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Some Effects of Methyl Green on Expression of Damage Induced in G1 *Xenopus* Cells by Ultraviolet Light

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SAGE THRASHER (*OREOSOPTES MONTANUS*), A NEW STATE RECORD

On 24 November 1979, Cheryl Lavers and I were looking for birds in the Farville area, about 6 miles NE of Jonesboro, Craighead County, when we discovered a Sage Thrasher (*Oreoscoptes montanus*). The bird was rather Mockingbird-like (*Mimus polyglottos*) in shape, plain gray-brown crown and back, and bright yellow eye with a black pupil. Other characters differed from a Mockingbird in that it was smaller and shorter tailed and it had a white throat with a black malar stripe. The underparts, with a ground color of warm buff, were densely streaked with black chevrons arranged in length-wise rows. There was a white wingbar, and a dark tail with white outer corners. The bill was slender, very slightly decurved, and dark bluish-black in color, as were the rather short legs. The bird ran along the ground in open places, or under brush and stayed on or near the ground. It was observed catching, decapitating, then swallowing ground crickets (*Acheta* sp.). We have both observed Sage Thrashers in several western states. An examination of standard field guides (Peterson, R. T. 1961. A field guide to western birds. Houghton Mifflin Co., Boston; Robbins, C. S. et al. 1966. Birds of North America. Golden Press, N.Y.) further confirmed our identification. A description and slides of the bird have been sent to Charles Mills, Curator, the Arkansas Audubon Society, and Dr. Douglas James, Department of Zoology, University of Arkansas at Fayetteville. This is a first recorded instance of the Sage Thrasher in Arkansas.

In its normal range, the Sage Thrasher breeds in the sagebrush (*Artemisia*) deserts of western North America, coming as far east as western Oklahoma. It winters in the southern part of its range and Mexico, occurring as far east as central and southern Texas, with a small isolated winter colony in extreme southwestern Louisiana (A.O.U. check-list of North American birds, 1957). The species is seldom recorded as a vagrant. The following extralimital occurrences to the east of its range have been recorded: Florida, 2; Illinois, 2; Maryland, 1; New York, 2; North Carolina, 1; South Dakota, 1.

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SOME EFFECTS OF METHYL GREEN ON EXPRESSION OF DAMAGE INDUCED IN G1 *XENOPUS* CELLS BY ULTRAVIOLET LIGHT

Methyl green shows a high degree of specificity for DNA and is a component of many nuclear stains. Kurnick (1952) observed that this basic dye was readily bound by polymerized DNA; two amino groups of the dye binding to two phosphoric acid groups of DNA. Errera (1951) demonstrated that the affinity of DNA for methyl green is influenced by radiation and other agents which depolymerize DNA, or alter its internal configuration. Doudney and Haas (1958) showed that methyl green significantly influenced metabolic activities, such as DNA and RNA synthesis, in *Escherichia coli*. These results suggested that appropriate experiments, involving methyl green treatments coupled with germicidal UV exposures, might aid in describing the expression and repair of UV-induced lethal and mutational damage in prokaryotic cells. Experiments of this nature were carried out by investigators, such as Witkins (1961), which led to the notion that UV induces lesions in bacterial DNA that either are removed by repair systems or are converted to permanent structural changes during the first DNA synthetic period following the exposure. We report here an extension of such experiments to eukaryotic cells, in which some effects of methyl green on the repair of UV (254 nm)-induced damage in G1 phase *Xenopus* cells are examined.

Routine maintenance of log phase A8W243 *Xenopus* cultures, incubations, cell synchronizations, irradiations, mitotic index determinations, survival determinations (colony counts), and chromosome analysis have been described in detail (Griggs and Bender, 1973; Payne and Griggs, 1977; Griggs and Orr, 1979).

The basic dye used, methyl green, was obtained from Difco.

Figure 1 shows results of a series of mitotic index experiments performed to examine the effects of methyl green on progression of UV irradiated G1 cells through interphase and the first succeeding mitosis, (M1). These data described the appropriate time intervals for collection of the sets of mitotic cells analyzed for effects of methyl green on UV-induced aberration production (Table 1). Concentrations of the dye in the range 0.0 - 0.003 g/l appeared to induce little delay in progression of the irradiated cells above that induced by the UV alone. The similarity in average height and width of these mitotic peaks indicated that the dye did not significantly reduce the number of irradiated cells that reached G1.

The average cell cycle for non-irradiated *Xenopus* cells was approximately 26 hours (not shown); eight hours G1, 13 hours S, three hours G2, and two hours M1. Payne and Griggs (1977) carried out autoradiographic studies which indicated that early G1 phase cells, exposed to low doses of UV (0 - 150 ergs mm⁻² range), are not delayed in their progression through G1, but experience rather lengthy S phase delays. These facts, coupled with the data of Figure 1 and Table 1, indicate that chromatid aberration frequencies, resulting from UV exposure of early G1 cells, are significantly altered by methyl green only when the dye is in contact with the exposed cells as they pass through early S phase. Some relationship between the aberrant intracellular mechanism, by which methyl green augments chromatid type aberration production, and DNA synthesis is suggested by the fact that both mechanisms appear to function with peak efficiency in early S phase.

The data of Figure 2 indicate that a methyl green concentration of 0.003 g/l has virtually no effect on the expression of UV-induced lethal damage, no matter where in the cell cycle the dye is applied. These data suggest that the mechanism which expresses UV-induced aberrational damage in *Xenopus* cells differs significantly from the mechanism which expresses lethal damage, supporting results of previous studies of the overlap of UV-induced lethal and aberrational lesions in *Xenopus* cells (Griggs and Orr, 1979; Payne and Griggs, 1977).

Consideration of the data associated with the 0, 8, and 10 hr points of Table 1 suggests that the marked increase in chromatid aberrations observed could have resulted from methyl green inhibition of an early S phase mechanism which repairs UV-induced damage (possibly pyrimidine dimers) in DNA that leads to chromatid aberrations. A previous study (Griggs and Bender, 1973) has shown that UV-induced pyrimidine dimers in the DNA of G1 *Xenopus* cells lead to chromatid aberrations. Both UV-induced dimers and chromatid aberrations in *Xenopus* cells appear to be removed by enzymatic photoreactivation (PR). Furthermore, pyrimidine appears to be the only substrate for PR enzyme. Thus, if the mechanism inhibited by methyl green decreases aberration frequencies by repairing UV-induced dimers, an appropriate PR treatment applied in conjunction with the UV and methyl green treatment should reduce the methyl green effect. Table 2 contains results of one attempt to explore this notion. Immediately after the cultures of early G1 cells were exposed to 90 ergs mm⁻² UV, they were photoreactivated with the doses indicated and then incubated in methyl green until fixed for chromosome analysis. Comparison of the data of Tables 1 and 2 clearly shows that chromatid aberration frequencies resulting from UV + PR + methyl green treatment are significantly lower than the frequencies resulting from the UV + methyl green treatment, and reduction in aberration frequency is dependent on PR dose.

The present study is far from conclusive and further experimentation is certainly indicated. Nevertheless, we feel that the data strongly suggest the existence of a dark (non-PR) radiation repair mechanism in early S phase *Xenopus* cells. This mechanism is probably closely associated with DNA synthesis and capable of repairing a significant fraction of the UV-induced lesions that lead to chromosomal aberrations. The operation of such a mechanism would tend to explain results of previous studies indicating that doses of UV between 0 and 90 ergs mm⁻², which kill from 0-80 percent of the *Xenopus* cells exposed, do not produce aberration frequencies above control levels (Griggs and Orr, 1979). Research supported by National Cancer Institute Grant CA-18809-03.

Table 1. Effects of methyl green on aberration frequencies resulting from a UV exposure of 90 ergs mm⁻² to early G1 phase cells.

concentration of methyl green (µg per liter)	time methyl green added to cultures (hrs after UV)*	time range over which mitotic cells selected (hrs after UV exposure)	number cells scored	chromatid aberrations deletions	chromosome exchanges	chromosome aberrations deletions	standard error
0		55-75	500	4	2	2	1
.001	0	55-80	500	62	33	1	1
.002	0	55-80	500	67	35	2	2
.002	10	55-80	500	45	28	1	1
.002	14	55-80	500	24	7	2	0
.002	20	55-80	500	5	3	2	0

*Methyl green was added to the cultures at the times indicated and not removed until mitotic spreads were prepared for chromosome analysis.

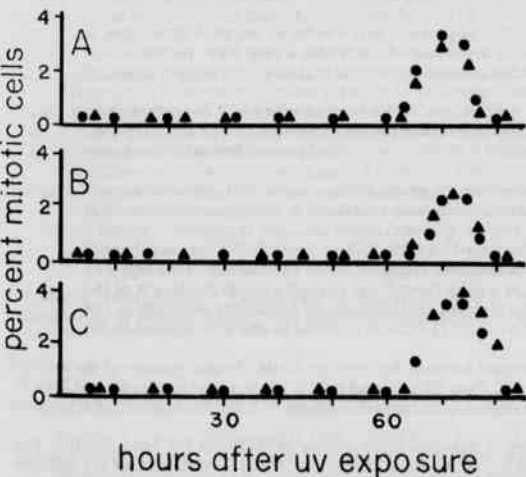


Figure 1. Some effects of methyl green on progression of UV irradiated early G1 cells through interphase and the first succeeding mitosis. Synchronous cultures were exposed to 90 ergs mm⁻² UV one hour after mitotic selection. A (circles) represents UV alone. In the other experiments, the following concentrations of methyl green were added to the cultures immediately after UV exposure; A (triangles) 0.001 g/l, B (circles) 0.002 g/l, B (triangles) 0.003 g/l, C (circles) 0.004 g/l, C (triangles) 0.005 g/l. The dye remained in the cultures until the experiment was terminated.

Table 2. Effects of methyl green on aberration frequencies produced in early G1 phase cells by exposures of 90 ergs mm⁻² UV, followed by varying doses of PR light.

concentration of methyl green (µg per liter)	time methyl green added to cultures (hrs after UV)*	PR dose (ergs mm ⁻² sec)**	time range over which mitotic cells selected	number cells scored	total chromatid aberrations	standard error
0		0	55-80	500	5	0.224
0		4x10 ³	25-35	500	8	0.258
.002	0	0	55-80	500	64	4.204
.002	0	1x10 ³	55-80	500	62	2.773
.002	0	2x10 ³	25-40	500	34	1.521
.002	0	4x10 ³	24-35	500	22	0.984
.002	0	4x10 ³	25-35	500	23	1.030

*Methyl green was added to the cultures at the times indicated and not removed until mitotic cells were prepared for chromosome analysis.

**Cultures received the indicated doses of PR light immediately after UV exposures, before methyl green was added to the cultures.

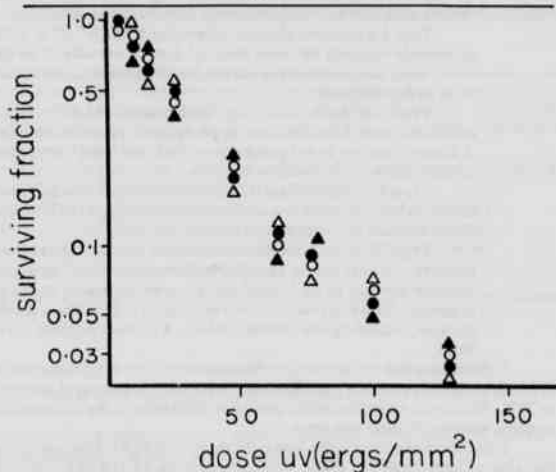


Figure 2. Some effects of methyl green on survival of UV irradiated G1 cells. Synchronous cultures of early G1 cells were exposed to the indicated doses of UV one hour after mitotic selection. The open circles represent UV alone. In the other experiments, methyl green (0.003 g/l) was added to the cultures at the following times after the UV exposures; 0 hours (open triangles), 10 hours (filled triangles), 16 hours (half-filled triangles). The dye remained in the cultures until the experiment was terminated.

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HEMOGREGARINES IN THE RED-EARED SLIDER, *CHRYSEMYS SCRIPTA ELEGANS* (WIED) FROM ARKANSAS

Twenty-five red-eared sliders, *Chrysemys scripta elegans* (Wied), were examined for hemogregarines. Turtles were purchased in late October, 1979 and early May, 1980 from Anderson's Minnow Farms, Lonoke County, Arkansas (T. 1N, R. 9W, Sec. 35). Blood samples (2.0 ml) were obtained by puncturing the heart with a 21 gauge needle fitted on a 10 ml syringe. Thin blood smears were air dried, fixed in absolute methanol for 2-3 minutes, stained with Giemsa for 25 minutes, dipped briefly in buffered water (pH 7.0 @ 25°C) and allowed to dry. Blood smears were examined under oil immersion and infected erythrocytes were located to avoid the possibility of confusing ex-erythrocytic forms of the parasite with normal blood cells, especially young thrombocytes. The staining characteristics, cytoplasmic characteristics, and general shape and form of the parasitic forms were noted. Measurements were taken with an eyepiece micrometer. No attempt was made to study the tissue stages of the hemogregarines through histological preparations.

All of the *C. s. elegans* examined were infected with intraerythrocytic forms. Parasitemias ranged from approximately 0.5 percent of the erythrocytes in one turtle to barely detectable infections in the majority of the turtles. Following a conservative strategy, no attempt was made to relegate parasitic forms to particular life cycle stages. Rather, individual forms were assigned to one of four morphological types as follows (measurements in micrometers [μm], the range follows in parentheses):

Type I is crescent shaped, measuring $8.9 (9.8-7.8) \times 2.3 (2.9-2.0)$. The parasite, as is true for all morphological types, is apparently encased by some form of cyst or vacuole. The cytoplasm is flocculated and stains a light blue. Inclusions are rarely seen, and a nucleus is not readily discernable. The nucleus of the invaded erythrocyte is at most only slightly displaced from its normal position.

Type II is characteristically "bean shaped" $13.4 (14.1-12.7) \times 4.2 (4.9-2.9)$, with a short recurved tail. The cytoplasm is somewhat more basophilic and appears more dense in some specimens. Acidophilic inclusions are common in the cytoplasm. A distinct nucleus is not generally evident, but a dark irregularly shaped structure is sometimes present instead. The parasite usually displaces the nucleus laterally.

Type III parasites are $13.6 (14.1-12.7) \times 5.6 (6.8-3.9)$ and vary from "bean shaped" to nearly oval. Cytoplasmic and nuclear characteristics are similar to those of Type II. The acidophilic inclusions are prominent in this type, and the nucleus of the invaded cell is displaced toward one end.

Type IV is the most distinctive of the morphological types. The large $17.5 (19.5-15.6) \times 5.9 (7.8-4.9)$ "banana-shaped" parasite occupies nearly all of the turtle erythrocyte. The erythrocyte nucleus is displaced to the extreme end of the cell. The parasite appears to be formed by two arms or bodies at the ends, as a clear "canal" or "groove" extends the length of the organism. The flocculated cytoplasm stains a light blue. Acidophilic granules characteristically border the periphery of the parasite, including the central "canal". A distinct nucleus $6.0 (6.8-5.9) \times 2.2 (2.9-2.0)$ is apparent in one arm adjacent to the bend.

A determination of the relative frequencies of the morphological types could be made for only one turtle. Twenty percent of the infected erythrocytes contained the Type I parasite. Twenty-nine percent carried Type II. Type III occurred in 21% of the parasitized cells, and Type IV in 30%. There was no statistically significant difference in the occurrence of morphological types (Chi square = 3.28). No evidence of schizogony or other nuclear division was seen.

This report represents the first published record of a hemogregarine from an Arkansas turtle species. Mohammed and Mansour (*Bull. Fac. Sci. Cairo Univ.* 35:39-51, 1959); Ball (*J. Protozool.* 18:198-210, 1967); Ball et al., (*J. Parasitol.* 53:897-909, 1967) have stated that the establishment of the taxonomic status of hemogregarines at both the generic and specific levels is dependent upon a thorough examination of all stages of the life cycle in both the vertebrate and invertebrate hosts; however, in the absence of such data, the exact determination of the taxonomic status of hemogregarines in Arkansas turtles awaits further study.

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