induced genetic damage may be fixed as self-replicating mutational events without the involvement of protein (Altenberg and Browning, 1961; Muller _et al._, 1961). On the other hand, another kind of lesion may be stabilized

by being converted into self-replicating mutations involving protein dependent metabolic steps (Wolff, 1960; Kimball, 1968).

Various factors like the initial radiosensitivity of the genetic material, efficiency of the repair process, time of mutation fixation and the radiosensitivity of the repair system can affect the mutation response of a system (Sobels, 1963). Depending on the metabolic state of the cell, the pre-mutational lesion may encounter different possible fates:

a. the damage may not be fixed and may be detected genetically as a dominant lethal or cytologically as an unhealed chromosome break;

b. the lesion may be repaired fully to its original state; and

c. the lesion may also undergo "repair" ("misrepair"?--Kimball, 1965) with a genetic change detectable as a recessive lethal event.

Hence, given a certain amount of pre-mutational radiation damage, a proportion of it does not result in mutation. This recovery due to normal repair processes can be altered by the use of exogenous chemicals that may favour or antagonize the repair processes. In an attempt to explain the reduction of radiation-induced genetic damage by the nucleosides in the present experiments, three possible mechanisms of action may be considered:

a. Action as protective compounds during irradiation,

help reducing the initial radiation damage;

b. Making some additional energy available to the repair processes; and

c. Physically being involved in the chemical repair of the radiation damage.

DNA precursors have been known to repair radiation induced chromosome breaks in *Tradescantia* microspores (Beatty and Beatty, 1967). By subjecting the microspores to pre- and post-irradiation treatments, the authors concluded that the effect of the nucleosides was not by protection from the initial radiation damage, but rather by a facilitation of the recovery process.

The second mode of action involves a possible supply of excess energy made available for the chromosome repair by some stimulus to some metabolic pathway, probably carbohydrate catabolism (Matsudaira et al., 1970) that would favour the production of more adenosine triphosphate (ATP). Availability of exogenous glucose increased the rejoining of X-ray induced breaks in the DNA of Ehrlich ascites tumor cells by increasing cellular ATP which aided in the synthesis of DNA, RNA and protein as well as thymidine triphosphate (TTP). Addition of adenine to rat thymocyte cell suspensions increased the cellular ATP content and restored the cells from radiation-induced cell death (Ohyama and Yamada, 1970). ATP favoured chromosome rejoining (Matsudaira *et al.*,1970)

and decreased the radiation-induced chromosomal aberrations (Beatty and Beatty, 1967). Either aerobic or anaerobic energy metabolism was found necessary for the repair of the pre-mutational damage in Drosophila mature sperms and early spermatids (Sobels, *et al.*, 1967). However, by depleting the cellular ATP in the anthers of Tradescantia and later supplying AdR, it was not possible to decrease the chromosomal aberrations; When ATP was supplied at this stage instead of AdR, the aberration yield was decreased (Beatty and Beatty, 1967). These observations indicated that while adenine was converted into ATP to provide energy for repair (Ohyama and Yamada, 1970), AdR was not converted into ATP; but it had another role in the radiation repair for which ATP was also necessary (Beatty and Beatty, 1967).

The third consideration is that the nucleosides could probably be involved in the chemical repair of the radiation-induced genetic damage. It is well established in the mitotic systems that exposure to U.V. radiation (Rasmussen and Painter, 1964), X-radiation (Hill, 1967) and radiomimetic chemicals (Roberts *et al.*, 1971) can stimulate incorporation of nucleosides in the DNA in the non-S-phase cells in an attempt to repair the damage in the DNA. DNA precursors have been known to repair chromosome breaks (Beatty and Beatty, 1967) or decrease the damage to chromosomes in mammalian cells in culture (Smets *et al.*, 1967), and restore the cell from radiation-induced cell death (Petrovic and

Nias, 1966). Equimolar solution of the four nucleosides (TdR, CdR, AdR and GdR) showed maximal restoration of irradiated cells (Ferle-Vidovic *et al*., 1965). Addition of depolymerized RNA (Rounds and Slick, 1962) or highly polymerized DNA (Miletic *et al*., 1964) has resulted in increased survival of irradiated cells. The 'protective' effect of the polymerized DNA was also reflected in the organism level by an increase in the survival of irradiated rats. This radioprotective effect of the nucleosides and nucleic acids has been attributed to a possible enhancement of the nucleic acid metabolism and incorporation of the exogenous nucleosides, probably repairing the sublethal and potentially lethal damage (Petrovic, 1968). The repair of DNA damage may take place in the non-S-phase in a non-semi-conservative fashion called unscheduled DNA synthesis (also called repair incorporation or repair replication) or during the S-phase of the cell called repair synthesis (Elkind and Whitmore, 1967).

In *D. melanogaster* testis, one is dealing with a meiotic system, where the spermatogenous cells are in different stages of maturation and so in different degrees of metabolic activity. In the pre-meiotic cells, the major episode of DNA synthesis (from 2C to 4C stage) occurs in the pre-meiotic interphase (Swift, 1950) and so in the radiobiological studies with *D. melanogaster* males, it is usually assumed that no further DNA synthesis takes place in the post-

meiotic cells until after fertilization (Sobels, 1963; Fahmy *et al*., 1966). By pre-treatment with protein synthesis inhibitors like chloramphenicol, ribonuclease, streptomycin and actinomycin-D, the initial radiosensitivity of the spermatids could be decreased by reducing the metabolic activity (Sobels, 1963; Clark, 1963 and Mukherjee, 1965). By post-treatment with chloramphenicol or 2-4-dinitrophenol, the efficiency of the repair system could be retarded due to lack of protein synthesis yielding an increase in the recessive lethal frequency (Sobels, 1963). Since the inhibition of protein synthesis increased the radiation-induced chromosomal, and hence, genetic damage, there prevails a strong tendency to believe that repair involves only the structural proteins in the chromosomes (Wolff, 1960; Sobels, 1963). Since the post-meiotic cells are neither growing nor mutiplying and have no apparent DNA synthetic activity (Chandley and Bateman, 1962), the repair of DNA damage as a factor in the modification of radiation genetic damage has been implicitly ruled out (Sobels, 1963; Wolff, 1966). From this approach, one might draw the erroneous inference that the lesion was in protein, which may not be the case, because a genetic defect has to have DNA as the physical basis of alteration and not the structural protein. It might very well be that in the presence of the protein synthesis inhibitors, the formation of an enzyme or enzymes involved in the repair process was inhibited or retarded in which case a radiation-

induced lesion in the DNA might become a lethal event (Wolff, 1960; 1966; and Sinclair, 1967).

The lack of inhibitory effect of FUdR, a potent DNA synthesis inhibitor (Cohen et al., 1958), on the chromosomal reunion or restitution has also been cited as an evidence that only the synthesis of structural protein (Wolff and Scott, 1969) and not of DNA (Beatty and Beatty, 1967; Wolff, 1966) is involved in the chromosome repair. However, the effect of FUdR on the repair replication should be interpreted with caution. The inhibitory effect of FUdR may depend on the concentration of FUdR as well as the duration of the treatment before irradiation. It is likely that very small quantities of nucleosides already present in the system would be enough to carry out the DNA repair process (Cleaver, 1969). Treatment with FUdR itself can induce chromosome breaks even without irradiation (Taylor et al., 1962). Prolonged FUdR treatment may not allow restitution after irradiation (Yamaguchi and Yamamoto, 1969). The induction of chromosome breaks by FUdR as well as the persistence of radiation-induced chromosome breaks in the presence of FUdR could be avoided by exogenous TdR (Taylor, 1963; Yamaguchi and Yamato, 1969).

In the absence of an understanding of the macromolecular organization (DNA, RNA and protein) of the eukaryotic chromosomes, it is reasonable to assume that the morpholo-

gical integrity of the chromosome as a cellular structure is probably maintained by its protein component (with the probable exception of some protein or other macromolecules) with a regulatory function) and the functional integrity of the chromosome in terms of heredity by the DNA component. While the synthesis of the structural protein may be involved or even be necessary for the reunion or restitution of the chromosome breaks (Wolff and Scott, 1969), it seems logical to assume that the repair of heritable damage (including potential chromosome breakage) and point mutations involving DNA damage) must necessarily involve DNA repair to restore the original state of DNA as much as possible (Painter, 1970). It has been shown that the repaired DNA can subsequently undergo normal semi-conservative replication (Rasmussen *et al.*, 1970; Painter *et al.*, 1970) suggesting a complete functional recovery is possible. The 'healing' of broken ends of chromosome bridges as well as the formation of ring chromosomes was accompanied by the incorporation of labelled thymidine in HeLa cells as shown by autoradiography and the grains were not present in the autoradiographs prepared after DNA-ase treatment (Seed, 1971). Since in HeLa cells the chromosome abnormalities arise spontaneously, it would be interesting to study this phenomenon in other cell systems following irradiation.

If DNA repair was one of the factors in reducing the genetic damage, this repair may take place in the post-meio-

tic germ cells before mating or alternatively the nucleosides may be carried inertly by the sperms and the damage may be repaired during the first DNA synthetic phase after fertilization. This possibility was revealed by the capacity of the exogenous DNA injected in the male to induce mutations in the marked X chromosomes carried by the female parent (Fahmy and Fahmy, 1965). The delayed effect of certain mutagenic chemicals on the treated sperms stored in the female also indicate such a possibility (Watson, 1966). It is also known from work on *Drosophila* (Muller, 1940; Kaufman, 1941), the mouse (Russell *et al*., 1958; Russell, 1960) and sea urchin (Failla, 1962) that sperm may undergo repair after fertilization.

There is a paucity of information on the probable involvement of unscheduled DNA synthesis in the recovery process in the post-meiotic germ cells. In the autoradiographic studies by Chandley and Bateman (1962) the injection of tritiated TdR was followed by an exposure time of 21 days, which did not reveal any evidence of DNA synthetic activity in the post-meiotic cells in *D. melanogaster* testis. However, recently Riley and Bennet (1971) detected low level of DNA synthetic activity in all the stages of the meiotic division in wheat anthers using long exposure periods (200 days). Similar observations have been reported in newt and in mammals (Riley and Bennett, 1971). Irradiation is known to stimulate DNA polymerase activity (Keiding and Westergaard, 1971).

It is possible that irradiation may stimulate repair of DNA damage by enhancing nucleic acid metabolism in the presence of the exogenous nucleosides as was found in the somatic cells (Petrovic, 1968). Radiation-stimulated incorporation of tritiated TdR into diplotene oocytes of the guinea-pig (Crone, 1970), spermatogenous cells (spermatocytes, spermatids and cells undergoing spermeogenesis) of mouse (Kofman-Alfaro and Chandley, 1971) and of man (Chandley and Kofman-Alfaro, 1971) have been reported very recently. On the other hand, unscheduled DNA synthesis in the somatic cells has been correlated with the radiosensitivity of the meiotic cells of the organism; a desynaptic strain of barley was found to be more radiosensitive to chromosomal aberrations in mitotic cells (Riley and Miller, 1966). Results suggesting a similar relationship between repair synthesis and events during meiosis have been observed in human beings also (Pearson *et al.*, 1970).

The use of several anti-metabolites has been widely employed to understand the mechanism of the repair processes in the cellular (Cleaver, 1969) and organism (Sobels, 1963) levels. Iodoacetamide (glycolysis inhibitor) that can inhibit DNA repair process (Boling and Setlow, 1962) and radiosensitize cells had a tendency to sensitize the post-meiotic cells to radiation damage and increased the frequency of lethal mutations in *D. melanogaster* males; but the results were not very significant (Mukherjee and Sobels, 1968). Sodium

fluoride that strongly sensitizes the spermatids and mature sperms (Mukherjee and Sobels, 1968) is very likely an inhibitor of normal and repair DNA synthesis. Actinomycin-D capable of inhibiting semi-as well as non-semi-conservative DNA synthesis would be expected to sensitize the spermatids and mature sperms (Elkind *et al*., 1964). On the contrary, actinomycin-D had a protective effect on spermatids and no effect on spermatozoa (Mukherjee, 1965). This antibiotic inhibits protein synthesis at the transcription level (mRNA formation). Hence, the protective effect could be explained by the continued progress of lysine rich protein synthesis in these cells in the presence of the antibiotic, indicating that the post-meiotic cells have pre-synthesized long-living stable RNA (Brink, 1968). Presumably actinomycin-D acts as a "super inducer" preventing the breakdown of the existing RNA molecules (Tomkins *et al*., 1969). This is consistent with the fact that no RNA synthesis was observed in the post-meiotic cells though a detectable amount of RNA was present till the end of spermeogenesis (Olivieri and Olivieri, 1965). Since actinomycin-D is known to inhibit repair synthesis and sensitize the cell (Elkind *et al*., 1964; Cleaver, 1969) and the post-meiotic germ cells showed a protective effect in the presence of actinomycin-D, it is likely that some of the stable RNA were responsible for the repair of the genetic damage at the DNA level also. Since the repair is enhanced by the DNA precursors, repair incorporation seems to be a

likely explanation; the present studies, however, are not designed to confirm this. The probable involvement of protein synthesis in this process cannot be completely ruled out, because CdR is known to induce DNA as well as basic protein synthesis (Lou and Kong, 1971) preferentially arginine rich histones (Kong, 1971), that replace lysine rich histones in very late periods of the sperm maturation process in D. melanogaster (Sobels, 1966).

In the light of these observations, the reduction of radiation-induced genetic damage in the post-meiotic germ cells observed in this study raises the question of possible repair DNA synthesis playing a role in the genetic recovery. Since the present study was not designed to elucidate the mechanism of the repair by the nucleosides, it is suffice to say that the nucleoside pre-treatment reduces the yield of the pre-mutational lesionsby an enhancement of the efficiency of the repair processes active in irradiated cells; the actual mechanism may involve (a) an enhancement of the energy available for the repair, (b) stimulation of structural protein synthesis or (c) stimulation of unscheduled DNA synthesis or repair replication; very likely the nucleosides may act in more than one way simultaneously.

SUMMARY

The mutagenic and the radiosensitizing properties of the halogenated pyrimidine nucleosides BUdR and BCdR and their normal nucleosides TdR and CdR were studied using the genetic system in the different stages of the spermatogenous cells in *D. melanogaster*. The effect of the exogenous nucleosides and their analogues on the radiation-induced genetic damage in the pre-meiotic germ cells (post-injection DNA synthesis cells) were studied by irradiating (1.2 kr gamma radiation) the nucleoside or analogue treated cells before (1-Day Gamma A series)or after (1-Day Gamma E series) meiotic division. The effect of these chemicals on the radiation damage in the post-meiotic cells (pre-injection DNA synthesis cells) was studied by irradiating (1.2 kr gamma radiation) the different stages of the post-meiotic cells (mature and immature sperms, and spermatids in 1-Day Gamma A series brs A, B and C) and on the mature sperms by using 7-day old virgin males and different radiation doses (7-Day Gamma A series: 2.4 kr and 3.6 kr gamma radiation).

The halogenated analogues BUdR and BCdR did not induce dominant lethality, non-disjunction, chromosome loss, translocations or lethal mutations in the different stages of the spermatogenous cells. When the pre-meiotic germ cells (post-injection DNA synthesis) sampled in brs E and F were irradia-

ted before meiosis (1-Day Gamma A series), BUdR and BCdR pre-treatments showed an increase in the radiation-induced genetic damages over saline pre-treatment like (a) relative dominant lethality, (b) non-disjunction, (c) chromosome loss, and (d) sex-linked recessive lethal mutations. TdR and CdR pre-treatments had no such effects. The radiosensitizing property was evident with BCdR even when the post-injection DNA synthesis cells were irradiated after meiosis (1-Day Gamma E series); but not with BUdR pre-treatment. Eventhough, the tendency of radiosensitization by BUdR and BCdR was evident, apparently due to their incorporation in the DNA of the pre-meiotic cells (The spermatocytes and the late gonial cells sampled in brs E and F), studies under conditions favouring a higher degree of analogue incorporation and autoradiography were considered useful.

When irradiation was preceded by the injection of nucleosides or analogues, the radiation genetic damage in the post-meiotic germ cells (pre-injection DNA synthesis cells) was lower than in the saline treated irradiated control. This reduction in the radiation-induced genetic damage in the post-meiotic cells due to the nucleoside pre-treatment was evident in (a) the relative dominant lethality (with TdR and CdR), (b) the frequency of chromosome loss (XO males), (c) translocations (not significant) and (d) lethal mutations and was conspicuous in the mature sperms (br A). The anti-radiation effect of the nucleosides on the mature sperms was

confirmed using 7-day old virgin males and different radiation doses. The frequency of lethal mutations was lowest when irradiation was preceded by an injection of equimolar solution of all the four nucleosides (TdR, CdR, AdR and GdR). The factor of protection decreased with increase in radiation dose.

Different possible mechanisms for the radio-protective action of the nucleosides were considered. They were (a) reduction of the initial radiation damage, (b) increase in the available ATP for repair, and (c) being physically involved in the chemical repair of the DNA damage. DNA precursors have been known to repair the radiation-induced chromosome breaks or reduce the yield of chromosome aberrations and increase the cell as well as organism survival. Radiation-stimulated incorporation of the exogenous nucleosides and DNA repair mechanisms are known to exist in the mitotic and meiotic systems. Furthermore, for the repair of genetic damage, DNA repair was considered necessary, eventhough the synthesis of structural protein was also necessary for the chromosome repair or reunion. It was concluded that the radiation-induced genetic damage in the post-meiotic cells may be repaired before mating or after fertilization, probably involving protein as well as DNA repair synthesis. The usefulness of different antimetabolites in the study of the mechanism of repair was discussed.

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APPENDIX

Table 1. Percent mortality among the treated *D. melanogaster* males through the successive broods in 1-Day GA series.

Treatments	Days				after				treatment			
	0		2		4		6		8		10	
	No Sur	% Mort	No Sur	% Mort	No Sur	% Mort	No Sur	% Mort	No Sur	% Mort	No Sur	% Mort
I S	61	-	57	6.6	44	22.8	40	9.9	36	9.9	28	22.2
II S + 1.2 GA	53	-	48	9.9	41	14.6	36	12.2	29	19.4	26	10.3
III TdR	49	-	43	12.2	33	23.3	32	7.0	24	25.0	21	12.5
IV TdR + 1.2 GA	62	-	56	9.7	53	5.4	43	19.9	34	20.9	34	0.0
V BUdR	69	-	65	5.8	55	15.4	46	16.4	38	17.4	34	10.5
VI BUdR + 1.2 GA	67	-	60	10.4	47	21.7	38	19.1	33	13.2	29	12.1
VII CdR	58	-	56	4.4	47	16.1	38	19.1	30	21.1	28	8.7
VIII CdR + 1.2 GA	58	-	53	8.6	45	15.1	38	15.6	32	15.8	27	15.6
IX BCdR	54	-	53	1.9	41	22.7	37	9.8	31	16.2	27	12.9
X BCdR + 1.2 GA	112	-	102	8.9	77	24.5	66	14.3	56	15.6	51	8.9

Table 2. F_1 progeny data (fertility, non-disjunction and chromosome loss) on the nucleoside or analogue injected unirradiated control experiments (1-Day old males--Gamma A series).

Br	Treatment	F_1 Progeny Total (T)	Mean/Male ± S.D.	No. XX Females	No. XY Males (m)	Sex ratio $=\frac{100m}{T}$	XXY Females No.	%	XO Males No.	%
A	S	5656	283 ± 67	2948	2708	47.9	2	0.08	7	0.26
	TdR	7603	272 ± 48	3907	3696	48.6	1	0.03	6	0.16
	BUdR	6421	247 ± 64	3335	3086	48.1	0	0.00	1	0.01
	CdR	7036	271 ± 55	3728	3308	47.0	2	0.05	4	0.12
	BCdR	6722	280 ± 70	3439	3283	48.8	1	0.03	8	0.24
B	S	4806	267 ± 53	2256	2550	53.1	1	0.04	5	0.20
	TdR	5957	284 ± 57	3025	2932	49.2	0	0.00	5	0.30
	BUdR	6216	282 ± 56	3222	2994	48.2	1	0.02	3	0.10
	CdR	6782	295 ± 48	3593	3189	47.0	2	0.06	4	0.13
	BCdR	7160	298 ± 66	3732	3428	47.8	7	0.19	6	0.18
C	S	6296	315 ± 72	3447	2849	45.3	4	0.12	5	0.18
	TdR	5311	312 ± 53	2754	2557	48.2	3	0.11	1	0.04
	BUdR	5431	285 ± 56	2810	2621	48.8	3	0.11	3	0.11
	CdR	5840	278 ± 37	3087	2753	47.1	2	0.06	4	0.15
	BCdR	6742	306 ± 69	3595	3147	46.7	8	0.22	11	0.35
D	S	5232	262 ± 87	2766	2466	47.1	3	0.11	5	0.20
	TdR	4503	281 ± 48	2339	2164	48.1	0	0.00	4	0.19
	BUdR	4149	259 ± 62	2124	2025	48.8	1	0.05	4	0.20
	CdR	5598	311 ± 54	2920	2678	47.8	1	0.03	3	0.11
	BCdR	6679	318 ±100	3454	3225	48.3	5	0.15	9	0.28
E	S	4021	237 ±108	2015	2006	49.9	3	0.15	5	0.25
	TdR	2880	240 ± 56	1361	1419	49.8	0	0.00	1	0.07
	BUdR	2872	221 ± 46	1567	1305	45.4	2	0.13	4	0.31
	CdR	4192	246 ± 41	2228	1964	46.9	1	0.05	3	0.15
	BCdR	4344	229 ± 62	2295	2049	47.2	5	0.22	8	0.39
F	S	2857	204 ± 53	1297	1374	51.4	0	0.00	2	0.15
	TdR	2220	202 ± 48	1131	1089	49.6	1	0.09	3	0.28
	BUdR	2291	208 ± 76	1217	1074	46.9	1	0.08	2	0.19
	CdR	3062	191 ± 52	1637	1425	46.5	1	0.06	3	0.21
	BCdR	3206	178 ± 70	1681	1525	47.6	2	0.12	5	0.33

Table 3. F_1 progeny data (fertility, non-disjunction and chromosome loss) on the nucleoside or analogue injected and irradiated (1.2 kR) experiments (1-Day old males--Gamma A series).

Br	Treatment	F_1 Progeny Total (T)	Mean/Male ± S.D.	No. XX Females	No. XY Males (m)	Sex ratio ∓100m/T	XXY Females No.	XXY Females %	XO Males No.	XO Males %
A	S + 1.2 GA	6789	212 ± 63	3614	3175	46.8	1	0.03	20	0.63
	TdR + 1.2 GA	6460	208 ± 48	3428	3032	46.9	1	0.03	12	0.40
	BUdR + 1.2 GA	10549	234 ± 50	5757	4792	45.4	4	0.07	23	0.48
	CdR + 1.2 GA	7454	249 ± 44	3851	3603	48.3	2	0.05	6	0.12
	BCdR + 1.2 GA	14837	291 ±100	7847	6990	47.1	2	0.03	32	0.46
B	S + 1.2 GA	7317	261 ± 58	3900	3417	46.8	7	0.18	27	0.79
	TdR + 1.2 GA	7530	279 ± 94	4144	3386	46.3	1	0.02	28	0.83
	BUdR + 1.2 GA	10283	271 ± 50	5561	4722	46.0	5	0.09	21	0.45
	CdR + 1.2 GA	7625	318 ± 83	4068	3557	46.7	0	0.00	11	0.31
	BCdR + 1.2 GA	9127	234 ± 42	4818	4309	47.2	3	0.06	22	0.51
C	S + 1.2 GA	4626	154 ± 49	2634	1992	43.1	2	0.08	39	1.96
	TdR + 1.2 GA	4800	200 ± 53	2542	2258	47.0	2	0.08	42	1.86
	BUdR + 1.2 GA	4615	171 ± 61	2573	2042	44.2	4	0.16	41	2.01
	CdR + 1.2 GA	3708	185 ± 34	1993	1715	46.3	3	0.15	21	1.23
	BCdR + 1.2 GA	5769	160 ± 40	3284	2485	43.1	5	0.15	46	1.85
D	S + 1.2 GA	1814	70 ± 24	959	855	47.1	6	0.63	40	4.68
	TdR + 1.2 GA	1117	59 ± 26	558	559	50.0	1	0.18	13	2.33
	BUdR + 1.2 GA	1717	72 ± 26	961	756	44.0	14	1.46	41	5.42
	CdR + 1.2 GA	1109	58 ± 26	574	535	48.2	4	0.70	18	3.36
	BCdR + 1.2 GA	2246	58 ± 23	1187	1059	47.2	9	0.76	58	5.48
E	S + 1.2 GA	2624	131 ± 48	1408	1219	46.5	6	0.43	13	1.07
	TdR + 1.2 GA	1863	124 ± 31	970	893	47.9	2	0.21	8	0.90
	BUdR + 1.2 GA	2690	117 ± 44	1402	1288	47.9	10	0.71	19	1.48
	CdR + 1.2 GA	2405	150 ± 58	1304	1101	45.8	3	0.23	6	0.55
	BCdR + 1.2 GA	4202	140 ± 37	2227	1975	47.0	18	0.91	33	1.67
F	S + 1.2 GA	2907	171 ± 80	1573	1334	45.9	0	0.00	3	0.23
	TdR + 1.2 GA	2335	156 ± 48	1189	1146	49.1	1	0.08	3	0.26
	BUdR + 1.2 GA	4109	179 ± 68	2192	1917	46.7	10	0.46	16	0.83
	CdR + 1.2 GA	2263	162 ± 61	1205	1058	46.8	6	0.50	6	0.57
	BCdR + 1.2 GA	4481	172 ± 78	2379	2102	46.9	8	0.34	14	0.67

Table 4. Effect of exogenous pyrimidine nucleosides and their brominated analogues on the radiation induced dominant lethal frequency (% egg hatchability) in the different stages of <u>D. melanogaster</u> male germ cells.

Treatment pairs \ Broods	A		B		C		D		E		F	
	No. eggs	% hatc.	No. eggs	% hatc.	No. eggs	% hatc.	No. eggs	% hatc.	No. eggs	% hatc.	No. eggs	% hatc.
	1,965	80.7	2,499	81.3	3,972	78.3	3,109	67.2	2,383	71.0	1,600	73.1
S + 1.2 GA	2,079	69.9	1,668	63.1	3,595	34.0	2,615	23.7	1,684	57.0	2,081	67.2
I RDL		13.4		22.4		56.6		64.7		19.7		8.1
TdR	1,700	80.9	2,052	78.9	2,062	80.3	1,751	72.9	1,662	71.8	1,652	75.4
TdR + 1.2 GA	1,990	75.2	2,178	66.0	2,848	34.6	2,336	30.4	1,948	61.2	1,509	78.2
II RDL		7.0		16.3		56.9		58.3		14.8		-3.7
BUdR	2,034	81.9	2,002	80.5	2,154	77.8	1,882	69.9	1,804	71.9	1,858	76.6
BUdR + 1.2 GA	2,516	70.7	2,812	65.5	2,816	36.9	1,836	28.0	1,577	48.4	1,233	76.1
III RDL		13.7		18.6		52.6		59.9		32.7		0.7
CdR	1,351	82.9	1,607	81.9	1,810	80.2	1,540	70.8	1,400	74.8	1,419	79.7
CdR + 1.2 GA	1,553	74.8	1,770	70.4	1,821	34.9	1,627	29.9	1,537	60.0	1,523	76.9
IV RDL		9.8		14.0		56.5		57.8		19.8		3.5
BCdR	2,458	81.3	2,630	80.2	2,270	79.0	1,803	68.6	1,251	69.3	1,253	71.6
BCdR + 1.2 GA	2,273	68.9	1,943	60.7	2,344	36.6	1,617	27.0	1,467	43.6	1,636	58.6
V RDL		15.3		24.3		53.7		60.6		47.1		18.2
Signific. test	X^2_1 DF	P	X^2_1 DF	P	X^2_1 DF	P	X^2_1 DF	P	X^2_1 DF	P	X^2_1 DF	P
I vs II	26.64	≪.001	9.81	<.01	0.00	>.99	1.08	>.20	6.26	<.02	--	--
I vs III	0.02	>.80	3.81	>.05	1.00	>.30	0.47	>.30	21.60	≪.001	55.89	≪.001
I vs IV	6.12	<.02	18.47	≪.001	0.00	>.99	1.02	>.30	0.00	>.99	20.44	≪.001
I vs V	1.44	>.20	0.58	>.30	0.44	>.50	0.30	>.50	69.62	≪.001	41.10	≪.001
II vs III	30.54	≪.001	1.98	>.10	1.06	>.30	0.06	>.80	53.75	≪.001	--	--
IV vs V	13.34	≪.001	27.63	≪.001	0.28	>.50	0.14	>.70	67.29	≪.001	100.06	≪.001

> = not significant; < = significant; ≪ = Highly significant

Table 5. Effect of exogenous pyrimidine nucleosides and their brominated analogues on the radiation-induced frequency of $XX^{c2}Y$ females in the post-meiotic (brs A, B and C), meiotic (br D), and pre-meiotic (brs E and F) stages of D. melanogaster male germ cells.

Germ line sectors / Treatment	Post-meiotic brs A, B & C No			Meiotic br D No			Pre-meiotic Brs E & F No		
	No	No	%	No	No	%	No	No	%
I S + 1.2 GA	10148	10	0.10	959	6	0.63	2981	6	0.20
II TdR + 1.2 GA	10118	4	0.04	558	1	0.18	2159	3	0.15
III BUdR + 1.2 GA	13891	13	0.09	961	14	1.46	3594	20	0.56
IV CdR + 1.2 GA	9912	5	0.05	574	4	0.70	2159	6	0.28
V BCdR + 1.2 GA	15949	10	0.06	1187	9	0.76	4606	26	0.56

Signific. test	χ^2_1 DF	P	χ^2_1 DF	P	χ^2_1 DF	P
I vs II	1.76	>.10	0.70	>.30	0.04	>.80
I vs III	0.00	>.99	2.40	>.10	4.32	<.05
I vs IV	0.97	>.30	0.03	>.80	0.07	>.70
I vs V	0.62	>.30	0.01	>.99	4.81	<.05
II vs III	1.71	>.10	4.57	<.05	4.86	<.05
IV vs V	0.02	>.80	0.02	>.98	1.97	>.10

> = not significant; < = significant; << = highly significant.

Table 6. Effect of exogenous pyrimidine nucleosides and their brominated analogues on the radiation-induced frequency of XO males in the post-meiotic (brs A, B and C), meiotic (br D) and pre-meiotic (brs E and F) stages of D. melanogaster male germ cells.

Germ line sectors / Treatment	Post-meiotic brs A, B & C			Meiotic br D			Pre-meiotic brs E & F		
	No XY	No XO	% XO	No XY	No XO	% XO	No XY	No XO	% XO
I S + 1.2 GA	8584	86	1.00	855	40	4.68	2553	16	0.62
II TdR + 1.2 GA	8676	82	0.94	559	13	2.33	2039	11	0.54
III BUdR + 1.2 GA	11556	85	0.74	756	41	5.42	3205	35	1.09
IV CdR + 1.2 GA	8875	38	0.42	535	18	3.36	2159	12	0.56
V BCdR + 1.2 GA	13784	100	0.72	1059	58	5.48	4077	47	1.15

Signific. test	$X^2_{1\ DF}$	P	$X^2_{1\ DF}$	P	$X^2_{1\ DF}$	P
I vs II	0.08	>.70	4.22	<.05	0.04	>.80
I vs III	3.77	>.05	0.28	>.50	2.94	>.05
I vs IV	19.28	≪.001	1.01	>.30	0.02	>.80
I vs V	4.48	<.05	0.42	>.50	4.00	<.05
II vs III	2.36	>.10	6.51	<.02	3.70	>.05
IV vs V	7.31	>.01	2.76	>.05	4.66	<.05

< = significant; > = not significant; ≪ = highly significant.

Table 7. Effect of exogenous pyrimidine nucleosides and their brominated analogues on the spontaneous frequencies of translocations (II, III and / or Y chromosomes) and sex-linked recessive lethals in the different stages of D. melanogaster male germ cells.

Treatment \ Brs	A No Te	A L/Tr	B No Te	B L/Tr	C No Te	C L/Tr	D No Te	D L/Tr	E No Te	E L/Tr	F No Te	F L/Tr	All broods pooled No Te	No L/Tr	% L/Tr
I S:															
Leth.	838	1	715	2	919	1	632	-	509	1	636	1	4249	6	0.14
Tran.	721	-	609	-	614	-	579	-	609	-	-	-	3132	-	-
II TdR:															
Leth.	550	1	543	1	614	1	525	1	501	-	582	-	3315	4	0.12
Tran.	631	-	538	-	516	-	542	-	535	-	-	-	2762	-	-
III BUdR:															
Leth.	650	1	535	1	566	2	568	1	631	2	603	1	3553	7	0.20
Tran.	538	-	510	-	522	-	577	-	625	-	-	-	2772	-	-
IV CdR:															
Leth.	584	1	534	-	521	1	551	1	624	-	672	2	3386	5	0.15
Tran.	529	-	519	-	537	-	550	-	557	-	-	-	2692	-	-
V BCdR:															
Leth.	710	-	725	1	635	1	576	-	629	3	607	-	3882	5	0.13
Tran.	762	-	521	-	576	-	566	-	548	-	-	-	2973	-	-
VI Four Nucleosides:															
Leth.	621	-	-	-	-	-	-	-	-	-	-	-	621	-	-

Table 8. Effect of exogenous pyrimidine nucleosides and their brominated analogues on the radiation-induced translocation (II, III and / or Y chromosomes) frequencies in the different stages of *D. melanogaster* male germ cells.

Broods		A			B			C			D			E		
Treatment		No Te	No Tr	% Tr	No Te	No Tr	% Tr	No Te	No Tr	% Tr	No Te	No Tr	% Tr	No Te	No Tr	% Tr
I S +	1	373	9		158	6		132	15		227	7		114	-	
1.2 GA	2	270	4		372	14		278	29		328	3		230	-	
	3	-	-		-	-		154	17		-	-		189	-	
Total		643	13	2.0	520	20	3.9	564	61	10.8	555	5	0.9	533	-	-
II TdR +	1	239	2		260	9		194	20		198	6		248	1	
1.2 GA	2	230	1		320	10		186	19		253	7		283	-	
	3	158	1		-	-		173	15		115	3		112	-	
Total		627	4	0.6	580	19	3.3	553	54	9.8	566	16	2.8	643	1	0.2
III BUdR +	1	324	4		240	5		281	30		185	7		238	-	
1.2 GA	2	288	3		236	5		274	24		252	7		177	-	
	3	-	-		103	2		114	12		141	5		123	-	
Total		611	7	1.2	579	12	2.1	669	66	9.9	578	19	3.3	536	-	-
IV CdR +	1	391	3		421	9		199	12		250	7		298	1	
1.2 GA	2	216	1		-	-		377	29		279	1		238	-	
Total		604	4	0.7	430	9	2.1	535	41	7.7	515	14	2.7	535	1	0.2
V BCdR +	1	222	2		198	4		267	29		266	4		197	-	
1.2 GA	2	221	2		130	3		409	38		324	4		245	-	
	3	184	1		203	5		-	-		-	-		132	-	
Total		627	5	0.8	531	13	2.5	676	67	9.9	590	8	1.4	574	-	-

Signific. te.	X^2_1 DF	P	X^2_1 DF	P	X^2_1 DF	P	X^2_1 DF	P	X^2_1 DF	P
I vs II	3.61	>.05	0.08	>.70	0.22	>.50	4.66	<.05	-	-
I vs III	1.02	>.30	2.28	>.10	0.20	>.50	6.66	<.01	-	-
I vs IV	2.18	>.10	1.75	>.10	4.34	<.05	3.83	>.05	-	-
I vs V	2.60	>.10	1.59	>.10	0.18	>.50	0.20	>.50	-	-
II vs III	0.42	>.50	1.18	>.20	0.00	>.99	0.08	>.30	-	-
IV vs V	0.00	>.99	0.02	>.80	1.47	>.30	1.96	>.10	-	-

> = not significant; < = significant; ≪ = highly significant.

Table 9. Effect of exogenous pyrimidine nucleosides and their brominated analogues on the radiation induced lethal frequencies in the different stages of *D. melanogaster* male germ cells.

Treatments \ Broods		A			B			C			D			E			F		
		No Ch. T	No L	% L	No Ch. T	No L	% L	No Ch. T	No L	% L	No Ch. T	No L	% L	No Ch. T	No L	% L	No Ch. T	No L	% L
I S + 1.2 GA	1	274	9		240	8		140	10		226	8		208	2		323	1	
	2	633	19		443	20		306	24		405	20		550	7		479	3	
	3	250	7		-	-		255	24		-	-		177	1		181	1	
	Total	1157	35	3.0	683	28	4.1	701	58	8.3	631	28	4.4	935	10	1.1	983	5	0.5
II TdR + 1.2 GA	1	266	3		227	4		212	12		217	4		254	5		416	3	
	2	402	7		105	2		233	15		324	6		150	2		233	2	
	3	348	5		235	7		342	22		-	-		264	3		-	-	
	Total	1016	15	1.5	576	13	2.3	787	49	6.2	541	10	1.9	668	10	1.5	649	5	0.8
III BUdR + 1.2 GA	1	385	7		326	8		267	15		354	12		488	14		257	4	
	2	217	4		236	4		171	9		137	6		311	9		292	4	
	3	461	9		144	3		211	14		258	10		123	5		142	2	
	Total	1063	20	1.9	70.6	15	2.1	649	38	5.6	749	28	3.7	922	28	3.0	691	10	1.5
IV CdR + 1.2 GA	1	414	8		485	12		388	21		250	7		266	3		367	3	
	2	274	5		-	-		257	19		316	8		319	5		424	4	
	Total	688	13	1.9	485	12	2.3	645	40	6.2	566	15	2.7	585	8	1.4	791	7	0.9
V BCdR + 1.2 GA	1	325	3		463	15		358	23		264	10		181	7		238	2	
	2	326	6		209	6		279	19		209	6		309	8		223	2	
	3	292	4		-	-		324	20		248	9		253	5		189	2	
	Total	943	13	1.4	672	21	3.1	961	62	6.5	721	25	3.5	743	20	2.9	650	6	0.9

Signific. test	X^2_1 DF	P	X^2_1 DF	P	X^2_1 DF	P	X^2_1 DF	P	X^2_1 DF	P	X^2_1 DF	P
I vs II	5.10	<.05	2.64	>.10	2.03	>.10	5.98	<.02	0.27	>.20	0.12	>.70
I vs III	2.54	>.10	3.88	<.05	2.63	>.10	0.42	>.70	8.00	<.01	3.02	>.05
I vs IV	1.77	>.10	0.42	>.70	1.84	>.10	2.64	>.10	0.06	>.80	0.44	>.30
I vs V	5.59	<.02	1.32	>.20	1.74	>.10	0.83	>.83	5.32	<.05	0.48	>.30
II vs III	0.30	>.50	0.00	>.99	0.03	>.80	3.28	>.05	3.28	>.05	0.84	>.30
IV vs V	0.38	>.50	0.23	>.50	0.01	>.90	0.47	>.30	2.18	>.10	0.04	>.80

> = Not significant; < = significant; ≪ = highly significant.

Table 10. Effect of exogenous pyrimidine nucleosides and their brominated analogues on the radiation-induced lethal frequencies in the pre-injection DNA synthesis cells (brs A, B, C and D) and in the post-injection DNA synthesis cells (brs E and F) in the gonad of *D. melanogaster* males.

Germ line sector	Pre-injection DNA synthesis brs A, B, C and D				Post-injection DNA synthesis brs E and F			
Treatment	No Te	No L	% L	MF*	No Te	No L	% L	MF*
I S + 1.2 GA	3172	149	4.7		1922	15	0.8	
II TdR + 1.2 GA	2831	107	3.7	0.78	1317	15	1.1	1.38
III BUdR + 1.2 GA	3066	91	3.0	0.68	1575	38	2.4	3.00
IV CdR + 1.2 GA	2316	80	3.5	0.74	1363	15	1.1	1.38
V BCdR + 1.2 GA	3287	111	3.4	0.72	1393	26	1.8	2.25

Signif. test	χ^2_1 DF	P	χ^2_1 DF	P
I vs II	3.90	$<$.05	0.74	$>$.30
I vs III	12.14	$\ll$.001	14.38	$\ll$.001
I vs IV	4.86	$<$.05	0.58	$>$.30
I vs V	6.94	$<$.01	6.14	$<$.02
II vs III	1.98	$>$.10	5.78	$<$.02
IV vs V	0.00	$>$.99	1.86	$>$.10

*MF = Modification Factor (% lethals in nucleoside or analogue + 1.2 GA / % lethals in S + 1.2 GA)

$>$ = not significant; $<$ = significant; $\ll$ = highly significant.

Table 11. Effect of exogenous pyrimidine nucleosides and their brominated analogues on the radiation-induced translocation (II, III and / or Y chromosomes) and lethal frequencies, when the post-injection DNA synthesis cells (brs E and F) were irradiated in the spermatozoan stage in D. melanogaster (1-Day GE series).

Broods	E						F					
	Lethals			Trans.			Lethals			Trans.		
Treatment	No Te	No L	% L	No Te	No Tr	% Tr	No Te	No L	% L	No Te	No Tr	% Tr
I S + 1.2 GE	557	12	2.2	532	9	1.7	668	22	3.3	594	12	2.0
II TdR + 1.2 GE	560	15	2.7	514	9	1.8	516	13	2.5	531	15	2.8
III BUdR + 1.2 GE	569	14	2.5	607	9	1.5	507	14	2.8	511	15	3.0
IV CdR + 1.2 GE	547	11	2.0	521	8	1.5	721	15	2.1	508	10	2.0
V BCdR + 1.2 GE	520	27	5.2	509	13	2.6	495	27	5.5	492	18	3.7

Signific. test	χ^2_1	DF P	χ^2_1	DF P	χ^2_1	DF P	χ^2_1	DF P
I vs II	0.14	$>.70$	0.02	$>.98$	0.36	$>.50$	0.46	$>.30$
I vs III	0.02	$>.80$	0.00	$>.99$	0.12	$>.50$	0.62	$>.30$
I vs IV	0.00	$>.99$	0.00	$>.99$	1.53	$>.20$	0.02	$>.98$
I vs V	6.26	$<.02$	0.56	$>.30$	2.78	$>.05$	2.11	$>.10$
II vs III	0.00	$>.99$	0.01	$>.90$	0.00	$>.99$	0.00	$>.99$
IV vs V	6.96	$<.01$	0.88	$>.30$	9.03	$<.01$	2.04	$>.10$

$>$ = not significant; $<$ = significant; $\ll$ = highly significant.

Table 12. Effect of exogenous nucleosides and analogues on the radiation induced lethal frequency in the mature sperms of 1-day and 7-day old *D. melanogaster*.

Pre-treatment	1-Day old males 1.2 kR No Te	No L	% L	MF	7-Day old males 2.4 kR No Te	No L	% L	MF	7-Day old males 3.6 kR No Te	No L	% L	MF
I Saline	1157	35	3.0		733	55	7.5		934	100	9.3	
II TdR	1016	15	1.5	0.50	904	49	5.4	0.72	-	-	-	
III BUdR	1063	20	1.9	0.63	1161	83	7.2	0.96	-	-	-	
IV CdR	688	13	1.9	0.63	-	-	-		-	-	-	
V BCdR	943	13	1.4	0.46	773	46	6.0	0.80	-	-	-	
VI 4 Nuc*	524	6	1.2	0.40	939	29	3.1	0.41	962	58	6.0	0.64

Signi. Te.	X^2_1 DF	P	X^2_1 DF	P	X^2_1	P
I vs II	5.26	< .05	2.61	> .10	-	-
I vs III	2.54	> .10	0.04	> .80	-	-
I vs IV	1.77	> .10	-	-	-	-
I vs V	5.59	< .02	1.21	> .20	-	-
I vs VI	4.60	< .05	15.90	≪ .001	12.96	≪ .001

* 0.1% Equimolar solution of TdR, CdR, AdR and GdR in saline.

= not significant; = significant; = highly significant.