REPAIR REPLICATION AND PHOTOREPAIR OF DNA IN LARVAE OF DROSOPHILA MELANOGASTER

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ABSTRACT

Repair replication of DNA has been studied in first instar larvae of Drosophila melanogaster with isopycnic centrifugation techniques. Larvae were fed BUdR, FUdR, streptomycin, penicillin, and Fungazone for two to four hours prior to exposure to UV, X-rays, MMS, or EMS. Feeding was continued for four hours in the presence of 3HBUdR and DNA was isolated from whole larvae. Repair replication is stimulated by each of these agents. MMS is about 10 times as potent as EMS in stimulating repair synthesis. A dose of 200 ergs/mm² largely saturates the level of repair replication observed after UV irradiation. Repair replication rises between 0 and 80,000 R of X-rays before falling off. Semiconservative synthesis is seriously inhibited above a dose of 40,000 R of X-rays. Photorepair has been detected as a reduction in repair synthesis resulting from post-irradiation exposure to photoreactivating light. The same treatment has no detectable effect on X-ray-stimulated repair replication. Repair replication is insensitive to the presence of caffeine or hydroxyurea during the final incubation, although semiconservative synthesis is strongly inhibited by these agents. A mixture of BUdR and ³HTdR can be used to replace ³HBUdR in detecting repair replication.

Our understanding of the mechanisms of mutation, genetic recombination and DNA repair in microorganisms has advanced rapidly within the past few years primarily due to the development of methods for isolating mutants affecting these processes (Setlow and Setlow 1972). The relationship of these same processes in higher eukaryotes is less well understood as a result of a corresponding lack of appropriate mutants. The heritable human disorder xeroderma pigmentosum provides the only example of a mutation in higher eukaryotes that has been shown to affect a specific step in DNA repair (Cleaver 1968; Cleaver 1969b; Setlow et al. 1969; Cleaver and Trosko 1970; Weerd-Kastelein, Keijzer and Bootsma 1972). Drosophila offers unique genetic advantages for further pursuit of such studies. Mutants affecting recombination (Gowen and Gowen 1922; Sandler et al. 1968; Baker and Carpenter 1972), repair

Abbreviations:

FUdR 5-fluorodeoxyuridine
BUdR 5-bromodeoxyuridine
³HBUdR 5-bromodeoxyuridine-6-³H
³HTdR thymidine methyl-³H
SSC 0.15 M sodium chloride—0.015 M sodium citrate
Tris tris(hydroxymethyl) aminomethane
UV ultraviolet radiation—predominantly 254 nm
MMS methyl methanesulfonate
EMS ethyl methanesulfonate
(Ghiliovitch 1966; Watson 1972; Ogaki and Nakashima-Tanaka 1966; Smith 1972; Parsons and Macbean and Lee 1969; Westerman and Parsons 1972) and mutation (Green 1970) are currently available for biochemical studies. More importantly, the newer selection systems used to isolate some of these mutants have greatly extended the potential of Drosophila for studies in this area.

The approach to this problem reported here involves a demonstration of repair replication in Drosophila melanogaster with the technique of Hanawalt as modified for higher eukaryotes by Painter and Cleaver (1969). When the heavy thymine analog bromouracil is substituted for thymine during semiconservative DNA synthesis, the increased density of the daughter helices is sufficient to permit their separation from unreplicated DNA by isopycnic centrifugation. Repair processes that replace thymine with bromouracil in short random nucleotide sequences, however, do not increase the density of the DNA sufficiently to permit such a separation. This distinction provides a basis for measuring repair replication in the presence of simultaneous semiconservative synthesis. Repair replication is detected as DNA synthesis that gives rise to a normal density product in the presence of the heavy precursor. Identification of newly synthesized DNA is accomplished by using a precursor which carries a radioactive label as well as a density label (3HBUdR). This technique has permitted the detection of photorepair and repair replication in Drosophila and has provided a system for exploiting the genetics of the organism in studies of DNA repair. An abstract of this work has been published (Boyd and Presley 1972).

MATERIALS AND METHODS

Egg Isolation: Eggs of Drosophila melanogaster (Oregon R) were collected during a 16 hr period from large population cages as described by Mitchell and Mitchell (1964). The egg suspension was filtered through a 400 micron nylon screen and collected on a 180 micron screen (TET-Kressilk, Los Angeles). Dechorionation with 2.6% sodium hypochlorite for 2 min was followed by washing with 70% ethanol. The eggs were then incubated in flasks containing 1.5% agar and 1% sucrose at 28° for 24 hr.

Larval Isolation: Live larvae were obtained free of eggs and other debris by forcing them to crawl up through several layers of nylon screen. The larvae and eggs were first collected on 53 micron screens and then covered with one 180 micron screen and two 250 micron screens. All screens were kept water-saturated. The 250 micron screens were periodically freed of larvae by swirling in water. Pure larvae were subsequently collected on 53 micron screens washed with 70% ethanol and weighed. Larval mortality was monitored by counting the percentage of larvae that moved in dilute acetic acid.

Larval incubation: A maximum of 0.3 g of larvae per shell vial was incubated under humid dark conditions. The first incubation was carried out for 3.5 hrs at 28° in vials containing 80 mg of Type 19 cellulose (Sigma, St. Louis) and 175 μl of Solution A: 5 × 10^{-4} M FUdR (gift from W. E. Scott of Hoffmann-La Roche), 0.1 mg/ml streptomycin (Squibb), 0.03 mg/ml penicillin (Squibb), 1.25 μg/ml Fungazone (Grand Island) and 200 μg/ml BUdR (Sigma). The larvae were then isolated under red light on nylon screens and either fed MMS for one hour in Solution A or irradiated on damp filter paper. The final incubation of 4 hrs at 28° was carried out with 40 mg of cellulose, 10 μCi 3HBUdR (New England Nuclear, 26 Ci/mM), and 88 μl of Solution B (identical to Solution A with a BUdR concentration of 150 μg/ml). Larvae were isolated, monitored for mortality and frozen at -80°. Very little mortality was encountered during the final incubation with 3HBUdR. In all reported experiments mortality never exceeded 33% for the duration of the experiment. Feeding had no adverse effect on the survival of unirradiated larvae. About 80% of fed or unfed larvae were capable of developing to adult flies.
Variations in the duration of the first incubation, which are reported for some of the experiments, had little effect provided BUdR was present for at least two hours. Concentrations of $5 \times 10^{-4}$ to $2 \times 10^{-3}$ M FUdR gave equivalent results as did 150–300 $\mu$g/ml BUdR in the first incubation. A minimum of 150 $\mu$g/ml BUdR was required in the second incubation for good analog substitution. In addition to reducing problems of contamination, the antibiotics and Fungazone contributed to a better substitution of thymine by BU. This effect is presumably due to the Fungazone, which is known to stimulate an increased transport of nucleosides across cell membranes (RAIKOW and Fristrom 1971).

**DNA Isolation:** Up to 0.3 g of frozen larvae were ground with pre-crushed glass beads in a mortar and pestle cooled in dry ice. The cold powder was added to 4 ml of MUP solution containing 25 $\mu$l of diethyloxydiformate (Eastman, Rochester). MUP solution contains: 8.0 M urea (reagent grade, purified), 0.24 M Tris, 0.01 M EDTA, 1.0 M NaClO, and 1% SDS at pH 7.8 (R. Shleser, personal communication). The urea was purified by treating a 10 M solution with Norite A at 60° for 5 min. Norite was subsequently removed by filtration.

The tissue suspension was shaken 10 min. at room temperature. Four ml of CHCl₃: 1-octanol (24:1) were added and shaking was continued for 15 min. The aqueous phase recovered after centrifugation at 27,000 × g was mixed with two volumes of 95% ethanol and spun at 16,000 × g for 10 min. The precipitate was washed twice with 70% ethanol, dissolved in 0.1 × SSC, and incubated for 1 hr at 37° in 1 × SSC containing 130 $\mu$g RNase/ml (Worthington-RAF). The RNase had been preheated at 90° for 5 min in SSC adjusted to pH 5.0. The sample was then shaken with an equal volume of SSC-saturated phenol containing 0.1% 8-hydroxyquinoline, precipitated with ethanol, and redissolved in SSC. The resulting nucleic acid preparations contained approximately 70% DNA as determined by absorbance measurements and diphenylamine reactions (Burton 1956). Absorbance ratios were typically: $230/260 = 0.40; 260/280 = 1.92$.

**Isopycnic centrifugation** was performed according to the procedure of Cleaver (1970a). 5.80 g of CsCl (American Potash and Chemical Co.) were added to samples of 10 to 100 $\mu$g DNA in 4.5 ml SSC. Radioactivity was measured in 0.2 ml aliquots of each 0.25 ml fraction according to the procedure of Bollum (1966).

The relative amount of DNA in each sample was determined by measuring the area under the optical density peaks. Most optical density curves fell wholly within the linear range of the ISCO UA-2 analyzer (0–100 $\mu$g DNA/gradient). Repair and semiconservative replication were estimated from the areas under the radioactive profiles. The dividing line between the two forms of synthesis was taken as the low point between the two peaks of radioactivity. In the control gradients this separation was made at the same density as that used in the treated samples. The values were normalized for each sample to the average amount of DNA used in that particular experiment. Normalized values of different experiments, therefore, cannot be compared.

**Irradiation:** X-rays were produced from a Machlett OEG-60 tube at 50 KV and at a dose of 160 R/s (Snow and Korch 1970). Ultraviolet radiation was obtained from two 15 watt General Electric germicidal lamps which produced 12.7 ergs/mm²/sec. Calibration was performed with an IL200 UV-visible photometer (International Light, Newburyport, Mass).

**Contamination:** Parallel experiments performed under completely sterile conditions produced gradient profiles similar to those reported here. The observed repair replication, therefore, does not arise from microbial contamination.

**RESULTS**

Repair replication was detected in Drosophila larvae with the procedure described by Cleaver (1970a) for studying repair in mammalian tissue culture. Newly hatched larvae were fed unlabelled BUdR, irradiated or treated with MMS, and then incubated with labelled BUdR. Typical profiles obtained by isopycnic centrifugation of larval DNA are presented in Figure 1. The position of the total DNA in the gradients is indicated by optical density measurements. DNA synthesized during the last incubation is labelled with tritium. The newly synthesized DNA in the unirradiated control sample has a peak density of 1.748
Figure 1.—Isopycnic centrifugation of larval DNA. Purified DNA was centrifuged to equilibrium at 25° in a 40 rotor of the Beckman L2-65B. The UV and control patterns were obtained under standard conditions with the treated larvae receiving 95 ergs/mm². The X-ray treated larvae were incubated for 2 hr with Solution A containing no BUdR and then for 2 hr with Solution A prior to receiving a dose of 20,000 R. The MMS treated sample was incubated with Solution A for 2.5 hr and Solution A containing 0.02 M MMS for 1 hr prior to incubation with 3HBUdR. The density profile is typical of all gradients. Absorbance (-----), Radioactivity (●—●), Density (—–—–).

assuming a density of 1.699 (Travaglini, Petrovic and Schultz 1972) for normal density DNA. This density shift corresponds to a 48% substitution of thymidine by BUdR (Simon 1961). The width of the radioactive peak in the unirradiated control reflects actual density heterogeneity of the DNA. This conclusion is drawn from data obtained with parallel alkaline cesium gradients as well as from experiments in which the normal-density DNA from such a gradient was reband in a second gradient. Neither of these approaches eliminated the label from the normal-density region of the gradient.

Repair replication, which is detected by the presence of a second peak of radioactivity in DNA of normal density, is observed after the three indicated treatments (Figure 1). About ten times the given MMS concentration is required to produce equivalent results using EMS. Each of these agents simultaneously reduces the level of semiconservative DNA synthesis to varying degrees. Although repair replication has been detected with MMS concentrations ranging
from 0.005 M to 0.02 M, survival is reduced by the higher concentrations. For the duration of the experiment mortality is unaffected by either form of radiation.

Dosage and Kinetic Studies

Dosage studies were performed to permit optimum detection of repair replication (Figure 2). Estimates of the relative amounts of semiconservative and repair replication were obtained by measuring the areas under the peaks of radioactive profiles. Such estimates are only qualitative due to the incomplete separation of the two peaks, and the radiation-induced reduction of thymidine substitution by BUdR (Figure 1). Repair replication following UV treatment is seen to rise rapidly to a dose of about 200 ergs/mm². A more gradual increase is observed above this dose. Semiconservative synthesis is inhibited by UV radiation at all doses used, although these data are less reproducible than those obtained for repair replication.

In contrast to repair replication induced by UV treatment, repair observed after X-ray administration continues to rise beyond doses which have badly

![Figure 2.-DNA synthesis as a function of radiation dose. Incorporation is presented as the normalized areas under the peaks of the radioactive profiles (see MATERIALS AND METHODS for details). The data for UV-induced repair replication represent the averages of duplicate experiments that were normalized to comparable incorporation levels. The variation between the duplicate values is indicated for each average. In one of the two experiments, incubation was carried out for 1 hr with Solution A lacking BUdR and then for 2 hrs with Solution A prior to irradiation. The points for zero dose represent that portion of the unirradiated control peak which has a normal density. The X-ray dose curve was obtained under conditions described for the X-ray treated sample in Figure 1.](image-url)
disrupted semiconservative synthesis. The final drop in repair at the extreme dose is attributable to a 50% mortality in that sample during the final incubation. By contrast, the mortality at 60,000 R and 80,000 R was 10% during the same period. The dip encountered in the repair curve at 60K is probably due to a decrease of the incorporation in the repair peak that is contributed by semiconservative synthesis at the lower doses.

Kinetic studies presented in Figure 3 provide further criteria for optimizing the detection of repair replication. Since the concentration of BUdR in the precursor pool must vary during the final incubation, these data do not provide an absolute measure of synthetic rates. They do, however, indicate the relative rates of the two forms of synthesis. A four-hour final incubation is optimum for the detection of repair replication after treatment with either form of radiation. The increase in repair replication induced by both agents begins to level off at this time.

Inhibitors

The presence of 0.01 M hydroxyurea during the final incubation inhibits up to 85% of the semiconservative DNA synthesis but has very little effect on UV-induced repair replication (unpublished data). This selective effect on semiconservative replication makes it possible to detect UV-induced repair replication at levels well below the half-lethal dose of about 150 ergs/mm² (Donini, personal communication) (Figure 4). In the experiment presented in Figure 5 caffeine is shown also to inhibit semiconservative replication by over 50% without significantly affecting the level of repair replication. This observation supports those of Troshko and Wilder (1973) on excision repair in Drosophila as well as related studies of excision repair (Regan, Troshko and Carrier 1968), and unscheduled DNA synthesis (Cleaver 1969a) in mammalian cells.

![Figure 3](image-url)

**Figure 3.**—Kinetics of DNA synthesis following irradiation. Larvae received either 380 ergs/mm² of UV radiation or 40,000 R of X-ray treatment. The time of the final incubation was varied as indicated.
Figure 4.—Detection of low levels of DNA repair replication with hydroxyurea. Two larval samples were incubated under standard conditions with 0.01 M hydroxyurea present in the final incubation. One sample (■—■) received 30 ergs/mm² and the control (○—○) was not irradiated. DNA recovered from the two larval samples was subjected to isopycnic centrifugation on separate gradients. The absorbance (—–) profile was taken from the control gradient.

Figure 5.—The effect of caffeine on DNA synthesis in UV-treated larvae. Two larval samples were irradiated with 380 ergs/mm². The treated sample was exposed to 0.02 M caffeine during the final incubation.

**Photorepair**

Exposure of larvae to visible light immediately after UV irradiation reduces the amount of repair replication that is subsequently detected (Figure 6). Quantitative variations in semiconservative synthesis, such as those seen in Figures 4
Larvae were incubated 2 hr with Solution A lacking BUdR and then 2 hr with Solution A prior to treatment with 950 ergs/mm². One half of the sample was held in the dark for 50 min on damp filter paper while the other half was exposed to two 15W cool white fluorescent tubes at a distance of 32 cm through a plastic petri dish cover. The plastic did not transmit wave lengths below 290 nm. The final four hour incubation was standard. DNA isolated from the larvae was subjected to isopycnic centrifugation.

and 6, are within the normal experimental fluctuations encountered between samples. The result with visible light implies that a photorepair process has reduced the number of UV lesions that stimulate repair replication and is consistent with the observation of Krishnan and Painter (1973) that both photoreactivation and excision repair mechanisms respond to pyrimidine dimers in another eukaryote. In our experiment (950 ergs/mm²) a 50% reduction in repair replication was observed after 50 minutes of fluorescent light exposure, and a significant effect has been observed by 20 minutes. A 10 min light exposure following a UV dose of 380 ergs/mm² reduced the observed repair replication by half. The fact that the survival of UV-treated larvae is increased by subsequent exposure to visible light (unpublished data) suggests that the observed photorepair is of biological significance. No significant effect of visible light has been observed on X-ray treated larvae.

Substitution of ³HTdR for ³HBUDR

Density gradient patterns obtained after substituting ³HTdR for ³HBUDR in the final incubation (Figure 7) are very similar to patterns obtained with the standard incubation conditions in a parallel sample (not shown). In this experiment the ratio of repair to semiconservative replication was 0.41 when ³HBUDR was employed and 0.36 with ³HTdR. There is, therefore, little if any significant difference between the two precursors with respect to their relative utilization for repair and semiconservative synthesis. However, both forms of synthesis have about an 8-fold preference for ³HTdR over ³HBUDR. This conclusion was obtained by comparing the specific activities of DNA preparations obtained from
Figure 7.—Detection of DNA repair replication with a mixture of $^3$HTdR and BUdR. Larvae were incubated 1 hr with Solution A lacking BUdR and then 2 hr with Solution A. The UV-treated sample received 760 ergs/mm$^2$. In the final incubation $^3$HBUdR was replaced with 2μCi of $^3$HTdR (18 Ci/mM, New England Nuclear) and the BUdR concentration was maintained at 150 mg/ml. In the parallel experiments in which $^3$HBUdR was used (not shown) the curves had almost identical profiles to those shown here. In these two experiments the specific activities of the precursors in Ci/mM of total nucleoside were 0.117 and 0.023 for $^3$HBUdR and $^3$HTdR respectively. In the latter experiment the molar ratio of TdR to BUdR was 1/840. The specific activities of the isolated DNA samples in cpm/μg nucleic acid were: $^3$HTdR control 19.9, $^3$HTdR UV 27.5, $^3$HBUdR control 13.1, and $^3$HBUdR UV 16.7. The ratio of BUdR to TdR incorporation was, therefore, 0.658 in the control samples and 0.607 in the UV-treated samples. Since random incorporation of the two precursors predicts a ratio of 5.09, $^3$HTdR is preferred over $^3$HBUdR by a factor of 7.7 in the control samples and 8.4 in the UV-treated sample. These ratios are subject to fluctuations in feeding and small variations in the RNA contamination of the DNA sample.

Larvae fed BUdR and $^3$HTdR or $^3$HBUdR. The specific activity of DNA obtained from larvae fed BUdR + $^3$HTdR was 8 times that expected from a random utilization of the two precursors. This result parallels observations made in Tetrahymena (Brunk and Hanawalt 1969), but stands in contrast to others made in mammalian systems in which no preference for one substrate over the other has been observed (Cleaver 1970b).

DISCUSSION

To exploit the genetic advantages of Drosophila for studies of excision repair in higher eukaryotes, it was first necessary to establish that this organism exhibits the expected features of this form of repair. Toward this end Valencia and Plaut (1969) have documented the existence of unscheduled DNA synthesis in the salivary glands of irradiated larvae; Troshko and Wilder (1973) have shown that thymine-containing dimers are excised from the DNA of UV-treated tissue culture cells; and in this report we have demonstrated that whole larvae are capable of repair replication after treatment with radiation or alkylating agents. Each of these criteria provides an independent test for excision repair.
Their agreement clearly establishes the existence of this phenomena in Drosophila.

One of the motivations for developing this method was to provide a relatively simple system for monitoring repair replication in potentially affected mutants. Such tests are performed most efficiently on whole animals, although their use seriously limits the quantitative features of the assay. A portion of the synthesis which we measure as repair may be due to a radiation-induced shift of semiconservative synthesis to a lighter density. In addition, feeding problems introduce further quantitative variability into the method. In spite of these quantitative limitations, repair replication is clearly detected under these conditions by the induction of a second radioactive peak by irradiation. More quantitative work in this area will be possible with tissue cultures derived from Drosophila.

Detection of repair replication should prove particularly valuable for monitoring available mutants, because it can be applied to whole animals. Starved first instar larvae offer three advantages over other stages of development for such work: (1) They are free of gross contamination, because they can be maintained on sucrose after hatching. (2) They represent the smallest feeding stage of the organism and, therefore, permit maximum radiation penetration. In spite of their small diameter (approximately 150 microns [DOANE 1967]), UV radiation probably does not penetrate to the center of a larva. KALTHOFF (1971b) has shown that the intensity of UV light is approximately halved after passage through a 4-micron layer of the crushed eggs of another insect. If this same magnitude of absorption were found in Drosophila larvae, tissues in central or ventral positions of the larvae would not receive substantial UV radiation. (3) Because the larvae are not growing, they synthesize very little DNA. The optical density profiles indicate that less than 1% of the total larval DNA is replicated during the course of an experiment. This situation prevents semiconservative DNA synthesis from swamping low levels of repair replication in the CsCl density gradients. Under the conditions reported here, repair replication is undetectable at other stages of development due to our inability to separate it from the predominant semiconservative synthesis.

The use of whole young larvae also entails some disadvantages. Simultaneous analysis of numerous tissue types, which undoubtedly possess different precursor pools, probably accounts for the heterogeneous substitution of BUdR for thymidine in the DNA. Nevertheless, the homogeneity of the substitution reported here is higher than that reported by RIZKI, DOUTHIT and RIZKI (1972). This difference is due in part to the fact that those investigators used FU instead of the more effective FUdR used in our experiments to inhibit endogenous production of thymidine monophosphate (unpublished observation). Analysis of certain mutant stocks would be facilitated by a capability for separating larvae by sex or phenotype. Since this is difficult with small larvae, methods are being developed for detecting repair replication in 3rd instar larvae.

Dosage studies (Figure 2) have permitted the determination of optimum doses for the detection of repair replication. A dose of about 200 ergs/mm² of UV radiation virtually saturates repair replication. This is close to the dose of 100–200 ergs/mm² which saturates unscheduled DNA synthesis in Chinese hamster cells.
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(CLEAVER 1970b). Since the LD₅₀ of young larvae is about 150 ergs/mm², the stimulation of repair replication by doses as low as 30 ergs/mm² of UV radiation (Figure 4) strengthens the argument that this form of synthesis is biologically meaningful. At 20,000 to 40,000 R of X-radiation the cellular machinery responsible for both repair and semiconservative replication appears to be intact. Although the LD₅₀ for first instar larvae is 1000-2000 R (EICHE 1970), this system may still provide meaningful information on the X-ray repair capacity of various Drosophila strains as it has in mammalian cell lines (PAINTER and YOUNG 1972).

The UV dose curve (Figure 2) indicates that the excision repair mechanism being monitored in these experiments is probably saturated above a dose of about 200 ergs/mm². Below this point the level of detected repair is presumably a reflection of the amount of repairable damage the DNA has suffered. Hence, within a restricted range the level of repair replication can be taken as a rough estimate of the “substrate” available to the excision repair mechanism. This situation has permitted us to detect photorepair in Drosophila larvae. A lower level of repair is observed when UV-treated larvae are exposed to fluorescent light than when they are held in the dark prior to incubation with radioactive substrate (Figure 6). This observation suggests that the fluorescent light removed some of the UV damage that would otherwise have been acted on by the excision repair system. This conclusion is further substantiated by the observation (unpublished) that exposure to fluorescent light increases the survival of previously UV-irradiated larvae. Our detection of photorepair in larvae complements earlier reports of photoreactivation of UV-induced mutations or inactivation in Drosophila (MEYER 1951; BROWNING and ALTENBURG 1962; GHLELOVITCH 1966). TROSKO and WILDER (1973) have more recently reported that pyrimidine dimers disappear from the DNA of Drosophila tissue culture cells as the result of photoreactivating illumination. Their results together with our observation that X-ray damage is not affected by visible light leads to the conclusion that the observed repair replication is stimulated primarily by pyrimidine dimers.

Several aspects of this study reveal a close parallel between repair replication in Drosophila and repair observed in mammalian tissue culture cells. In mammalian cells:

a. Repair replication is stimulated by UV (PAINTER and CLEAVER 1969), X-ray (PAINTER and YOUNG 1972), and the alkylating agents MMS and EMS (ROBERTS et al. 1971).

b. Repair replication is insensitive to moderate doses of caffeine and hydroxyurea, although a semiconservative synthesis is strongly inhibited by both (CLEAVER 1969a).

c. Repair replication is more readily observed after exposure to biologically reasonable UV doses than it is after moderate X-ray doses (PAINTER and YOUNG 1972).

d. Repair replication is saturated at moderate UV doses but continues to increase at extreme X-ray doses (CLEAVER 1970b; PAINTER and YOUNG 1972).

e. MMS is a much more potent inducer of repair replication than is EMS (ROBERTS et al. 1971). The close parallel between these observations and the re-
results reported here indicate that Drosophila will provide a valid system for studying general features of eukaryotic repair and its relationship to mutation and genetic recombination.

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LITERATURE CITED


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