Repair of Ultraviolet and Gamma-Ray Induced Lethal Damage in an Insect Tissue Culture Cell Line

Craig Endres  
*John Brown University*

Susan Kulp  
*John Brown University*

H. Gaston Griggs  
*John Brown University*

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REPAIR OF ULTRAVIOLET AND GAMMA-RAY INDUCED LETHAL DAMAGE IN AN INSECT TISSUE CULTURE CELL LINE

We have recently performed a series of preliminary radiation experiments which indicate that the IPL-22 insect tissue culture cell line constitutes another fruitful system for study of the roles played by intracellular repair mechanisms in the radiation resistance of eukaryotic cells. The effects of repair processes on the kinetics of ultraviolet (UV) and gamma-ray induced cell killing (loss of colony forming ability) are briefly described here.

The IPL-22 line was cloned from the IPL-21 insect line (Spodoptera frugiperda), which was obtained from Dr. Troy Orr of the Southwest Foundation for Research and Education in San Antonio, Texas. The line was routinely maintained in IPL-41 medium (Kansas City Biological) in plastic tissue culture flasks (Falcon). Log phase monolayer cultures with plating efficiencies near 0.70 were selected for each experiment. All experimentation was carried out at 26 degrees Celsius. Gamma-ray was administered with a custom designed Mark IV Cesium 137 irradiator at a dose rate of 40 rads/minute. Techniques employed for UV irradiations, photoreactivation (PR), cell fusions, caffeine treatments, single cell plating of treated cells, incubations, and survival determination (assays for colony forming ability) were essentially the same as those described previously for Xenopus cells (Griggs and Bender, 1973; Griggs and Orr, 1979; Haetten, McGuinness and Griggs, 1982).

The UV LD₅₀ (lethal dose to 50 percent of the cells) for IPL-22 cells can be estimated from the UV-alone data of Figure 1 to be near 200 ergs/mm², indicating a significantly higher resistance to the lethal effects of UV than that observed for established vertebrate tissue culture lines such as the AR Xenopus line (LD₅₀ near 60 ergs/mm²) and the V79 hamster line (LD₅₀ near 75 ergs/mm²) (Griggs and Bender, 1972). The UV-alone data (Figure 1) constitutes a sigmoid or threshold curve, suggesting a multihit single target, multithread single hit, or multithread multithread relation (Elkind and Whitemore, 1967). However, as indicated by the UV + caffeine data of Figure 1, caffeine significantly alters the UV curve by reducing the shoulder or threshold segment. These data suggest that the threshold results, at least in part, from the operation of a caffeine sensitive intracellular repair mechanism, perhaps similar to the caffeine sensitive recombination like repair mechanism observed in V79 hamster cells (Cleaver, 1974; Haetten et al., 1982).

IPL-22 cells photoreactivate a small fraction of the lethal damage induced by UV doses in the range 0-400 ergs/mm² (Figure 2). Direct enzymatic repair is indicated, since the reactivation light effectively diminishes the UV dose (Rupert and Harm, 1966). It is interesting that IPL-22 cells do not appear to possess an efficient PR mechanism, as do many microorganisms (Rupert and Harm, 1966) and some vertebrate cells (Griggs and Bender, 1972).

The gamma-ray survival curve for IPL-22 cells also indicates a threshold response with an L.D₅₀ near 1000 rads (gamma-ray alone points, Figure 3). This is a rather marked resistance to the lethal effects of gamma-ray as compared to the resistance shown by established mammalian cell lines (Elkind and Whitemore, 1967). The observed increase in resistance to a given dose when the dose is fractionated (Table 1) suggests that gamma-ray resistance is due in part to the operation of a dark repair mechanism, perhaps similar (or identical) to "Elkind recovery" (Elkind and Sutton, 1960).

Two experiments were carried out to explore overlap of UV and gamma-ray induced lethal lesions. As indicated by the UV + gamma data of Figure 3, UV exposures in the 0-40 ergs/mm² range actually reactivated some of the lethal damage induced by 500 rads of gamma-ray. This UV reactivation (UVR) appears to be similar to that observed in Xenopus cells (Cross and Griggs, 1978). Higher doses of UV may have an additive, or perhaps synergistic, effect with gamma-ray. The data of Table 2 are a result of an attempt at what could be termed "fusion reactivation" (FR). The synkaryons, produced by fusion of UV-irradiated parental cultures with gamma-irradiated parental cultures, exhibited a higher level of survival than the parental cultures (Experiment 3, Table 2). This "Reactivation" may result from a type of genomic complementation in which each viable synkaryon contains at least one undamaged copy of the essential genetic units. However, further investigation of the growth characteristics of viable synkaryons may indicate a more complex mechanism, perhaps involving some type of enzymatic repair.

The data described here indicate that a significant portion of the radiation resistance exhibited by IPL-22 insect cells is due to the functioning of dark (non PR) radiation repair mechanisms. These dark mechanisms appear to function more efficiently than similar repair mechanisms...
Figure 1. Survival of IPL-22 cells which were exposed to UV and then incubated in IPL-41 medium (circles) or IPL-41 medium containing 0.0008 moles/liter caffeine (triangles).

Figure 2. Survival of IPL-22 cells following UV (circles) and UV + PR light (3 x 10^4 ergs/mm^2) exposures.

Figure 3. Survival of IPL-22 cells following gamma-ray (circles) and gamma-ray + UV (triangles) exposures.

Table 1. Survival of IPL-22 cells following a gamma-ray exposure of 600 rads, administered in two fractions as indicated.

<table>
<thead>
<tr>
<th>First fraction (rads)</th>
<th>Second fraction (rads)</th>
<th>Interfraction time (hours)</th>
<th>Surviving fraction</th>
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<td>0.65</td>
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<td>0.76</td>
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<td>0.70</td>
</tr>
</tbody>
</table>

Table 2. Survival of IPL-22 cells which were exposed to UV or gamma-ray compared with survival of hybrid cells which were produced by fusion of UV exposed cells with gamma-ray exposed cells.

possessed by many vertebrate cells. To explore further relations between insect and vertebrate radiation repair mechanisms, we plan to study the extent and nature of interactions of insect and mammalian repair mechanisms, using mammalian-IPL-22 hybrid cell lines.

LITERATURE CITED


A PRELIMINARY REPORT ON THE ZYGOPtera (DAMSELFIES) OF ARKANSAS

Adams (1900) published the first list of Arkansas Odonata, reporting seven damselfly species. Subsequent papers (Needham and Heywood, 1929; Bick, 1939; and Houston, 1970) increased the species list to 18. Bick (1978) was apparently unaware of Houston's (1970) paper and restated these species as new records. This study provides a list for Arkansas damselfly species, their known flight seasons, and their distributions by county. Adults of most species occurring or possibly occurring in Arkansas can be identified using the keys of Johnson (1972). The exceptions, several Lestes species and Enallagma aspersum, are included in Walker's (1953) keys.

The data presented are a compilation of the contributions of all sources listed in the Acknowledgements, pertinent published records, and materials collected by myself. The museum collections at the University of Arkansas-Fayetteville and Little Rock were visited.

Treatment of captured specimens was as follows. While still alive, specimens were placed in paper triangles with wings in the normal resting position and heads rotated 90° to the left. The triangles were placed in science-grade acetone for a period of 18-24 hours. These may remain in acetone for up to five days with no detrimental effects. Next, each specimen was removed from the acetone, dried, identified, and transferred to a clear cellophane envelope with a 3 X 5 inch data card. These curatorial methods are advantageous in that they better preserve many colors than does air drying, storage space is minimized, and, since each envelope contains but one specimen, association of parts after breakup is facilitated.

Thirty-three damselfly species are currently recorded for Arkansas. The flight season for most of these extends from spring through summer months, and for many species persists into mid-autumn (Table 1). Temperature appears to be a major factor controlling their emergence. Some species vary from this generalization, however. Enallagma divagans seems to be a spring to early summer species, as was noted by Johnson (1972). Hetaerina titta in Arkansas appears in midsummer and flies through early autumn. Johnson (1972) stated that it is characteristically a spring form in central Texas. Species of Lestidae are highly adapted for life in temporary waters, and many have a diapause in the egg (Corbet, 1962). This imposes a characteristic massed, synchronized emergence on the species, followed by a relatively short flight period. Lestes disjunctus australis has an extended flight season in Arkansas, but L. inequilis and L. rectangulata are spring fliers, while Archilestes grandi adults are present during the late summer and autumn (Table 1).

Twenty-four Arkansas damselfly species (73%) are of the Eastern United States or Eastern U.S.-Tropics fauna, and seven species (21%) are Transcontinental or Transcontinental-Tropic in distribution. They are therefore likely to be found in any Arkansas county, provided suitable habitat is present. Ischnura posita and Anomalagrion hastatum are the most common forms, having been recorded in 69 and 54 counties, respectively (Table 2). These two species can be found in association with a variety of aquatic ecosystems, and they apparently have a wide range of tolerance for several environmental parameters.

Ischnura ramburii and I. verticalis are two species of the Eastern U.S. which reach a geographic limit in Arkansas. I. ramburii has been reported from Louisiana, Mississippi, Texas, and Oklahoma, but not from Kansas, Missouri, or Kentucky (Bick, 1957; Bick and Bick, 1957; Montgomery, 1967; Johnson, 1972; Huggins et al., 1976; Lago and Stanford, 1979). Its Arkansas distribution reflects that situation in that 19 of the 21 counties from which it is recorded are in the southern half of the state. Seemingly disjunct populations in Craighead and Washington Counties are the exceptions. I. verticalis has been reported from north of a line connecting Lake Texoma (Oklahoma-Texas state line) with extreme northeast Tennessee (Bick, 1957; Bick and Bick, 1957; Montgomery, 1967; Johnson, 1972; Huggins et al., 1976; Lago and Stanford, 1979; Johnson and Conley, 1980). Its Arkansas distribution concurs with those data, as the 10 counties listed are northerncentral and northwestern.

Argia plano apparently has a limited Central U.S. distribution, as it has only been reported from Texas, Oklahoma, and Kansas (Bick, 1957; Montgomery, 1967; Johnson, 1972; Huggins et al., 1976). Ten of the 11 Arkansas county records for this species are in the northern quarter of the state. Four of the six county collections made by me were from springs or spring-fed streams. I have not been able to determine the specific habitat for the remaining five county collections. Huggins et al. (1976) listed four of their seven county collections for this species in Kansas as being from springs.

Argia immunda has been reported from only Mexico, Texas, and Oklahoma (Bick and Bick, 1957; Johnson, 1972). Bick and Bick (1957) reported this species to be frequent and locally abundant in southern Oklahoma, but absent in the northern part of the state. The Washington County, Arkansas, record is the most northern and eastern location for A. immunda.

Johnson and Westfall (1970) have remarked that Ischnura kellicotti is one of the few temperate latitude odonates to have developed an apparently obligatory relationship with specific plants. The plant in this case is a water lily, Nuphar (spatter dock). My first collection of this species was on 27 July 1982 from Berg Lake at the western city limit of Camden, Ouachita County, Arkansas. This population was associated with Nymphapha odorata All., the sweet-scented water lily, and is the first record of this particular association. A subsequent collection of I. kellicotti was made on 6 September 1982 from a pond on the S side of U.S. Hwy. 270, 1 mi E of Poyen and immediately W of Frances Creek, in Grant County, Arkansas. This pond contained Nuphar.

A perusal of the damselfly species lists for neighboring states (Bick, 1957; Bick and Bick, 1957; Macklin and Cook, 1967; Montgomery, 1967; Johnson, 1972; Huggins et al., 1976; Lago and Stanford, 1979) reveals that at least eight additional species may be found in Arkansas. Those species include Calopteryx dimidiata (Burmeister), Lestes congener Hagen, L. dryas Kirby, L. forcipatus Rambur, Amphiagrion australianum (Burmeister), Chromagrion constrictorum (Hagen), Enallagma duxium Root, and Nehalemia integricollis Calvert.