Journal of the Arkansas Academy of Science

Volume 37 Article 29

1983

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

General Notes

LITERATURE CITED

ARAGÓN, F., R. BOLAÑOS, and O. VARGAS. 1977. Carbohidrates del veneno de *Bothrops asper* de Costa Rica. Estudio cuantitativo. Rev. Biol. Trop., 25(2):171-178.

BASU, A. S., R. PARKER and R. O'CONNER. 1970. Disc electrophoresis of glycoproteins in snake venoms. Toxicon 8:279-284.

HATTON, M. W. C. 1973. Studies on the coagulant enzyme from *Agkistrodon rhodostoma* venom. Isolation and some properties of the enzyme. Biochem. J. 131:799-807.

MARLAS, C. 1982. Purification and preliminary structure of a potent platelet aggregating glycoprotein isolated from the venom of *Crotalus durissus cascavella*. Toxicon 20:289-290.

MAWHINNEY, T. P., M. S. FEATHER, G. J. BARBERO, and J. R. MARTINEZ. 1980. The rapid quantitative determination of neutral sugars (as aldononitrile acetates) and amino sugars (as O-methyloxime acetates) in glycoproteins by gas-liquid chromatography. Anal. Biochem. 101:112-117.

OSHIMA, G., and S. IWANAGA. 1969. Occurrence of glycoproteins in various snake venoms. Toxicon 7:235-238.

RUFF, J. D., B. D. JOHNSON, and D. H. SIFFORD. 1980. A glycoprotein proteinase in *Agkistrodon bilineatus* venom. Proc. Ark. Acad. Sci. 34:130-131.

VARMA, R., R. S. VARMA, W. S. ALLEN, and A. H. WARDI. 1973. Gas chromatographic determination of neutral sugars from glycoproteins and acid mucopolysaccharides as aldononitrile acetates. J. Chromatogr. 86:205-210.

AHMED EL-SHERIF, PAUL D. GWINUP, BOB D. JOHNSON, and DEWEY H. SIFFORD, Departments of Chemistry and Biological Sciences, Arkansas State University, State University, AR 72467.

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 LD_{10} (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/mm^2, indicating a significantly higher resistance to the lethal effects of UV than that observed for established vertebrate tissue culture lines such as the A8 *Xenopus* line (LD_{10} near 60 ergs/mm^2) and the V79 hamster line (LD_{10} near 75 ergs/mm^2) (Griggs and Bender, 1972). The UV-alone data (Figure 1) constitutes a sigmoid or threshold curve, suggesting a multihit single target, multitarget single hit, or multitarget multihit relation (Elkind and Whitmore, 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/mm^2 (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 LD_{10} 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 Whitmore, 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/mm^2 range actually reactivate 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 have an additive, or perhaps synergestic, effect with gamma-ray. The data of Table 2 are results 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 either of 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 part 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

*Research supported by PHS Grant number CA-18809-07 awarded by NCI.

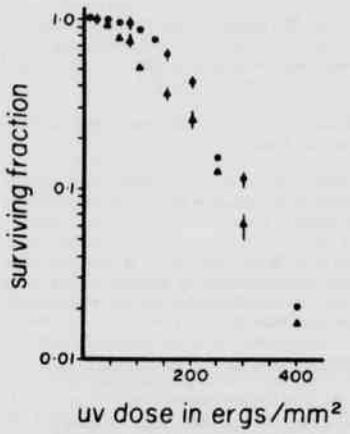

Figure 1. Survival of IPL-22 cells which were exposed to UV and then incubated in IPL-41 medium (circles) or IPL-41 medium containing 0.0008 moles/liter caffeine (triangles).

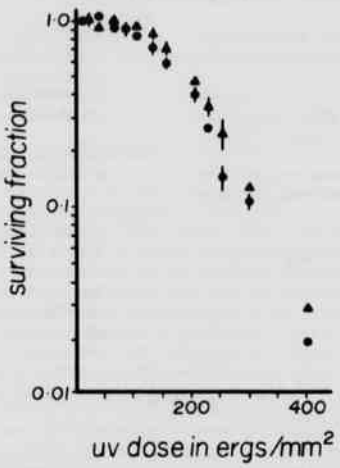

Figure 2. Survival of IPL-22 cells following UV (circles) and UV + PR light (3×10^3 ergs/mm²) exposures.

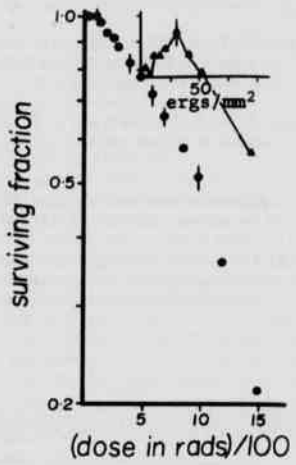

Figure 3. Survival of IPL-22 cells following gamma-ray (circles) and gamma-ray + UV (triangles) exposures.

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

First fraction (rads)	Second fraction (rads)	Interfraction time (hours)	Surviving fraction
600			0.65
300	300	0.5	0.66
300	300	1.0	0.76
300	300	1.5	0.80
300	300	2.0	0.84
300	300	3.0	0.76
300	300	4.0	0.70
300	300	5.0	0.70

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.

Experiment number	Description of cultures used	Treatment of cultures	Survival (number of colonies per 10^2 cells plated)
1	2×10^6 log phase cells in monolayers	cultures were exposed to 2000 ergs/mm² UV and the cells were plated for colony assays	3
2	2×10^6 log phase cells in monolayers	cultures were exposed to 2000 rads gamma-ray and the cells were plated for colony assays	8
3	10^7 cells in monolayers which had been exposed to 2000 ergs/mm² UV, and 10^7 cells in monolayers which had been exposed to 2000 rads gamma-ray	cell suspensions prepared from the UV exposed monolayers were fused with cell suspensions prepared from the gamma-ray exposed monolayers using P.E.G.1 and samples of cells from these fused cultures were plated for colony assays	47

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

CLEAVER, J. E. 1974. Repair processes of photochemical damage in mammalian cells, in J. T. LETT, H. T. ADLER and M. R. ZELLE (Eds). Advances in Radiation Biology. Academic Press, New York. 4:1-75.

CROSS, S. W., and H. G. GRIGGS. 1978. Ultraviolet light reactivation of gamma-ray-induced lethal damage in vertebrate cells. Proc. Ark. Acad. Sci. 32:37-38.

ELKIND, M., and H. SUTTON. 1960. Radiation response of mammalian cells grown in culture. I. Repair of X-ray damage in surviving Chinese hamster cells. Radiation Res. 13:556-593.

ELKIND M., and G. WHITMORE. 1967. In the radiobiology of culture mammalian cells. Gordon and Breach Science Publishers, New York. pp. 7-26.

GRIGGS, H. G., and M. A. BENDER. 1972. Ultraviolet and gamma-ray induced reproductive death and photoreactivation in a *Xenopus* tissue culture cell line. Photochem. and Photobiol. 15:517-526.

GRIGGS, H. G., and T. V. ORR. 1979. Chromosomal aberrations resulting from ultraviolet exposures to GI *Xenopus* cells. Photochem. and Photobiol. 30:363-368.

HAETTEN, R., M. MCGUINNESS, and G. GRIGGS. 1982. Dark repair of lethal damage induced in a hybrid mammalian tissue culture cell line by ultraviolet light. Proc. Ark. Acad. Sci. 36:89-91.

RUPERT, C. S., and W. HARM. 1966. Reactivation of photobiological damage. In Advances in Radiation Biology. Academic Press. 2:1-81.

CRAIG ENDRES, SUSAN KULP, and GASTON GRIGGS, John Brown University, Siloam Springs, AR 72761.

A PRELIMINARY REPORT ON THE ZYGOPTERA (DAMSELFLIES) OF ARKANSAS

Adams (1900) published the first list of Arkansas Odonata, reporting seven damselfly species. Subsequent papers (Needham and Heywood, 1929; Bick, 1959; and Houston, 1970) increased the species list to 18. Bick (1978) was apparently unaware of Houston's (1970) paper and relisted three 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 breakage 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 titia* 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. inaequalis* and *L. rectangularis* are spring fliers, while *Archilestes grandis* 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 Coney, 1980). Its Arkansas distribution concurs with those data, as the 10 counties listed are northcentral and northwestern.

Argia plana apparently has a limited Central U.S. distribution, as it has only been reported from Texas, Oklahoma, Kansas and Missouri (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 steams. 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 *Nymphaea odorata* Ait., 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 saucium* (Burmeister), *Chromagrion conditum* (Hagen), *Enallagma dubium* Root, and *Nehalennia integricollis* Calvert.

87