AN ABSTRACT OF THE THESIS OF

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Title: EFFECTS OF ENHANCED MID-ULTRAVIOLET RADIATION (290-315 NM) ON DEVELOPMENT AND SURVIVAL OF BOREAL TOAD (BUFO BOREAS BOREAS) TADPOLES

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Donald J. Kimeldorf

More than 3700 fertilized toad eggs were used in a series of studies designed to determine the effects of UV-B radiation (290-315 nm) on temporal developmental patterns, systematic development and viability prior to metamorphic climax. The eggs were exposed to radiation from either "white-light" fluorescent lamps (Vita-Lite, Duro-Test Corporation, North Bergen, New Jersey) or "white-light" fluorescent lamps plus fluorescent sunlamps (Westinghouse-FS40). Several radiation exposure schedules were utilized (chronic or varied, daily exposures) and, in all but one study, filters were utilized - Kodacel cut-off filters to transmit wavelengths longer than 290 nm and Mylar cut-off filters to transmit wavelengths longer than 315 nm. These filters provided a transmission "window" in the UV-B region.

Exposure of the developing tadpoles on a daily basis to eleven or more Sunburn Units of UV-B radiation per day resulted in the development of tadpole populations with lordotic posture, hyperplasia in the presumptive cornea and other dorsal epithelial tissue, increased pigmentation in the presumptive cornea,

desquamation of areas of the dorsal surface, and increased mortality. Exposure of the tadpoles on a daily basis to 4.4 Sunburn Units of UV-B radiation per day resulted in a significant lengthening of the time periods required to achieve metamorphic climax.

Biological photoreactivation of ultraviolet damage was demonstrable with three groups of tadpoles exposed to comparable daily exposures of UV-B radiation (11.0 Sunburn Units per day) at a comparable rate (1.1 Sunburn Units per hour) from similar sources. The critical differences between the groups was the duration of exposure to UV-A and visible radiation (0, 2 and 4 hours) following the termination of the UV-B exposure.

EFFECTS OF ENHANCED MID-ULTRAVIOLET RADIATION (290-315 NM) ON DEVELOPMENT AND SURVIVAL OF BOREAL TOAD (*BUFO BOREAS BOREAS*) TADPOLES

by

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INTRODUCTION

General Remarks

Hair, feathers, and pigmented skin usually can be expected to screen mature vertebrate organisms from the detrimental effects of ultraviolet radiation. On the other hand, early developmental stages and neonates may be vulnerable because of their size and less developed ultraviolet screens. Moreover, the rapidly proliferating and differentiating tissue mass of the individual during development represents a particularly radiosensitive period in the life cycle of the vertebrate organism. It has been found that these developmental stages in the toad can serve usefully as a model for radiation effects on the embryonic and larval stages of amphibian vertebrates.

Successful methods for inducing oviposition in anurans and for rearing the tadpoles in the laboratory have been devised (Dorsch, 1967; Rugh, 1935, 1962a; Sivula et al., 1972; Volpe, 1953, 1957), and the development of these embryos and larvae have been studied extensively with well documented and easily recognizable stages (Deuchar, 1966; Gosner, 1960; Rugh, 1951, 1962; Shumway, 1940; Taylor and Kollros, 1946). The fact that toads deposit their eggs in strings rather than masses

allows for a more uniform exposure geometry during experimental treatment when radiation is involved. Brattstrom (1962, 1963), Calef (1973), Karlstrom (1962), Mullally (1952), Mullally and Cunningham (1956), and Smith and Bragg (1949) have written excellent accounts concerning the behavior and the ecological niche of the developmental stages of certain anurans.

Effects of Ionizing Radiation on Embryonic Development

Many studies have been published regarding the effects of penetrating ionizing radiation on the embryological stages of various vertebrates. The results are summarized in comprehensive reports and reviews regarding the developmental effects produced in amphibians (Brunst, 1965), fish (Rugh and Grupp, 1959), chickens (Casarett, 1968), mice (Russell and Russell, 1954), hamsters (Harvey and Chang, 1962), rats (Hicks and D'Amato, 1963), mice and humans (Rugh, 1962b), humans (Rugh, 1958), mammals in general (Hicks and D'Amato, 1966), and other representative vertebrates (Rugh, 1959).

Effects of Ultraviolet Radiation On Embryonic Development

Anurans

Reports of studies regarding the effects of non-ionizing ultraviolet radiation on vertebrate development are far less available. Of the studies that have been made, investigators have made good use of amphibian models. This is because of the relative ease of rearing amphibian embryos in large numbers and the ease of radiation exposure of the developmental stages during ontogenesis. The studies extend from radiation effects on fertilized amphibian eggs through all stages of larval development and beyond. The energy spectrum of radiation utilized in these studies has been broad, ranging from the wavelengths of DNA sensitivity and protein denaturation, through to the longer wavelengths of the near-ultraviolet (UV-A)[1] region. As early as 1915, Baldwin (1915, 1919, 1921) published reports on the development of frog's eggs which had been irradiated with ultraviolet radiation emitted by an iron

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[1]The ultraviolet spectrum, which extends from 380 nm down to approximately 100 nm, may be divided into the following regions: UV-A, 380-315 nm; UV-B, 315-280 nm; UV-C, less than 280 nm.

electrode arc (a very broad emission spectrum in the ultraviolet region, ranging from less than 250 nm to a wide peak in the UV-A region). When the vegetal hemisphere or equator was irradiated, Baldwin could regularly induce embryos to develop spina bifida. When he irradiated the animal hemisphere of the freshly laid eggs, the exposure caused the development of U-shaped embryos. The cytological appearance of the tissues of the U-shaped embryos was normal upon histological examination, even though the myomeres in the region of the bend conformed to the flexure. Baldwin also irradiated embryos in the neurula stage and found that, after initial recovery, there was a thickening of the ectoderm in the area of exposure caused by an increase in the size of cells (hypertrophy) and an increase in the number of cells (hyperplasia). The cells in the thickened area assumed an irregular, "piled-up" distribution rather than the normal stratified arrangement. There was a temporary contraction of the chromatophores in the ectoderm of these embryos, followed by an increased number of pigmented cells. Subsequently, Smith (1966), after localizing the exposure of the fertilized frog's eggs to either the vegetal or animal hemisphere, reported that exposure of the vegetal hemisphere with

ultraviolet radiation (254 nm) at doses in excess of 770 $J \cdot m^{-2}$ resulted in the elimination of primordial germ cells, but normal tissue development otherwise. Irradiation of the animal hemisphere with equivalent doses produced no visible effects in the embryos by Gosner's (1960) developmental stage 25 (the stage preceding limb bud development). Utilizing a monochromator in another study, Smith (1966) exposed the vegetal hemisphere of the developing frog's eggs to narrowed wavelength regions of ultraviolet radiation centered at 278 nm and 302 nm. He found that a high percentage of abnormal embryos did develop with exposure to 278 nm radiation at doses in excess of 500 $J \cdot m^{-2}$; whereas, with equivalent doses of 302 nm radiation, almost all of the embryos appeared normal at stage 25. A more recent work, regarding the effect of exposure of the vegetal hemisphere of frog's eggs to ultraviolet radiation (254 nm) resulted in the conclusion that ultraviolet radiation destroys a cytoplasmic factor essential to neural induction (Grant and Wacaster, 1972). The resultant acephalic or aneural embryos suggested that the inductive capacity of the gray crescent (the future organizer of the embryonic neural axis) was destroyed. The exposures ranged from 5 to 100 $J \cdot m^{-2}$.

Malacinski et al. (1974) exposed the vegetal hemisphere of frog's eggs within 90 minutes after fertilization to 254 nm radiation and also found developmental lesions in neural morphogenesis. The abnormalities ranged from slight (750 $J \cdot m^{-2}$), through microcephalia and completely aneural morphology (1000 $J \cdot m^{-2}$), to developmental arrest prior to completion of neurulation (1500 $J \cdot m^{-2}$). The gray crescent was found to be the most sensitive area. They noted that incubation of the embryos at low temperatures following exposure would prevent many of these defects (cryoreversion).

Salamanders

Lethality studies with salamander larvae have shown that a dose of 640 $J \cdot m^{-2}$ from an intermediate pressure mercury lamp (measured by a sensor sensitive to wavelengths less than 315 nm), and given five times over a week, resulted in 50% lethality within 5-7 days (Blum and Matthews, 1952). Radiation sensitivity was inversely related to the stage of maturation. The older the larvae, the more resistant was the organism to ultraviolet irradiation. Exposure to short periods of UV-A and visible light, after potentially lethal or sublethal ultraviolet damage has occurred, improves the

probability of recovery (Blum and Mathews, 1950). Ultraviolet exposure from a low pressure mercury lamp in quartz (primarily 254 nm) has been found to have a marked effect on the development of limb buds as well. Irradiation of frogs or salamander limb buds can inhibit development or cause abnormal development of the limb (Blum et al., 1957; Cook, 1970; Rieck, 1954). The greater the degree of differentiation of the limb at the time of exposure, the greater was the incidence of abnormalities in the irradiated appendages. Partial recovery through photoreactivation was demonstrable with both the frog and the salamander. Ultraviolet irradiation has been observed to retard the regeneration of the stumps of an amputated salamander limb. These studies were done with intermediate pressure mercury lamps in quartz, with doses ranging from 10^4 to 10^5 $J \cdot m^{-2}$. In Xenopus tissue cultures, the loss of colony-forming ability of liver tissue has been observed when the cultures were exposed to ultraviolet radiation from germicidal lamps (254 nm, 0.5 $W \cdot m^{-2}$ with doses up to 25 $J \cdot m^{-2}$) (Griggs and Bender, 1972; Regan et al., 1968). These cells could be photoreactivated with a marked increase in the survival rate of the culture.

Fish

The effect of sunlight on the embryonic development of hatchery-reared trout has been studied in order to optimize the production of game fish. In nature, the eggs of trout and salmon are shielded from the direct rays of the sun by a cover of gravel. When trout or salmon eggs are exposed to the direct rays of the sun for more than a few minutes at a time, the eggs are killed (Leitritz, 1959). It is not known if one or more spectral regions play a major role producing this effect. Leitritz (1959, p. 43) offered the following opinion:

> Harmful effects have also been reported when fluorescent lighting was used over incubating eggs. It seems doubtful that the small amount of ultraviolet rays emitted by fluorescent lights is harmful to trout or salmon eggs, and there is nothing to really substantiate this claim. However, to be on the safe side, it seems best to use incandescent lights.

Perlmutter (1961, 1962) speculated that this effect is due to visible blue and violet light, but did not test the effect of the ultraviolet portion of the spectrum emitted by the fluorescent lamps. McCandless et al., (1969) have speculated that solar ultraviolet radiation absorbed by eye tissues of these fishes may cause corneal and lenticular lesions.

Keratoconus, ulcerative keratitis, and cataracts develop in hatchery-reared lake trout and, to a lesser degree, in rainbow trout. These conditions do not occur when the fish are shielded from the sun. The normal habitat of lake trout in nature is found at a depth of 100 feet or more; whereas rainbow trout usually inhabit much shallower water. Although the corneas of lake trout and rainbow trout absorb the same amount of radiant energy at 350 nm, the corneas of lake trout absorb more energy at the shorter wavelengths.

Chickens

A few studies have been made regarding ultraviolet radiation through egg-shell apertures on the development of chicken embryos. Davis (1944) demonstrated that the folding process in neural tube formation is affected when the embryo is irradiated with monochromatic ultraviolet radiation of wavelengths from 248 nm to 313 nm. The photochemical efficiency curves for inhibition of the neural folding process are similar to the absorption spectra for sterols, implicating the involvement of sterols in the folding process. The later development of chicken embryos has been shown to be very sensitive to ultraviolet irradiation (254 nm) by Lwin (1971). He has noted a deterioration of the vascular

system of five-day chicks as the most obvious gross pathological effect in the non-surviving embryos (3-30 $J \cdot m^{-2}$).

Beneficial Aspects of Ultraviolet Irradiation of Neonates and Adolescents

Lizards

Some observations have been made on the effects of exposing lizards to periods of ultraviolet irradiation. Kauffeld (1969) has found that certain lizards (e.g., the Iguanidae and the Agamidae) require exposure of their young to ultraviolet radiation for proper development. Commercial mercury-vapor sunlamps are the most commonly used source for the exposure. Moehn (1974) attributes the benefits derived from the sunlamps as possibly being due to the antirachitic factor.

Mammals

Although no reports were found describing effects of ultraviolet radiation on the embryonic development of mammals, some studies make reference to effects on the development of the neonate and adolescent. Gudkin and Tulupova (1970, as abstracted in Biological Abstracts, 1973) irradiated a group of piglets for 10 minutes on alternate

days with a PRK-2 ultraviolet lamp. When compared with the non-irradiated control group, it was found that the exposure "exerted a favorable effect on growth and development of suckling-piglets." The growth rate of several internal organs was increased and an increase of 23.4% in live-weights at weaning was accomplished by the irradiated group. Feller et al., (1971, as cited in Thorington et al., 1972) demonstrated that significant increases in gonadal development, skeletal development, body weight and sub-mandibular gland maturation resulted when young hamsters were exposed to deluxe cool-white fluorescent illumination containing enriched amounts of radiation in the UV-A region as opposed to hamsters exposed to standard cool-white fluorescent lamps. Wurtman and Weisel (1969) made a similar comparison of the relative effects of these radiation sources with newborn rats. They found that female rats reared under the UV-enriched radiation source had larger hearts and pineal glands than females exposed to the standard cool-white source, while males reared under the UV-enriched source had larger adrenals than those of the same sex reared under standard cool-white lamps.

Loomis (1970) cited an experiment in which two puppies were reared for six weeks, one in the sunlight from morning until evening and the other in the shade during the daylight hours. At the end of the study the puppy raised in the shade was found to be rachitic, with 36% less calcium in its bones than the puppy exposed to the sunlight. This experiment was followed by others which confirmed the beneficial aspects of exposure to sunlight. Loomis noted that, in the past, the children of entire towns were plagued with rickets when the sunlight was effectively attenuated by air pollution and narrow streets. He cited a typical report about a town which stated that the

> children develop thick joints, cease to be able to walk or have deformed legs. The head becomes large and even the vertebral column bends.

It was found that ultraviolet radiation from a mercury-vapor quartz lamp would cure rickets. The prohormone, vitamin D_3, is generated in the skin from 7-dehydrocholesterol under the influence of UV-B radiation. Conversion products of vitamin D_3 are required by most vertebrates for proper calcium mobilization. Loomis concluded that the cause of rickets

should not be considered a dietary lack of "vitamin D", but a lack of sufficient exposure to sunlight.

Specific Objectives of This Study

The lack of information regarding the effect of irradiating amphibian developmental stages with ultraviolet radiation in the specific range of 290-315 nm, coupled with a recent increase in interest regarding this region of radiant energy, prompted the present study.

Although the atmospheric ozone layer is only approximately one-third of a centimeter thick at STP, it is the principal absorber of solar radiation in the ultraviolet range from 220 nm to 320 nm. Because the absorption of ultraviolet radiation is exponential in nature, a small decrease in the thickness of the ozone layer would result in a relatively large increase in the global solar flux of radiation between 290 nm and 315 nm (a portion of the ultraviolet spectrum designated as the UV-B region). The fate of the ozone shield has recently become a topic of worldwide interest. Researchers have become aware, initially, that nitrogen oxides found in SST exhaust and, subsequently, that the photochemical degradation products of halomethanes (e.g., "Freons") can act as

catalytic agents for the destruction of ozone. The near-exponential growth during recent years in utilization of the chemically inert halomethanes as aerosol propellants and as refrigerants has resulted in an annual worldwide production of nearly 10^9 kilograms (Hammond and Maugh II, 1974). Partial destruction of the ozone shield by halomethane photoproducts could lead to undetermined biological consequences from the increased ultraviolet flux.

The present study is designed to determine the effects of increased intensity of UV-B radiation (290-315 nm) on the temporal developmental pattern, systemic development and viability prior to metamorphic climax in boreal toad (Bufo boreas boreas Baird and Girard) tadpoles.

MATERIALS AND METHODS

Experimental Animal

The boreal toad (Bufo boreas boreas) was first officially described by Baird and Girard (1852) while participating in the United States Exploring Expedition of 1852. This species of toad is presently the most common toad found in the Pacific Northwest, having a range extending from Colorado and California to southeastern Alaska (Stebbins, 1966). The adult averages 6-13 cm in length and can usually be distinguished from other toads by its light-colored, mid-dorsal stripe and the lack of cranial crests. The predominant color of the body is varied, being rust, brown, gray, or green with dark blotches (Figure 1).

Possessing a variety of habitats, the boreal toad is found in the vicinity of water in deserts, grasslands, woodlands, and mountain meadows. It is most active shortly after dusk, seeking shelter during periods of inactivity in burrows of other animals or in loose soil.

This species breeds in nature from March to September, depositing one or two rows of its eggs in strings along the margins of bodies of water. The ova may be black, brown,

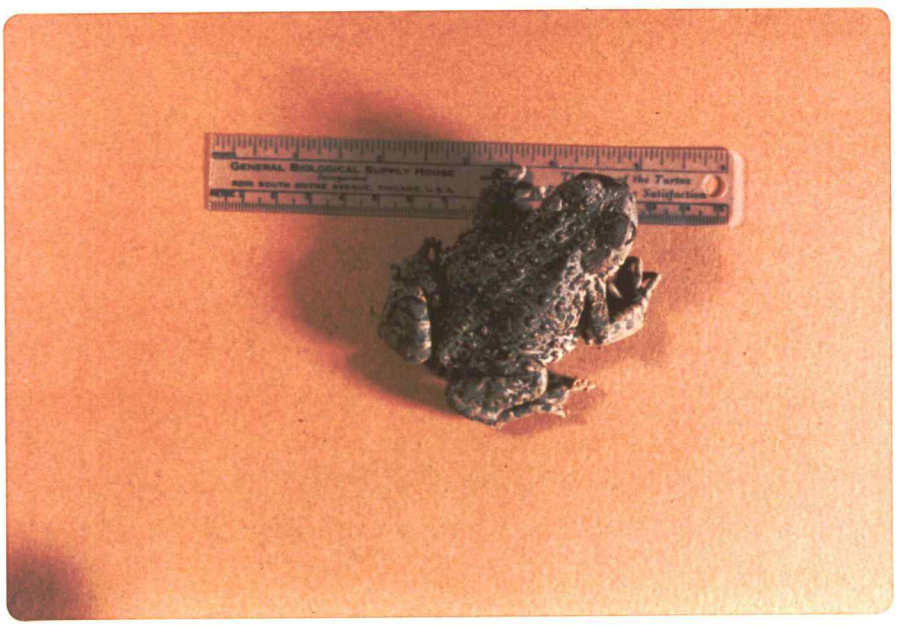

Figure 1. Adult boreal toad (Bufo boreas boreas).

or rust and they are encapsulated within two gelatinous layers (Stebbins, 1966). The size of the ova may range from 1.5 mm to 1.8 mm in diameter (Karlstrom, 1962).

Hatching usually occurs within one week of oviposition, although feeding does not begin until the mouthparts are fully developed. The color of the larvae is a uniform black, dark brown, or dark gray with transparent tail fins (Stebbins, 1966). Karlstrom (1962) reports that Bufo boreas boreas tadpoles may reach a maximum size of 40 mm, but typically are much smaller. The rate of development of the embryos and larvae is temperature dependent and, under natural conditions, metamorphosis is completed in approximately 46 days (Karlstrom, 1962).

Collection and Maintenance of Adults

Adult specimens of Bufo b. boreas were collected in the vicinity of Davis Lake (U.S.G.S. T23S, R7E) in the Deschutes National Forest, Deschutes and Klamath Counties, Oregon; Gold Lake (U.S.G.S. T22S, R6E) in the Willamette National Forest, Lane County, Oregon; and Todd Lake (U.S.G.S. T18S, R9E) in the Deschutes National Forest, Deschutes County, Oregon. One hour after sunset, during the summer and fall months

the specimens were abundant on the shore and along the margins of the lakes, or on neighboring paths and roads. Using a gas lantern for illumination, the toads could be caught with the hands or with a net. The specimens were returned to the Radiation Center in a cold styrofoam container and stored in a terrarium containing moist sand in the cold-room at 5°C.

Egg Production

Fertilized eggs of the boreal toad were obtained by inducing amplexus and oviposition with a pair of adult toads through a modification of Rugh's (1962a) technique. After injection of chorionic gonadotropin (either Antuitrin 'S' [Parke-Davis] or Chorisol [Burns-Biotec] and then fresh toad anterior pituitaries into the abdomens of the selected pair of adult toads (Table 1), the pair was placed into a small aquarium containing natural well water to a depth of approximately 6.5 cm. The aquarium was kept in the temperature-controlled (18°C), environmental laboratory until oviposition and fertilization occurred. Oviposition was usually accomplished within 48 hours of the final injection of the toad pituitaries. Of the females used for

TABLE 1. Typical timetable for induction of amplexus and oviposition with a pair of adult boreal toads (Bufo boreas boreas) using chorionic gonadotropin and toad anterior pituitaries.

Day/time	Female dose	Male dose
first day		
morning		750 Units
evening	750 Units	750 Units
second day		
morning	750 Units	
evening	750 Units	750 Units
third day		
morning	6 male anterior pituitaries	

egg production, success was achieved only with those collected in early spring prior to natural oviposition, or with those collected in late summer and fall, after öocyte development had occurred naturally.

Prior to first cleavage, strings of approximately ten toad eggs were cultured in shallow, polystyrene dishes (10.9 cm x 10.9 cm x 2.8 cm) containing the aquatic medium at a depth of 1.5 cm.

Aquatic Culture Medium

Natural well water, obtained from the Oregon State University Oak Creek Fisheries Laboratory, Corvallis, Oregon, was used to culture the developing tadpoles for most of the studies in this series. This medium has transmission characteristics within 1.5% of glass distilled water in the UV-B region. The other culture medium used during this series of studies was a synthetic pond water (amphibian Ringer's solution diluted to one-tenth standard concentration, Volpe, 1953) (See Appendix A).

Exposure Racks

Two steel-frame racks were constructed, each possessing four plywood (1/2" exterior A/C) shelves. The dimensions of each rack were 213 cm x 123 cm x 80 cm (Figure 2). Above each shelf were placed two white-porcelain, 2-tube fluorescent fixtures and, outside of these, two 2-tube fluorescent fixtures with asymmetric, aluminum reflectors. The asymmetric, aluminum reflectors optimized the homogeneity of the exposure field, as well as increasing the irradiance of the UV-B portion of the emission spectrum. At 300 nm, aluminum has a reflectance of 70-90%; whereas white porcelain has a

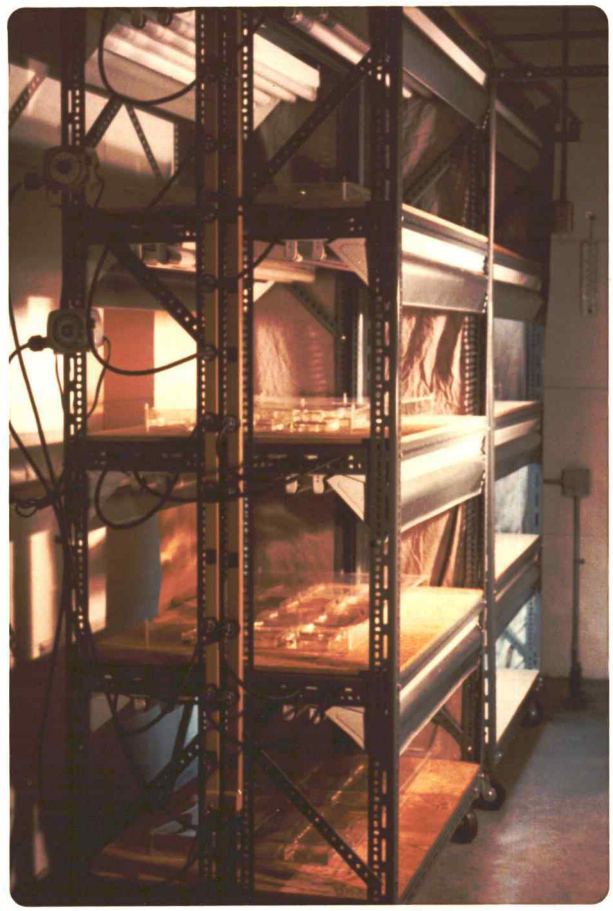

Figure 2. Exposure apparatus with protective side shielding removed for photographic purposes. Each shelf is 122 cm x 79 cm and has four two-lamp fluorescent fixtures located at 38 cm above the shelf. The outer lamp reflectors are asymmetric to optimize field homogeneity.

reflectance of 6% at the same wavelength (Koller, 1965). Each fixture was able to be controlled by individual interval timers, or many fixtures could be connected by way of Plugmold strips to single interval timers.

Radiation Sources

Sunlight-simulating fluorescent lamps (Vita-Lite, Duro-Test Corporation, North Bergen, New Jersey) were utilized in order to approximate the spectral balance of the UV-A and visible portions of sunlight reaching the surface of the earth (Figure 3). Where an increased flux of UV-B was required, fluorescent sunlamps (Westinghouse-FS40), having a peak emission at 310 nm, were used (Figure 4).

Filtration of Radiation

For the various studies to be described, 0.13 mm thicknesses of one or both of the following filters were used: (1) cellulose triacetate film, Kodacel-TA401:0.005" (Eastman Kodak Company), and (2) polyester sheets, Mylar 'S':0.005" (E.I. du Pont de Nemours and Company).

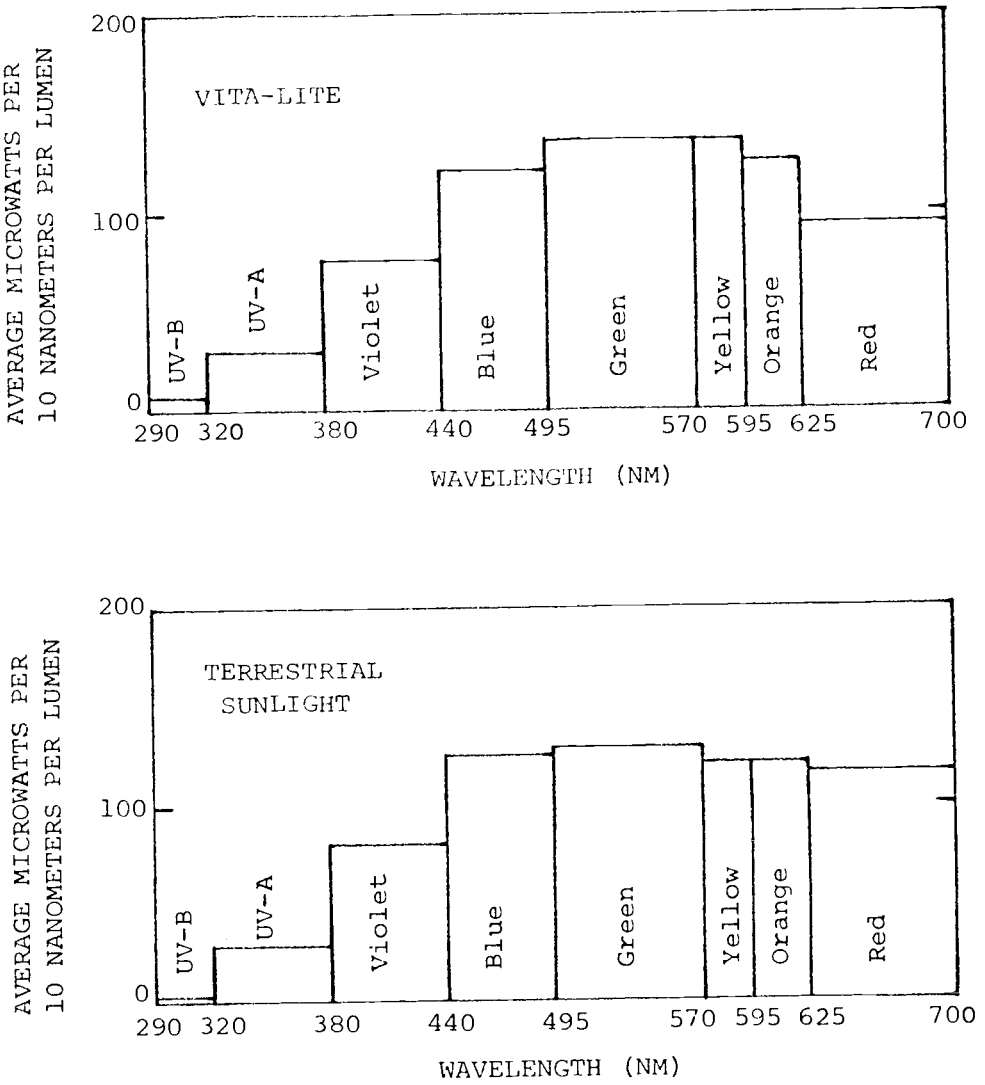

Fig. 3. Spectral energy distribution charts for Vita-Lite fluorescent lamps and terrestrial sunlight. The charts show the average amount of radiation generated in each color band by the source being measured. Charts are based on information supplied by the Duro-Test Corporation, North Bergen, New Jersey.

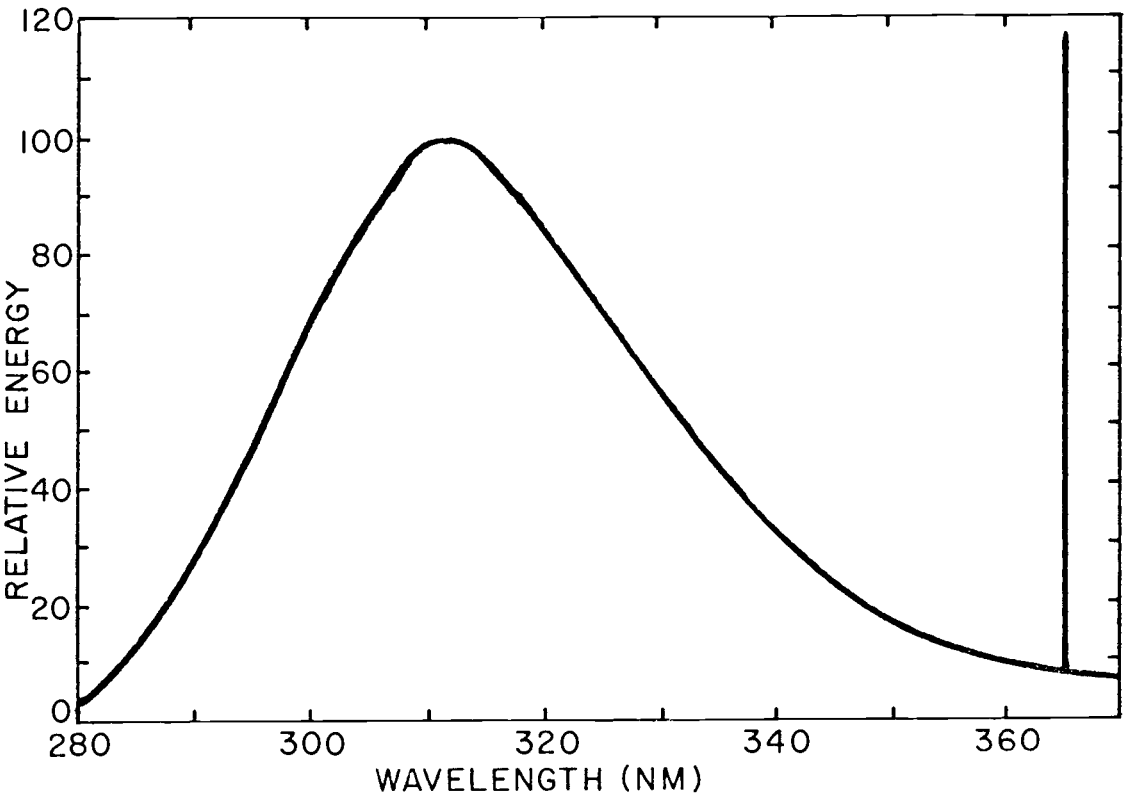

Figure 4. Emission spectrum of Westinghouse-FS40 fluorescent sunlamp. Closely spaced major emission lines of mercury at 366 nm are prominent, and are denoted as a single line. Spectrum based on information supplied by Westinghouse Electric Corporation.

The Kodacel acted as a cut-off filter, transmitting wavelengths longer than 290 nm, a realistic minimum wavelength found in the global solar spectrum. The Kodacel filters were presolarized for 24 hours and changed regularly in order to minimize variation in the transmission characteristics (See Figure 5 and Appendix B).

The Mylar acted as a cut-off filter transmitting wavelengths longer than 315 nm, and thereby effectively excluding the UV-B portion of the spectrum (Figure 6). Although the transmission characteristics of different production lots of Kodacel and Mylar have significant variability, the stability of Mylar transmission characteristics during prolonged exposure to ultraviolet radiation is far superior to that of Kodacel. It was not necessary to presolarize the Mylar.

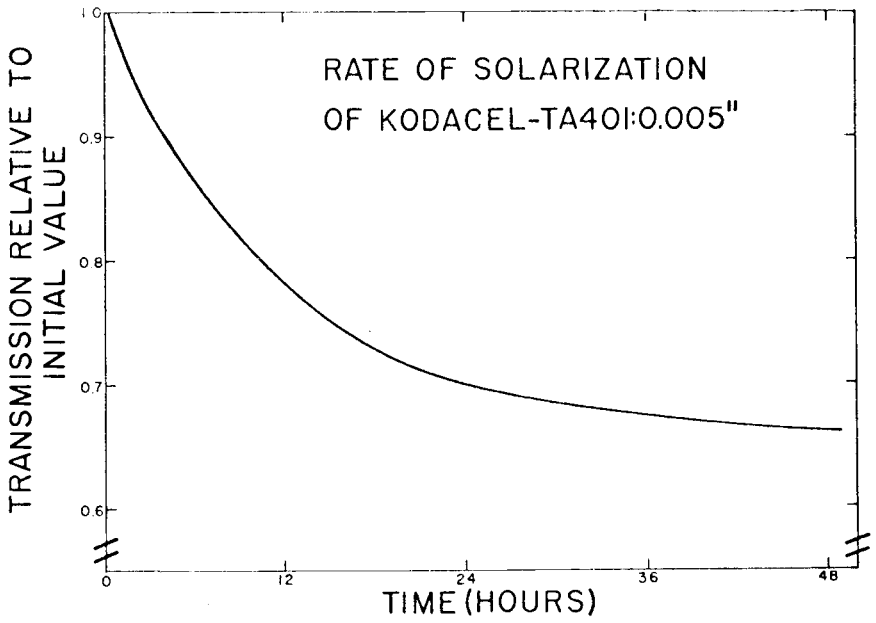

Figure 5. Effect of solarization on the transmission of Kodacel-TA401:0.005" in the UV-B region. Four FS40 sunlamps used as solarization source. Exposure rate without filter: three Sunburn Units per hour as measured with a UV-B Meter.

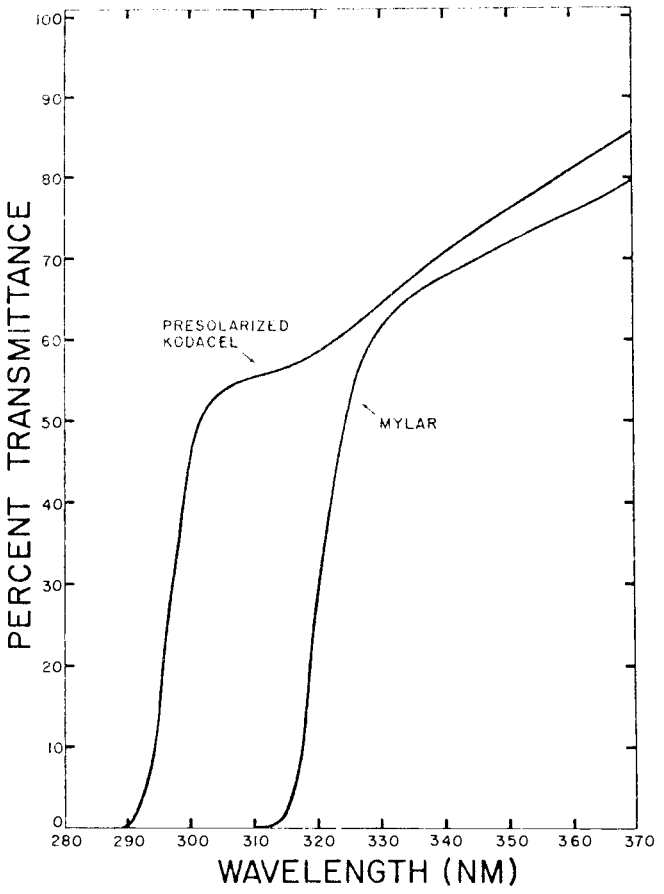

Figure 6. Transmission spectra for 0.13 mm thicknesses of 24-hour presolarized Kodacel-TA401:0.005" and nonsolarized Mylar 'S':0.005". This figure illustrates the transmission "window" between these two filters in the UV-B (290-315 nm) region.

Special Methods for Study #1

Effects of Exposure of Fertile Eggs to Sunlamps Without Added Filtration

In this study, following amplexus and oviposition, strings of approximately ten eggs were placed in culture dishes containing synthetic pond water (amphibian Ringer's solution diluted to one-tenth standard concentration). The dishes were divided into five groups, four being exposed continuously and the fifth being on a more natural, cyclic photoperiod (14L:10D) (Table 2). No filters were introduced between the lamps and the culture dishes. Radiant exposure rates were determined, utilizing a UV-B Meter (See Appendix C), and development of the embryos was followed.

Special Method for Study #2

Effects of Exposure of Fertile Eggs, Using Kodacel Filters, to White Lights with Added Sunlamp versus Exposure to White Lights only

Of the 1,111 toad eggs utilized, 98% were fertile (attained first cleavage). Prior to first cleavage, strings of approximately ten eggs were cultured in polystyrene dishes, one-half of the dishes containing natural well water, and the other half containing 0.1 amphibian Ringer's solution. Six

TABLE 2. Exposure schedule for the five groups of tadpoles irradiated without added filtration in Study #1. Note that Vita-Lite fluorescent lamps provided no significant energy in the UV-B region.

Group	Radiation source	Photoperiod	Radiant exposure rate ($SU \cdot h^{-1}$)
1	2 Vita-Lites + 4 FS40 sunlamps	continuous	2.7
2	2 Vita-Lites	continuous	0.0
3	4 Vita-Lites	continuous	0.0
4	6 Vita-Lites	continuous	0.0
5	2 Vita-Lites + 4 FS40 sunlamps	14L:10D	2.7

groups of 18 dishes each (a total of approximately 180 eggs in each group) were placed under Kodacel filters that had been presolarized for 24 hours.

Different groups of eggs (one-half of each group in well water and one-half in 0.1 amphibian Ringer's solution) were then exposed to two schedules of lighting periods in the temperature-controlled, environmental laboratory. To accentuate any potential effects, three groups were exposed to continuous radiation, while the remaining three groups were on a more natural daily lighting schedule. The continuous-treatment groups were exposed to either two

Vita-Lites (irradiance: 1.8 $W \cdot m^{-2}$ - YSI-Kettering Model 65 Radiometer), or four Vita-Lites (5.4 $W \cdot m^{-2}$), or two Vita-Lites plus six fluorescent sunlamps (5.9 $W \cdot m^{-2}$, of which approximately 1 $W \cdot m^{-2}$ was in the 290-315 nm region[2]). The exposures in Sunburn Units per hour ($SU \cdot h^{-1}$) received by the groups (as measured by a UV-B Meter) were 0.0 $SU \cdot h^{-1}$, 0.0 $SU \cdot h^{-1}$, and 2.1 $SU \cdot h^{-1}$, respectively (See Appendix C). For the groups on daily exposure schedules the photoperiod for the Vita-Lites was 14 hours "on" and then 10 hours "off". For the group exposed to supplemental ultraviolet radiation, the supplement was modulated by having two sunlamps cycled directly with the Vita-Lites, while the remaining four sunlamps started a "10 hours on - 14 hours off" cycle beginning at two hours after the start of the Vita-Lite "on" cycle (Table 3). The presolarized Kodacel filters were changed

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[2]By calculating the difference between irradiance through Kodacel-TA401:0.005" (290 nm cut-off filter) and irradiance through Mylar 'S':0.005" (315 nm cut-off filter), it was determined that approximately 1 $W \cdot m^{-2}$ of the irradiance through Kodacel was in the 290-315 nm region).

TABLE 3. Six-cell experimental design. Each experimental cell contains approximately 180 subjects, one-half cultured in natural well water and the other half cultured in amphibian Ringer's solution diluted to one-tenth standard concentration.

Radiation source	24-Hour (daily) light cycle		
	On-off cycle	On-off cycle	
High Intensity "white light (4 Vita-Lites)	24:0	14:10	
Low intensity "white" light (2 Vita-Lites)	24:0	14:10	
Low intensity "white" light with supplemental ultraviolet radiation (2 Vita-Lites + 6 FS40 sunlamps)	24:0	2 FS40's + 4 Vita-Lite	14:10
		4 FS40's	10:14

after every 70-96 hours of experimental exposure in order to minimize variation in transmission characteristics. Under these conditions, the water temperature reached a maximum of 25°C during periods of illumination, and dropped to a minimum of 17°C during the "off" periods of the cyclic exposure. The average temperature during the 24-hour cycle was 21°C.

The developing embryos remained in their original aquatic medium until time of hatching at approximately developmental stage 18[3]. Twice weekly during the free-swimming stages, the

[3]Staging of embryological development was done according to the method described by Gosner (1960).

larvae were placed in clean dishes containing a fresh supply of medium, and fed small pellets of Purina Commercial Rabbit Chow Checkers (see Appendix D). A chronological study of all groups was made to record morphological abnormalities, rates of development, and lethal effects. Representative specimens were removed periodically and fixed for microscopic examination. Serial sections were prepared and a trichrome stain was used to examine for histological abnormalities of the cornea and integument (See Appendix E).

Special Methods for Study #3

Effects of Exposure of Fertile Eggs to Enhanced UV-B Radiation: I.

Of the 1080 eggs utilized, 90% attained first cleavage. Prior to first cleavage, strings of ten eggs were cultured in the polystyrene dishes containing well water. Six groups of eighteen dishes were arranged so that half of each group (nine dishes) was placed under Mylar filters and the other half was placed under Kodacel filters (Figure 7). The tadpoles in the dishes beneath the Mylar filters served as controls for potential UV-B effects. Two schedules of lighting periods were employed using exposure apparatus

Figure 7. A group of eighteen culture dishes arranged on shelf of exposure apparatus, with nine dishes under Kodacel-TA401:0.005" and nine under Mylar 'S':0.005". Two sides of acrylic filter frame are raised to allow for ventilation.

similar to Study #2. All groups in this study were on cyclic exposure schedules, the photoperiod for the Vita-Lites being 14 hours "on" and then 10 hours "off". Each of the four groups exposed to radiation from fluorescent sunlamps was on a different schedule of supplemental exposure centered in the Vita-Lite "on" cycle (Figure 8 and Table 4). The presolarized Kodacel filters were changed after every 56-70 hours of peak exposure to the sunlamps, and the Mylar was changed after every 112-140 hours of peak exposure. Under these exposure conditions, the water temperature reached 25°C during periods of illumination and dropped to a minimum of 14°C during the "off" periods of the cyclic exposure. The average temperature during the 24-hour cycle was 21°C. The range of temperatures achieved during the cyclic photoperiods was independent of the type of filter utilized and length of peak exposure.

As in Study #2, the developing embryos were allowed to remain in their original aquatic medium until time of hatching at approximately developmental stage 18. At this time in this study, all but one of the larvae were removed from each of the culture dishes in order to minimize growth inhibition due to crowding (Licht, 1967; Richards, 1962). Three times weekly, during the free-swimming stages the larvae were placed in clean dishes containing a fresh supply of medium, and fed. As in

Figure 8. Radiation schedule schematics for tadpoles with regard to UV-B enhanced exposure schedule during daily 14-hour radiation periods alternated with 10-hour dark periods for Study #3. Other tadpoles were exposed concurrently on the same schedules beneath Mylar filters as controls for the effects of the UV-B spectral region. Source of radiation during exposure periods: two Vita-Lites plus six fluorescent sunlamps (), two Vita-Lites plus four fluorescent sunlamps (), two Vita-Lites plus two fluorescent sunlamps (), two Vita-Lites only (), four Vita-Lites only ().

Group

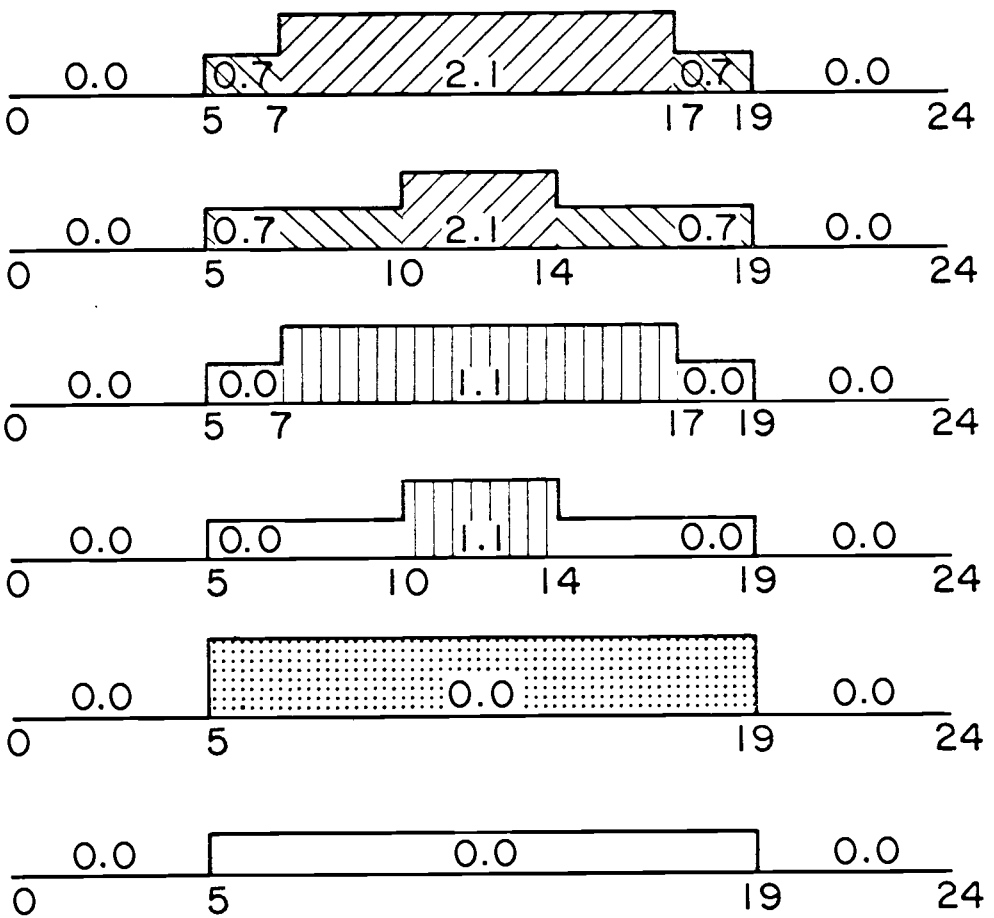

1 Exposure rate (SU·h⁻¹):
0.0
0.7
2.1
0.7
0.0
Hour of cycle:
0
5
7
17
19
24
2 Exposure rate (SU·h⁻¹):
0.0
0.7
2.1
0.7
0.0
Hour of cycle:
0
5
10
14
19
24
3 Exposure rate (SU·h⁻¹):
0.0
0.0
1.1
0.0
0.0
Hour of cycle:
0
5
7
17
19
24
4 Exposure rate (SU·h⁻¹):
0.0
0.0
1.1
0.0
0.0
Hour of cycle:
0
5
10
14
19
24
5 Exposure rate (SU·h⁻¹):
0.0
0.0
0.0
Hour of cycle:
0
5
19
24
6 Exposure rate (SU·h⁻¹):
0.0
0.0
0.0
Hour of cycle:
0
5
19
24

Table 4. Radiation conditions for Study #3. Sources of radiation: Vita-Lite fluorescent lamps and Westinghouse-FS40 fluorescent sunlamps. One-half of the tadpoles were cultured under Kodacel filters and the other half under Mylar filters.

Group	Radiation source	Filter	Total daily UV-B exposure (SU)
1	2 Vita-Lites + 6 FS40 sunlamps	Mylar	0.8
1	2 Vita-Lites + 6 FS40 sunlamps	Kodacel	23.8
2	2 Vita-Lites + 6 FS40 sunlamps	Mylar	0.5
2	2 Vita-Lites + 6 FS40 sunlamps	Kodacel	15.4
3	2 Vita-Lites + 4 FS40 sunlamps	Mylar	0.4
3	2 Vita-Lites + 4 FS40 sunlamps	Kodacel	11.0
4	2 Vita-Lites + 4 FS40 sunlamps	Mylar	0.2
4	2 Vita-Lites + 4 FS40 sunlamps	Kodacel	4.4
5	4 Vita-Lites	Mylar	0.0
5	4 Vita-Lites	Kodacel	0.0
6	2 Vita-Lites	Mylar	0.0
6	2 Vita-Lites	Kodacel	0.0

Study #2 chronological studies were made on all groups, and representative specimens were sectioned for miscroscopic examination.

Special Methods for Study #4

Effects of Exposure of Fertile Eggs to Enhanced UV-B Radiation: II.

Of the 1440 eggs utilized, 83% were fertile. Prior to first cleavage, strings of ten eggs were cultured in polystyrene dishes containing well water. Six groups of twenty-four dishes were arranged so that half of each group was placed under Mylar filters and the other half was placed under Kodacel filters. As with the previous study, the tadpoles in the dishes beneath the Mylar filters served as controls for potential UV-B spectral effects. All groups in this study were on cyclic exposure schedules, the photoperiod for the Vita-Lites being 14 hours "on" and then 10 hours "off". Each of the six groups was on a different schedule of supplemental exposure to radiation from the FS40 sunlamps (Figure 9 and Table 5). The presolarized Kodacel filters were changed after every 56-70 hours of peak exposure to the sunlamps, and the Mylar was changed after every 112-140 hours of peak exposure. Under these exposure conditions, the water temperature reached 25°C during periods of illumination, and dropped to a

Figure 9. Radiation schedule schematics for tadpoles with regard to UV-B enhanced exposure schedule during daily 14-hour radiation periods alternated with 10-hour dark periods for Study #4. Other tadpoles were exposed concurrently on the same schedules beneath Mylar filters as controls for the effects of the UV-B spectral region. Source of radiation during exposure periods: two Vita-Lites plus six fluorescent sunlamps (), two Vita-Lites plus four fluorescent sunlamps (), two Vita-Lites plus two fluorescent sunlamps (), two Vita-Lites only ().

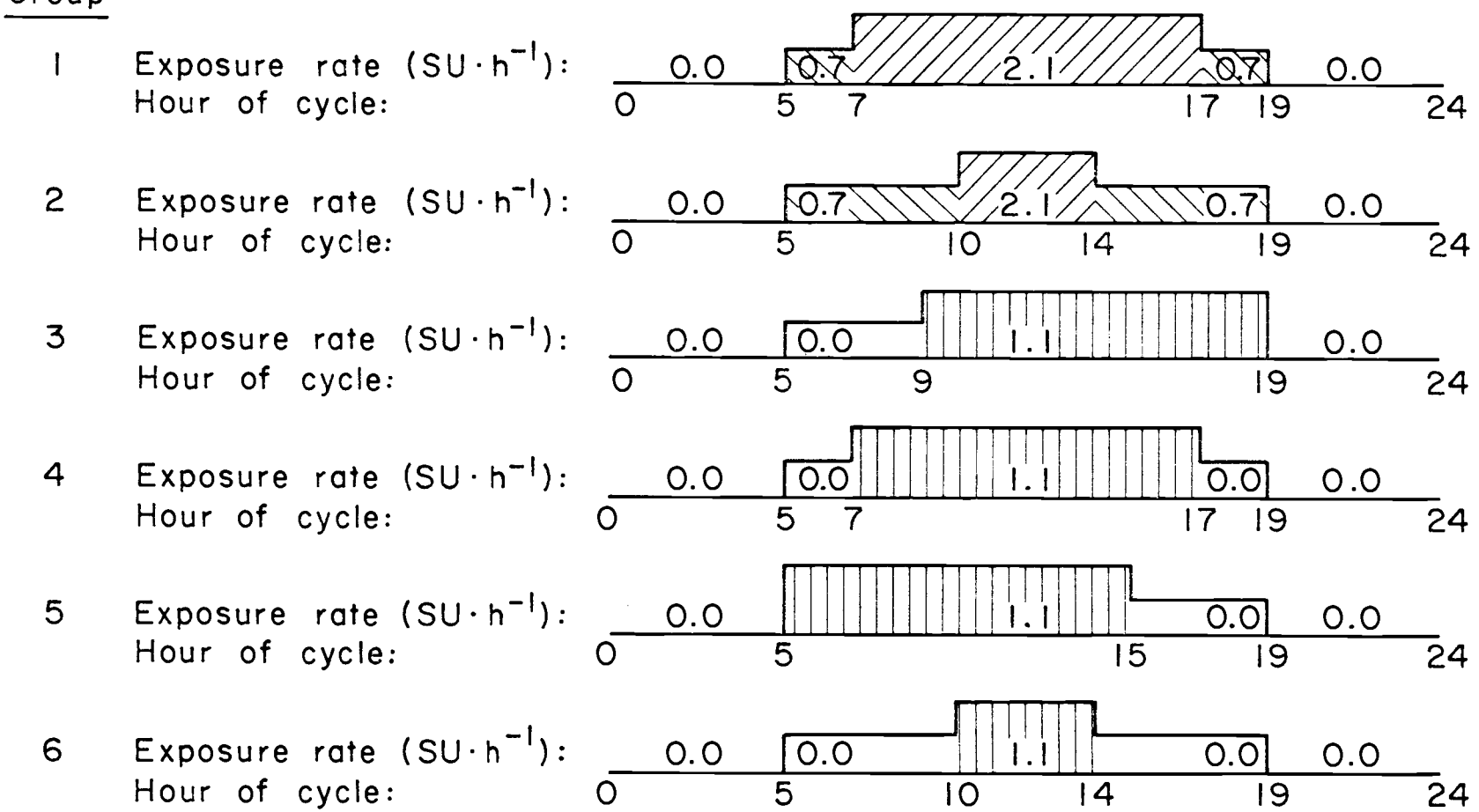

Group
1
Exposure rate (SU·h⁻¹):
Hour of cycle:
0.0
0.7
2.1
0.7
0.0
0 5 7 17 19 24
2
Exposure rate (SU·h⁻¹):
Hour of cycle:
0.0
0.7
2.1
0.7
0.0
0 5 10 14 19 24
3
Exposure rate (SU·h⁻¹):
Hour of cycle:
0.0
0.0
1.1
0.0
0 5 9 19 24
4
Exposure rate (SU·h⁻¹):
Hour of cycle:
0.0
0.0
1.1
0.0
0.0
0 5 7 17 19 24
5
Exposure rate (SU·h⁻¹):
Hour of cycle:
0.0
1.1
0.0
0.0
0 5 15 19 24
6
Exposure rate (SU·h⁻¹):
Hour of cycle:
0.0
0.0
1.1
0.0
0.0
0 5 10 14 19 24

Table 5. Radiation conditions for Study #4. Sources of radiation: Vita-Lite fluorescent lamps and Westinghouse-FS40 fluorescent sunlamps. One-half of the tadpoles were cultured under Kodacel filters and the other half under Mylar filters.

Group	Radiation source	Filter	Total daily UV-B exposure (SU)
1	2 Vita-Lites + 6 FS40 sunlamps	Mylar	0.8
1	2 Vita-Lites + 6 FS40 sunlamps	Kodacel	23.8
2	2 Vita-Lites + 6 FS40 sunlamps	Mylar	0.5
2	2 Vita-Lites + 6 FS40 sunlamps	Kodacel	15.4
3	2 Vita-Lites + 4 FS40 sunlamps	Mylar	0.4
3	2 Vita-Lites + 4 FS40 sunlamps	Kodacel	11.0
4	2 Vita-Lites + 4 FS40 sunlamps	Mylar	0.4
4	2 Vita-Lites + 4 FS40 sunlamps	Kodacel	11.0
5	2 Vita-Lites + 4 FS40 sunlamps	Mylar	0.4
5	2 Vita-Lites + 4 FS40 sunlamps	Kodacel	11.0
6	2 Vita-Lites + 4 FS40 sunlamps	Mylar	0.2
6	2 Vita-Lites + 4 FS40 sunlamps	Kodacel	4.4

minimum of 18°C during the "off" periods of the cyclic exposure. The average temperature during the 24-hour cycle was 22°C. As in the previous study, the range of temperature achieved during the cyclic photoperiods was independent of the type of filter used, length of peak exposure, or location of peak exposure period within the photoperiod.

The developing embryos were allowed to remain in their original aquatic medium until time of hatching, at which time all but three of the larvae were removed from each of the culture dishes. The three chosen were selected for a vigorous, normal appearance. Three times weekly during the free-swimming stages the larvae were placed in clean culture dishes containing a fresh supply of medium, and fed. Chronological studies were made of all groups, and representative specimens were sectioned for microscopic examination.

RESULTS

Study #1

For the first 36 hours of development all groups of tadpoles progressed at a comparable rate. Following that, cellular debris was observed on the surface of those embryos exposed to the sunlamps, and they survived less than 42 hours after oviposition. The protective jelly layers of the embryos exposed to the fluorescent sunlamps lysed eight hours prior to the groups not exposed to the sunlamps. This lysis may have contributed to the early death of the embryos (Rugh, 1962a, p. 21; See Appendix F).

Study #2

Exposure of the tadpoles (those cultured in natural well water) to the simulated sunlight with enhanced UV-B did not significantly alter the survival rates by stage 26 (the first of the "limb bud" stages). Survival for all groups ranged from 88% to 95%. A dramatic shift occurred in the survival pattern by stage 30 (the stage immediately preceding toe development). Control survival continued high (88-89%), but, in contrast, chronic exposure resulted in no survivors, while daily exposure significantly decreased survival by this stage (Table 6). Only one tadpole exposed to the daily, supplemental

Table 6. Percent survival by Gosner (1960) stages 26 and 30 with respect to radiation conditions, exposure periods, and culture medium. Controls were exposed to sunlight-simulating spectral conditions without added UV-B radiation. The results for the UV-B controls are pooled for both "high" and "low" intensity exposures since there were no significant differences between the results of these two conditions. The 0.1 amphibian Ringer's solution is amphibian Ringer's solution which has been diluted to one-tenth the standard concentration. Numbers in parentheses are numbers of subjects.

	Chronic exposure		Cyclic exposure	
Stage of development	With UV-B enhancement	Without UV-B enhancement	With UV-B enhancement	Without UV-B enhancement
Stage 26				
0.1 amphibian Ringer's solution	42%* (95)	89% (177)	65%* (91)	88% (180)
well water	93% (83)	95% (181)	88% (92)	90% (178)
Stage 30				
0.1 amphibian Ringer's solution	0%* (95)	83% (177)	31%* (91)	79% (180)
well water	0%* (83)	89% (181)	65%* (92)	88% (178)

* $p < 0.01$ (Test of Significance involving differences of proportions of two binomially distributed populations: the population with UV-B enhancement compared with the population at the same developmental stage and reared in similar culture medium, without UV-B enhancement)

UV-B achieved metamorphic climax (stage 42), and it perished shortly thereafter. Rearing the tadpoles in synthetic pond water appeared to accelerate the appearance of all symptoms. For a period of time prior to death, an anomalous development of the dorsal aspect of the body was observed in tadpoles exposed to either chronic or cyclic supplemental UV-B. Approximately ten days prior to death, 94% of the tadpoles in those two groups developed a concave curvature of the body axis, resulting in a lordotic posture (Figures 10 and 11).

Another anomalous development that occurred with supplemental UV-B irradiation involved the epidermal pigmentation of the presumptive cornea. The epidermis of the toad larva normally consists of two distinct cell layers. The outer layer is one cell thick and the inner layer is one or two cells thick (Chapman and Dawson, 1961). Cell division occurs in both layers of the epidermis, each undergoing mitotic activity independent of the other (Weed, 1934). Pigment granules, located in the epidermal cells throughout the body of a young tadpole, normally disappear from the ocular region at approximately stage 23, producing a transparent cornea (Figure 10). While this contribution to the presumptive cornea is losing its pigmentation, the outer epidermal layer appears to fuse with the inner layer, until only the inner layer of cuboidal or pyramidal cells is prominent (Figure 12).

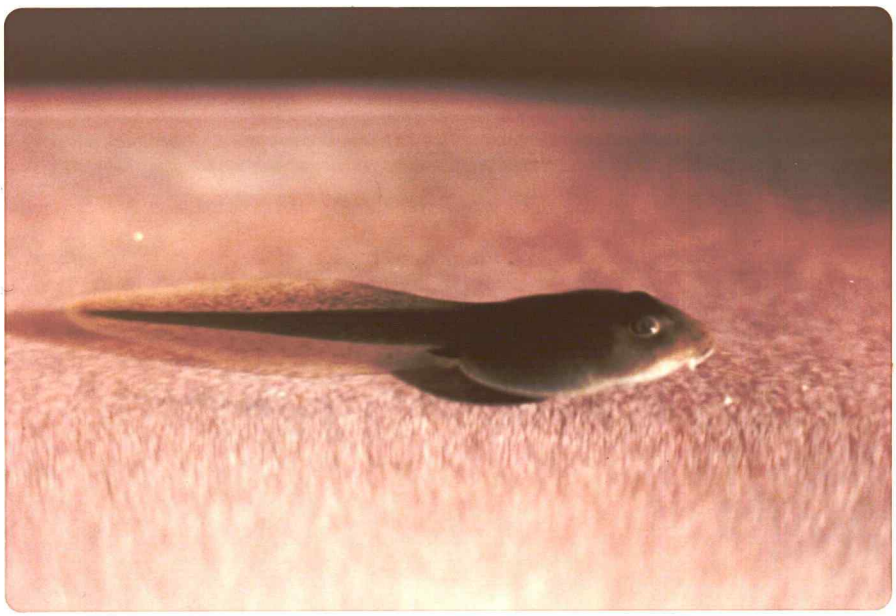


Figure 10. Control tadpole (stage 35, 37 mm) illustrating straight spinal column through body and tail, and transparent cornea. Cyclic exposure conditions: Mylar 'S':0.005" filter; two Vita-Lites plus two FS40 sunlamps - 14 hours "on" and then 10 hours "off"; four FS40 sunlamps - 10 hours "on" centered in the Vita-Lite "on" cycle.

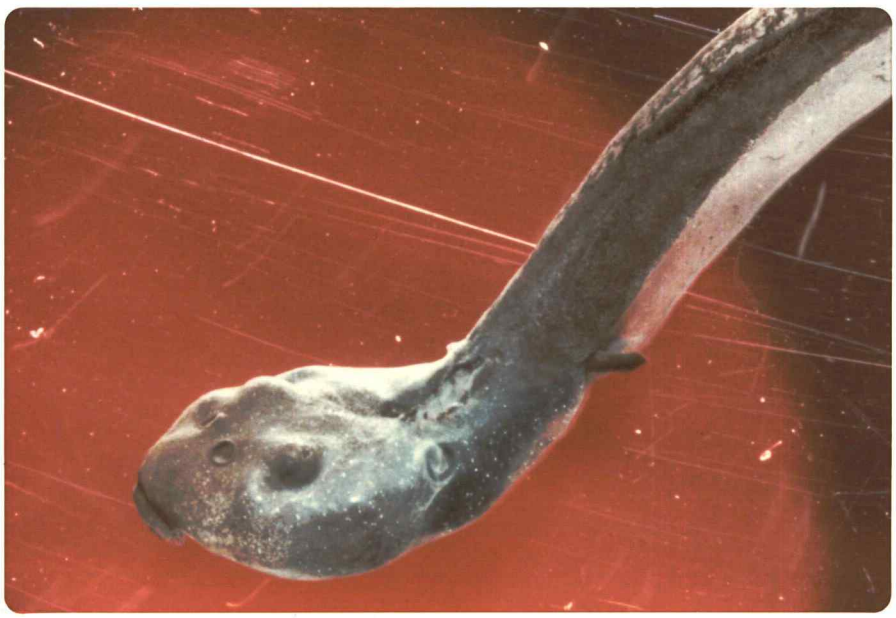


Figure 11. A tadpole (stage 34, 22 mm) illustrating anomalous condition typical of subjects exposed to enhanced UV-B radiation. This abnormally increased concavity in the curvature of the lumbar spine is termed "lordosis". Elapsed time since oviposition: 55 days. Cyclic exposure conditions: Kodacel-TA401:0.005" filter; two Vita-Lites - 14 hours "on" and then 10 hours "off"; four FS40 sunlamps - 10 hours "on" (1.1 $SU \cdot h^{-1}$) centered in the Vita-Lite "on" cycle.

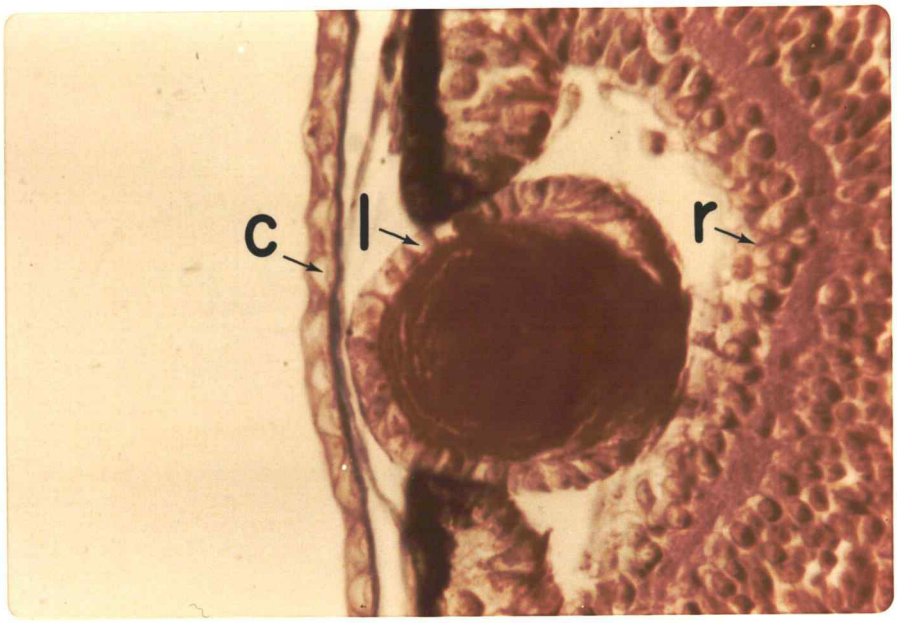


Figure 12. Section through eye of normal tadpole (stage 25) illustrating (c) fusion of epidermal layers of presumptive cornea, (l) lens, and (r) developing retina. Elapsed time since oviposition: 4 days.

The degree of pigmentation in the epidermis was enhanced by the exposure to supplemental UV-B radiation, with the entire corneal region remaining pigmented in those embryos continuously exposed to the sunlamps. In those embryos exposed cyclically to the UV-B supplemental light, only the dorsal area of the corneal region retained its pigmentation (Figure 13). This pigmentation persisted until death, which, for most of the tadpoles cultured in well water and on the daily exposure, occurred between stages 31 (the initiation of the "foot paddle" stages) and stage 38 (intermediate in premetamorphosis).

Histological studies demonstrated that the epidermal contribution to the presumptive corneas of subjects exposed to sunlamps did not develop in the normal fashion and, in some cases, areas of hyperplasia were evident (Figure 13). This increase in epidermal thickness also occurred in other regions of the tadpole's dorsal surface. Large areas were noted wherein the epidermis was four or five cells thick and the cells were arranged abnormally. Also, in many cases, figures of Eberth were absent from epidermal cells which normally should contain these fibrous, intracellular strands (Chapman and Dawson, 1961; Singer and Salpeter, 1961; Weed, 1934).

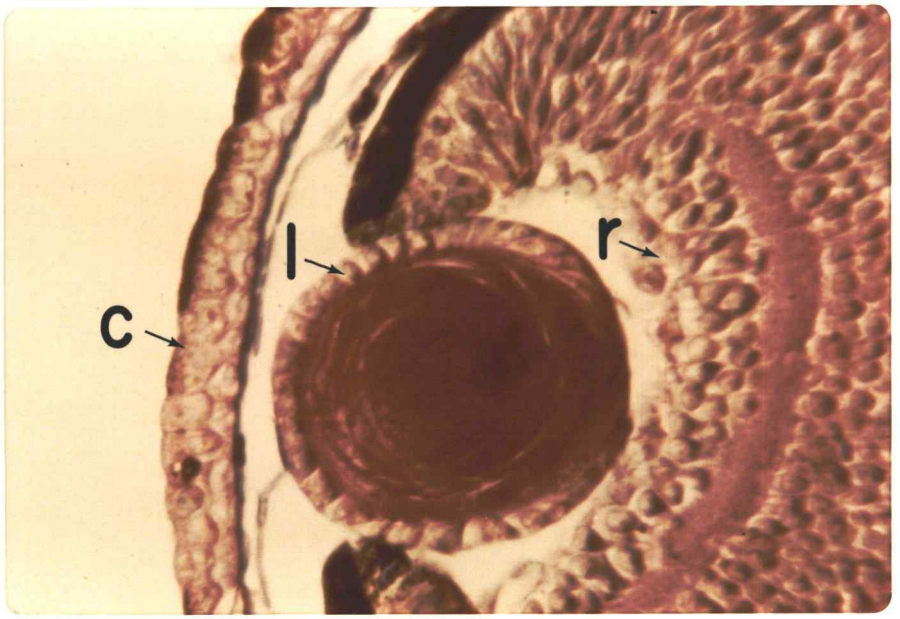


Figure 13. Section through eye of tadpole (stage 25) which had been continuously exposed through a Kodacel filter to two Vita-Lites plus six FS40 sunlamps. (c) Thickened presumptive cornea containing abnormal amount of pigment granules. (l) Lens. (r) Developing retina. Elapsed time since oviposition: 4 days.

Study #3

Exposure of the tadpoles to the simulated sunlight containing UV-B radiation under the exposure conditions of this study did not alter significantly the survival rates by stage 30 (Table 7): survival for all groups ranged from 89% to 100%. The two groups not exposed to supplemental radiation from the fluorescent sunlamps (Groups 5 and 6 of Figure 8) are excluded in Table 7, since there were no signifcant differences between the results of these two groups and the tadpoles screened from UV-B radiation by Mylar filters.

A significant shift in the survival pattern occurred by stage 35 (the last of the "foot paddle" stages) for the three groups receiving the greatest amount of UV-B radiation, Groups 1, 2, and 3 (see Figure 8). As seen in Table 7, survival remainded high in the controls (100%) and, also, in experimental Group 4 (78%), the group that received the smallest exposure to UV-B radiation of any of the experimental animals. No tadpole in the three groups accumulating the greatest UV-B exposure achieved metamorphic climax, with the population of Group 1 being depleted at the earliest date (Table 8). The groups of Mylar-filtered animals in this study had similar survival rates ($p > 0.05$, Fisher's exact test for

Table 7. Percent survival by Gosner (1960) stages 30 and 35 with respect to radiation conditions. Source of radiation: Vita-Lite fluorescent lamps plus Westinghouse-FS40 fluorescent sunlamps. Tadpoles under Kodacel filters were exposed to sunlight-simulating spectral conditions containing UV-B radiation. Tadpoles under Mylar filters were exposed to sunlight-simulating spectral conditions without UV-B radiation. N=9 in each column.

Exposure conditions (Fig. 8):	1		2		3		4	
	Kodacel filtered	Mylar filtered	Kodacel filtered	Mylar filtered	Kodacel filtered	Mylar filtered	Kodacel filtered	Mylar filtered
Accumulated daily dose (SU)+	(23.8)	(0.8)	(15.4)	(0.5)	(11.0)	(0.4)	(4.4)	(0.2)
Stage 30	89%	100%	100%	100%	100%	100%	89%	100%
Stage 35	22%*	100%	11%*	100%	44%**	100%	78%	100%

+ exposure as detected by a UV-B Meter (see Fig. 8 and Appendix C)

* $p < 0.01$ (Fisher's exact test for a 2 x 2 contingency table {Pearson and Hartley, 1966})

** $p < 0.05$ (Fisher's exact test for a 2 x 2 contingency table)

TABLE 8. Results of radiation conditions for Study #3. Sources of radiation: Vita-Lite fluorescent lamps and Westinghouse-FS40 fluorescent sunlamps. One-half of the tadpoles were cultured under Mylar filters and the other half under Kodacel filters.

Group (see Figure 8)	Filter	Daily radiant exposure (SU)+	Percent Survival
1	Mylar	0.8	89%
1	Kodacel	23.8	0%*
2	Mylar	0.5	89%
2	Kodacel	15.4	0%*
3	Mylar	0.4	89%
3	Kodacel	11.0	0%*
4	Mylar	0.2	78%
4	Kodacel	4.4	78%
5	Mylar	0.0	100%
5	Kodacel	0.0	100%
6	Mylar	0.0	89%
6	Kodacel	0.0	89%

+ exposure as detected by a UV-B Meter (see Appendix C)

* $p < 0.01$ (Fisher's exact test for a 2 x 2 contingency table)

a 2 x 2 contingency table [Pearson and Hartley, 1966]). As in the previous study, most tadpoles in the three groups with the greatest accumulation of UV-B exposure developed lordosis at an early stage. They also developed thick, pigmented corneas. The radiation damage to the dorsal surface in most of these larvae was severe and, in many, the insult resulted in desquamation and acute ulceration that exposed the deep muscle tissue (Figure 14). There was significant retardation of the rate of development in the experimental group receiving the smallest exposure to UV-B radiation: 67% of the control group had achieved metamorphic climax before any of this group achieved that end-point.

Study #4

The design of four of the six groups in Study #4 were duplications of the design of four groups in Study #3 (compare Figures 8 and 9), but with an increased number of subjects. As in Study #3, exposure of the tadpoles to the simulated sunlight containing UV-B radiation under the exposure conditions of this study did not alter significantly the survival rates by stage 30 (Table 9): survival for all groups ranged from 97% to 100%. A significant shift in the survival pattern occurred by stage 35 (the last of the "foot

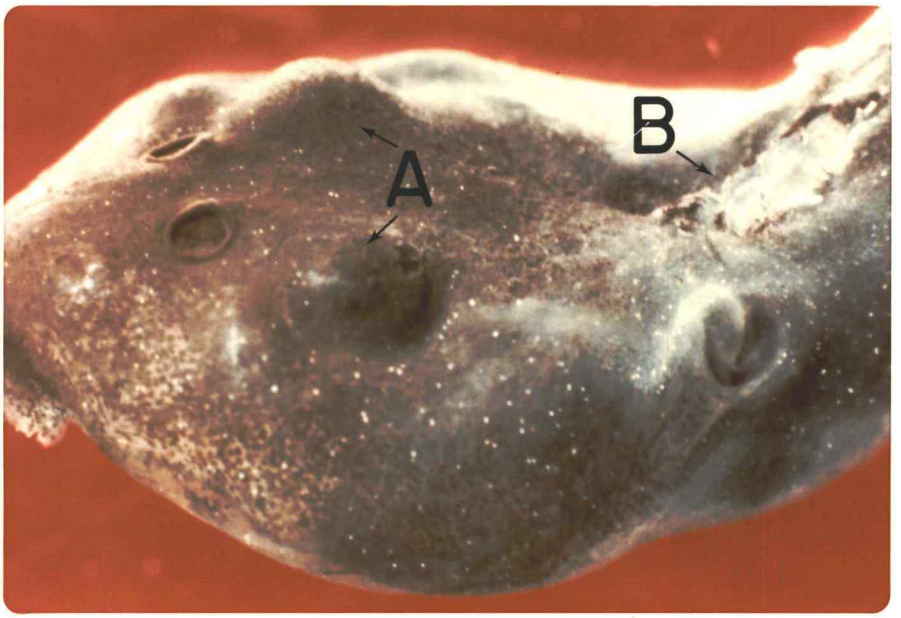


Figure 14. A tadpole (stage 34, 22 mm) demonstrating (A) thick, pigmented corneas and (B) severe ulceration of dorsal skin. Elapsed time since oviposition: 55 days. Cyclic exposure conditions: Kodacel-TA401:0.005" filter; two Vita-Lites - 14 hours "on" and then 10 hours "off"; four FS40 sunlamps - 10 hours "on" (1.1 $SU \cdot h^{-1}$) centered in the Vita-Lite "on" cycle.

Table 9. Percent survival by Gosner (1960) stages 30 and 35 with respect to radiation conditions. Source of radiation: Vita-Lite fluorescent lamps plus Westinghouse-FS40 fluorescent sunlamps. Tadpoles under Kodacel filters were exposed to sunlight-simulating spectral conditions containing UV-B radiation. Tadpoles under Mylar filters were exposed to sunlight-simulating spectral conditions without UV-B radiation. N = (34-36) in each column.

Exposure conditions (Fig. 9):	1		2		3		4		5		6	
	Kodacel filtered	Mylar filtered	Kodacel filtered	Mylar filtered	Kodacel filtered	Mylar filtered	Kodacel filtered	Mylar filtered	Kodacel filtered	Mylar filtered	Kodacel filtered	Mylar filtered
Accumulated daily dose (SU)+	(23.8)	(0.8)	(15.4)	(0.5)	(11.0)	(0.4)	(11.0)	(0.4)	(11.0)	(0.4)	(4.4)	(0.2)
Stage 30	97%	100%	100%	100%	100%	100%	100%	97%	100%	100%	100%	100%
Stage 35	0%*	97%	38%*	100%	0%*	100%	66%*	94%	92%**	100%	100%	100%

+ exposure as detected by a UV-B Meter (see Fig. 9 and Appendix C)

* $p < 0.01$ (one-tailed test of significance involving differences of proportions of two binomially distributed populations)

** $p < 0.05$ (one-tailed test of significance involving differences of proportions of two binomially distributed populations)

paddle" stages) for all experimental groups except the group receiving the smallest cumulative daily radiant exposure (Group 6). As seen in Table 9, survival remained high in controls (94-100%) and in experimental Group 6 (100%) which received the least exposure to UV-B radiation of any of the experimental animals. Of the four groups exposed to Kodacel-filtered radiation emitted by fluorescent sunlamps whose "on" cycle was centered in the "on" cycle of the Vita-Lite fluorescent lamps (Groups 1, 2, 4 and 6), there was an inverse relationship between cumulative daily exposure to UV-B radiation and both the percent survival and the mean elapsed time from oviposition until death if prior to metamorphic climax (Table 10 and Figure 15). The mean elapsed times to achieve metamorphic climax for the Mylar-filtered animals in these four groups were not significantly different from one another at the five percent level (two-tailed "t" test) (Table 10). Although the percent survival for the Mylar-filtered (97%) and Kodacel-filtered (86%) animals of Group 6 (low-irradiance group) were not significantly different from one another at the five percent level, it took the 30 Kodacel-filtered animals significantly longer to achieve metamorphic climax than the 34 Mylar-filtered animals in the same group (Table 10).

Table 10. Results of radiation conditions for Study #4. Sources of radiation: Vita-Lite fluorescent lamps and Westinghouse-FS40 fluorescent sunlamps. One-half of the tadpoles were cultured under Mylar filters and the other half under Kodacel filters. (See Appendix G for an itemized listing of the days required to achieve the two end-points: (1) metamorphic climax and (2) death, if prior to metamorphic climax.)

Group	Filter	Daily radiant exposure (SU)	End-point*	Mean elapsed time ±standard error(d)	Percent survival
1	Mylar	0.8	MC (34)	54.8±1.1	97%[a]
1	Mylar	0.8	D (1)	35	-
1	Kodacel	23.8	MC (0)	-	0%[a]
1	Kodacel	23.8	D (35)	30.6±0.6	-
2	Mylar	0.5	MC (34)	57.4±1.2	100%[b]
2	Mylar	0.5	D (0)	-	-
2	Kodacel	15.4	MC (0)	-	0%[b]
2	Kodacel	15.4	D (34)	50.9±1.3	-
3	Mylar	0.4	MC (34)	53.4±1.7	97%[c]
3	Mylar	0.4	D (1)	62	-
3	Kodacel	11.0	MC (0)	-	0%[c,f]
3	Kodacel	11.0	D (34)	40.9±0.9[g]	-
4	Mylar	0.4	MC (34)	53.9±1.4	94%[d]
4	Mylar	0.4	D (2)	21.5±2.5	-
4	Kodacel	11.0	MC (6)	51.7±1.3[h]	17%[d,f,j]
4	Kodacel	11.0	D (29)	55.6±2.0[g,k]	-
5	Mylar	0.4	MC (36)	53.8±1.3	100%[e]
5	Mylar	0.4	D (0)	-	-
5	Kodacel	11.0	MC (13)	66.0±3.3	41%[e,j]
5	Kodacel	11.0	D (19)	62.6±2.7[k]	-
6	Mylar	0.2	MC (34)	54.2±1.5[i,h]	97%
6	Mylar	0.2	D (1)	38	-
6	Kodacel	4.4	MC (30)	58.5±2.0[i]	86%
6	Kodacel	4.4	D (5)	68.8±4.1	-

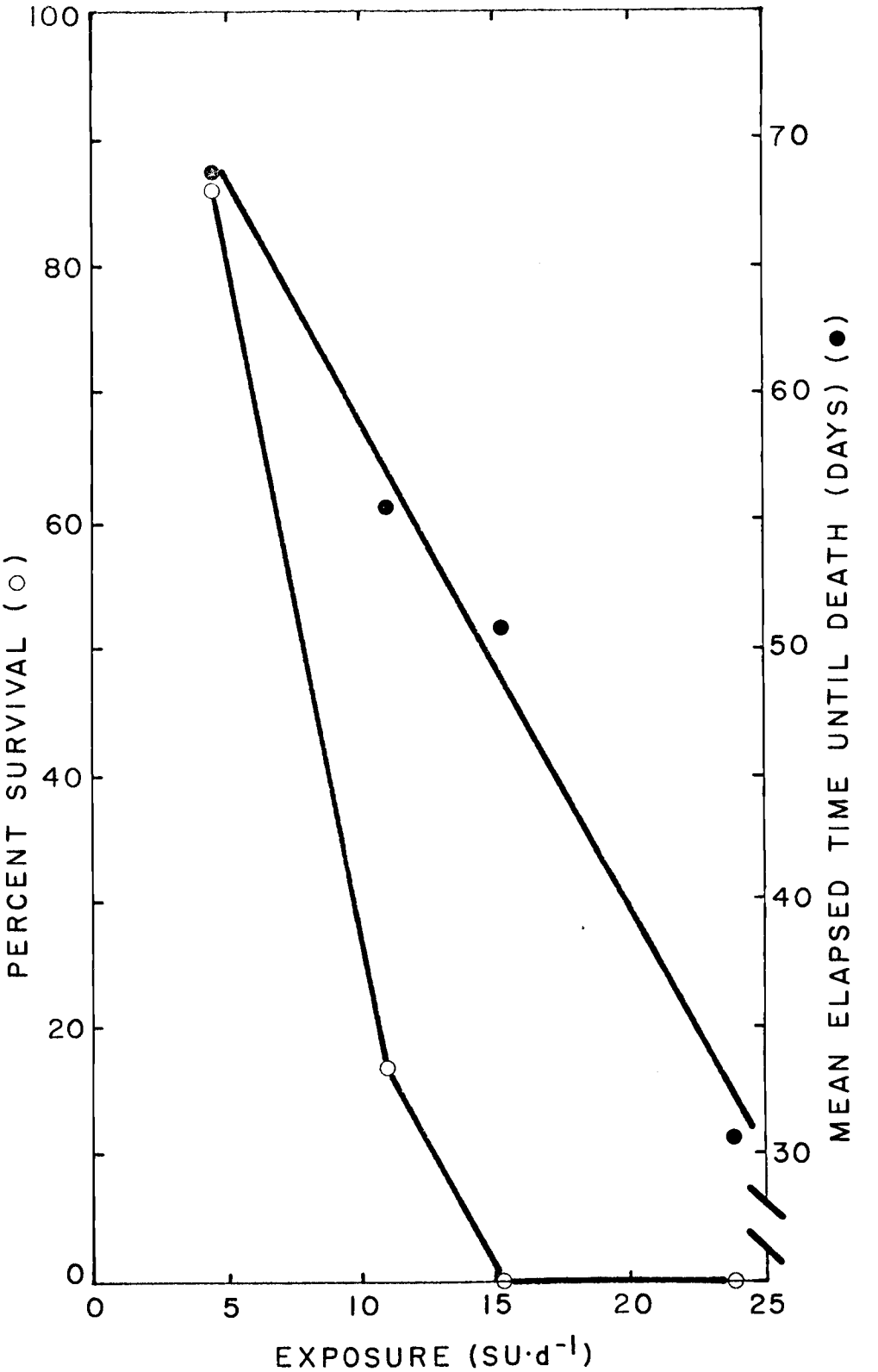
* MC = metamorphic climax (stage 42).

D = death prior to achieving metamorphic climax.

Numbers in parentheses are numbers of subjects.

Figures with similar alphabetical superscripts are significantly different (a-h: $p < 0.01$; i-k: $p < 0.05$).

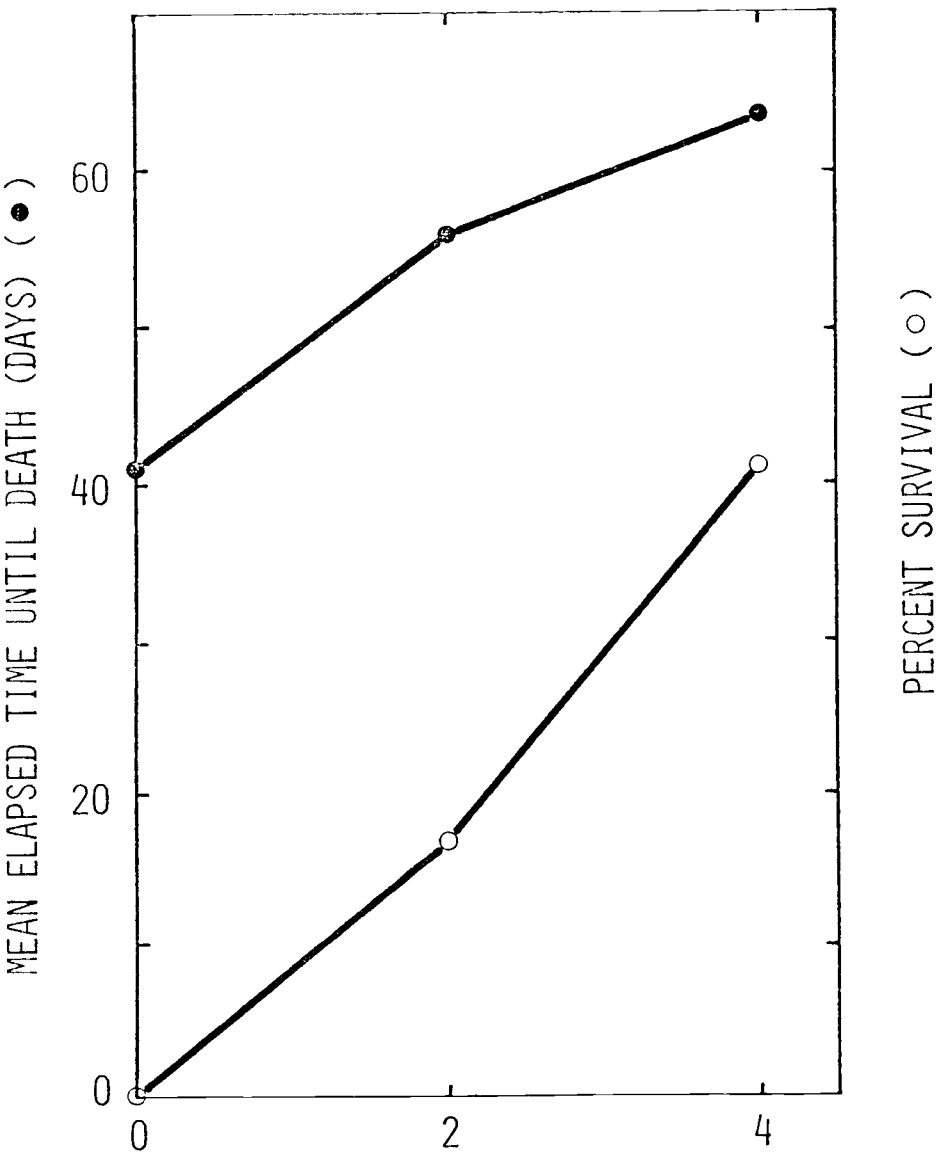
Figure 15. Percent survival, on the one hand, and mean elapsed time until death prior to metamorphic climax on the other, as related to accumulated daily dose of UV-B radiation. The four groups involved were those tadpoles in Study #4 who were exposed to Kodacel-filtered radiation emitted by fluorescent sunlamps in which the "on" cycle was centered in the "on" cycle of two Vita-Lite fluorescent lamps (Groups 1, 2, 4 and 6; see Fig. 9). The line drawn for the "Mean Elapsed Time until Death" is a least squares line of best fit and has a slope of -1.9.


100
80
60
40
20
0
PERCENT SURVIVAL (○)
0
5
10
15
20
25
EXPOSURE (SU·d⁻¹)
70
60
50
40
30
MEAN ELAPSED TIME UNTIL DEATH (DAYS) (●)

The Kodacel-filtered animals in the three groups (Groups 3, 4 and 5) were exposed to similar radiation sources and to similar daily radiant exposures (11.0 $SU \cdot d^{-1}$) at similar peak radiant exposure rates (1.1 $SU \cdot h^{-1}$). All three groups exhibited a direct relationship between the length of daily exposures to photoreactivating radiation following UV-B insult and both the percent survival and the mean elapsed time until death if prior to metamorphic climax (Figure 16). These three groups were exposed to varied time periods (0, 2 and 4 hours) of UV-A and visible radiation following the termination of the UV-B exposure. The percent survival and mean elapsed time until metamorphic climax for the Mylar-filtered animals of these three groups were not significantly different from one another at the five percent level (Table 10).

Most tadpoles in the four groups irradiated under the more severe conditions (Groups 1, 2, 3 and 4) developed lordosis at an early stage. They also developed thick, pigmented corneas. The radiation damage to the dorsal surface of most of the larvae which perished prior to metamorphic climax was severe, resulting in epidermal hyperplasia and desquamation.

Figure 16. Percent survival and mean elapsed time from oviposition until death prior to metamorphic climax for the three groups in Study #4 exposed to comparable daily exposures (11.0 $SU \cdot d^{-1}$) at a comparable rate (1.1 $SU \cdot h^{-1}$) from similar radiation sources. The critical difference between the groups was the duration of exposure to UV-A and visible radiation (0, 2 and 4 hours) following the termination of the UV-B exposure.


MEAN ELAPSED TIME UNTIL DEATH (DAYS) (●)
PERCENT SURVIVAL (○)
60
40
20
0
0
2
4
LENGTH OF DAILY EXPOSURE TO PHOTOREACTIVATING RADIATION FOLLOWING UV-B INSULT (HOURS)

DISCUSSION

Overview of Effects of Ultraviolet Radiation

Solar radiation acts as a two-edged sword. Among other benefits it provides us with essential energy required for the vital process of photosynthesis and vision, as well as a timing mechanism for many rhythmic processes of life. On the debit side, solar ultraviolet radiation can cause sunburn, skin cancer, skin aging, mutations and cell death. Organisms must be able to tolerate this damage in order to benefit from the essential aspects of solar radiation, a dichotomy which leads to the existence of a delicate balance between the damage and repair mechanisms (Smith, 1972).

Quality versus Quantity of Radiant Energy

This series of studies has demonstrated that ultraviolet radiation in the range from 290 nm to 315 nm does effect the development of boreal toad tadpoles. It appears as if spectral quality, rather than total irradiance, is the critical factor affecting the results. In the second study, for example, the group exposed to the light supplemented by radiation from sun-lamps displayed considerable damage when compared with the group exposed to high intensity "white" light; yet the total irradiances

received by these two groups were similar. The failure to find any significant differences in development between the tadpoles exposed to high intensity "white" light and those exposed to low intensity "white" light, where total irradiances were considerably different, further supports the concept that total irradiance is not the critical factor. In the third and fourth studies, groups receiving very similar total irradiances again displayed markedly different results when comparisons were made between those exposed to UV-B radiation and those not exposed to UV-B radiation. These results indicate that the presence or absence of UV-B radiation is a critical factor adversely affecting development in these studies.

Increased Sensitivity at Critical Phases of Development

A significant portion of the abnormalities and mortalities were observed during the limb-bud and foot-paddle stages (stages 26-35) regardless of the total radiant exposure received by the organisms in this series of studies prior to this critical phase of development. It is during this time period that true metamorphosis is initiated; that thyroid activity is stimulated, and tissue response can occur. The development of limb buds

signals the beginning of the transitions to a terrestrial form for those larval structures and physiological processes which are currently adapted to aquatic life. This transition results in alteration of biochemical and physiological mechanisms and anatomical structures of the tadpole. Deuchar (1966), Etkin (1955), Frieden (1961), and Kollros (1961) give excellent accounts of the tissue changes as well as the biochemical transformations which result, either directly or indirectly, from the increased thyroid activity and increased tissue sensitivity to thyroid secretions. It is conceivable that increased stress factors, such as exposure to increased UV-B radiation, could result in an increased number of abnormalities and mortality at this critical phase. In the studies utilizing synthetic pond water as a culture medium (Studies #1 and #2), the severity of the stress appeared to be augmented. This may have been due to a salt-concentration imbalance (Barth and Barth, 1974a; Barth and Barth, 1974b; Rugh, 1962a) or to a relatively high concentration of copper in the distilled water used in making the amphibian Ringer's solution (see Appendix A).

Exposure of the tadpoles to UV-B radiation through all stages of development might be expected to create a variety of abnormalities. Rugh (1953) has reported that when frog embryos at stage 18 were exposed to 50 kiloroentgen of x-rays

(184 kVp), they generally developed lordosis. When the embryos were exposed while at stage 25, they developed localized edema in the head region, but no lordosis. Rugh explained these, and other, results on the basis of varying regional sensitivities at different stages of differentiation. Exposure of the embryo to damaging radiation while the axial skeleton is being formed might be expected to produce structural abnormalities. It may be that, in the case of UV-B exposure, the exposure need be made only during some critical stage of development in order to produce particular abnormalities. If this is the case, the duration of exposure required may need to be only a fraction of what was employed in the present series of studies.

Any biological effects incurred by an organism through exposure to ultraviolet radiation are due to the absorption of specific wavelengths by particular molecules with resultant photochemical alteration (Environmental Studies Board, 1973). A delicate balance exists between this photochemical alteration and an organism's capacity to repair the damage. If the capacity to repair the damage is exceeded, the organism or injured cells will die. The most critical target (or chromatophore) in tissues is DNA. This is true because there are

relatively few copies of specific sequences of DNA in each cell; whereas other molecules exist in multiple copies, resulting in the possibility of little damage if only a few copies are damaged. As Caldwell (1971) indicates, the action spectra for animal cell response to UV-B radiation usually coincide with the corresponding tails of the absorption spectra of nucleic acids and proteins (Figure 17). The extreme lethality of the unfiltered sunlamps in Study #1 may be attributed to the presence of highly effective wavelengths shorter than 290 nm (Figure 18).

Biological Photoreactivation

The results of Study #4 indicate that exposure to UV-A and/or visible radiation following the UV-B insult mitigates the potentially lethal damage to the tadpole. Groups 3, 4, and 5 of Study #4 were all exposed to similar radiation conditions. The critical difference between the groups appeared to be the length of exposure to UV-A radiation and visible light preceding and following the UV-B insult. All indications (percent survival, mean elapsed time until metamorphic climax, and mean elapsed time until death if prior to metamorphic climax) point out that biological photoreactivation did occur (Table 10).

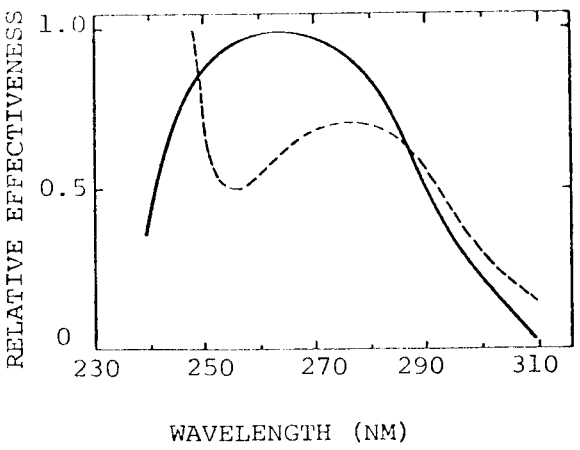


Fig. 17. Generalized absorption spectra for nucleic acids (solid line) and proteins (broken line). (After Caldwell, 1971)

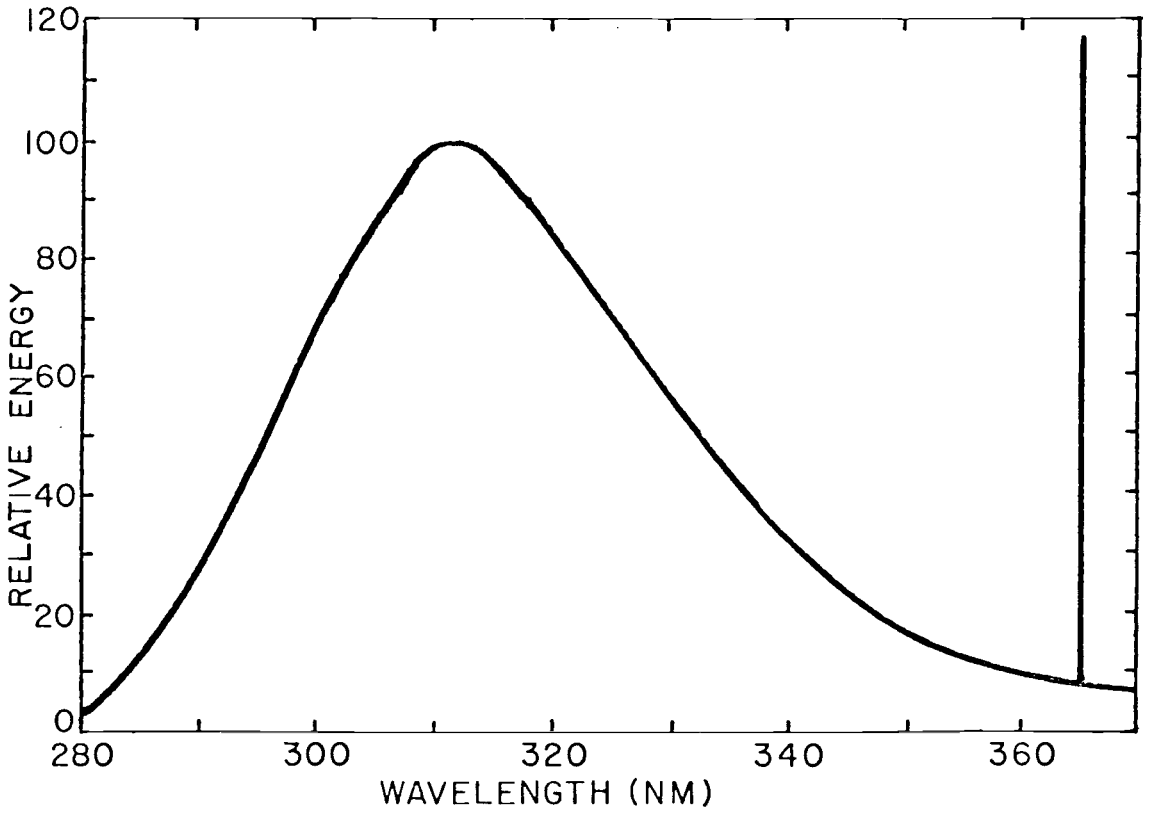


Figure 18. Emission spectrum of Westinghouse-FS40 fluorescent sunlamp. Closely spaced major emission lines of mercury at 366 nm are prominent, and are denoted as a single line. Spectrum based on information supplied by Westinghouse Electric Corporation.

The most commonly studied type of photoreactivation involves the monomerization of pyrimidine dimers in DNA by a photoreactivating enzyme. The dimers are formed by the absorption of short wavelengths of ultraviolet radiation by adjacent pyrimidines, creating cyclobutyl links. The pyrimidine dimers (commonly thymine dimers) are the only known substrate for the photoreactivating enzyme, which reversibly binds with the dimers until radiation of longer wavelengths (310-500 nm) is absorbed by the complex, at which time the dimers are split and the enzyme released. Under normal light conditions, this process may take from minutes to hours for maximum results. Among the vertebrates, photoreactivating enzyme activity has been identified in the fish (Cook and McGarth, 1967; Regan and Cook, 1967), toad, frog, chicken embryo (Cook and McGarth, 1967), South American Woolly opossum, Tasmanian rat kangaroo, North American opossum (Cook and Regan, 1969), and human leucocytes and fibroblasts (Sutherland, 1974; Sutherland et al., (1975).

Blum et al. (1949) and Marshak (1949) first described the photoreactivation phenomenon in animal cells (sea urchin eggs) in 1949. In 1950, Blum and Mathews (1950) described the photoreactivation of potentially sub-lethal ultraviolet damage to salamander larvae by UV-A and visible radiation, marking the first report of photoreactivation in a vertebrate. Zimskind and Schisgall (1955) describe the appearance, within 2-4 days, of pigmented streaks on the dorsal surface and melanization of the cornea after irradiating Rana pipiens (16-44 mm) and Rana catesbiana (45-85 mm) larvae with ultraviolet radiation emitted by an intermediate-pressure, mercury-vapor lamp (effective wavelengths less than 313 nm). The increased melanization could be avoided if the short-wavelength exposure was followed by illumination from a "daylight" fluorescent lamp, and Zimskind and Schisgall suggested the presence of a photoreactivation process. They determined that the pigment streak was a local process, not influenced by altered pituitary function. Daily sub-effective doses over an extended period would produce similar, localized melanization within 3-6 days, and could be photoreactivated in a manner similar to the single dose experiment.

Biological photoreactivation of eucaryotic organisms may be comprised of one mechanism or any combination of four different mechanisms (Cook, 1970):

> (a) Direct photoenzymatic repair of UV-induced lesions in DNA ...
> (b) Indirect photoreactivation ... 'in which the molecular site of action of the far ultraviolet radiation is not immediately and directly altered by the energy of the photoreactivating photon.'
> (c) Direct (nonenzymatic) photoreversal of UV lesions may in some cases be accomplished by wavelengths other than the one used for the initial radiation.
> (d) It is possible that biological photoreactivation may be observed if the 'reactivating' light induces a response antagonistic to the response induced by the first irradiation, thereby reducing the initial response without repairing the initial lesion.

With indirect photoreactivation (Mechanism b), pretreatment with photoreactivating radiation will be as effective as posttreatment. The results of Study #4 (Groups 3, 4, and 5) do not follow this pattern, indicating photoreactivation by another mechanism in this study. If the damage is due to the production of cyclobutyl pyrimidine dimers, then absorption of wavelengths in the vicinity of 240 nm could conceivably monomerize the dimers, resulting in a direct (non-enzymatic) photoreversal of the ultraviolet lesions (Mechanism c). The shortest wavelengths to which the tadpoles in Study #4 were exposed were in the UV-B region, wavelengths where dimers do not absorb strongly, thereby ruling out the direct (non-enzymatic) photoreactivation process in that study. This

leaves two remaining possibilities: (a) direct photoenzymatic repair of UV-induced lesions in DNA, and (d) the 'reactivating' light induced a response antagonistic to the response induced by UV-B exposure, thereby reducing the initial response without repairing the initial lesion.

Anthropogenic Assaults on Atmospheric Ozone

Nitrogen Oxides

It is conceivable that an increase of radiant exposure in the UV-B region may be occurring due to an anthropogenic assault on atmospheric ozone. Although the atmospheric ozone layer is only approximately one-third of a centimeter thick at STP, it is the principal absorber of solar radiation in the ultraviolet range from 220 nm to 320 nm (Gates, 1966; Green et al., 1974). Following absorption of ultraviolet radiation, the photochemical decomposition of ozone produces diatomic and singlet oxygen:

$$O_3 + h\nu \longrightarrow O_2 + O(^1D)$$

Ozone is concentrated primarily in a band at an altitude of 15-35 kilometers, with less ozone present at the equator than at the poles due to a flow of air toward the poles at

this altitude (Alyea et al., 1975; Crutzen, 1974a; Dütsch, 1974). Although the average ozone concentration in the atmosphere remains relatively stable, Johnston (1972) has calculated that the rate of ozone production is three times faster than the rate of ozone destruction based on "pure" air calculations (absorption of wavelengths shorter than 210 nm by oxygen leads to ozone formation). Johnston (1971) has calculated that one molecule of nitric oxide can, in time, destroy a large number of ozone molecules by means of a catalytic cycle:

$$(1)\quad NO + O_3 \longrightarrow NO_2 + O_2$$

$$(2)\quad O_3 + h\nu \longrightarrow O_2 + O$$

$$(3)\quad NO_2 + O \longrightarrow NO + O_2$$

$$\text{net result:}\quad (4)\quad 2\ O_3 + h\nu \longrightarrow 3\ O_2$$

The rate of reaction 3, the rate limiting reaction of the catalytic cycle, is 10,000 times faster than for the direct chemical destruction of ozone through combination with nascent oxygen (Johnston, 1972). The implication is that small increases in nitrogen oxide concentrations in the stratosphere will have marked effects on the rate of ozone destruction.

One of the major sources of nitric oxide in the stratosphere arises indirectly from the denitrifying bacteria, e.g., Pseudomonas denitrificans, which play an unheralded role in the nitrogen cycle. When there is an abundant supply of oxygen, the denitrifying bacteria use oxygen to oxidize organic molecules, but under oxygen deficient conditions nitrates and nitrites are utilized, yielding primarily nitrogen, and some nitrous oxide. The utilization of nitrates and nitrites occurs commonly in rich, wet soils and the ocean surface. The relatively inert nitrous oxide which is produced can slowly diffuse through the tropopause to altitudes of 20-30 kilometers where chemical and photochemical destruction occurs. This process may take from 10 to 70 years (Johnston, 1972). Singlet oxygen, which is usually deactivated by collisions with either nitrogen or oxygen, or by a chemical reaction with water, occasionally reacts with nitrous oxide. Approximately 50% of the time, this last reaction yields nitric oxide. Nitric oxide is very reactive and quickly reacts with ozone as shown in reaction 1. The nitrogen dioxide produced, besides being reconverted into nitric oxide, can react with hydroxyl radicals to produce nitric

acid (after dissolution in water). Some of the nitric acid diffuses through the tropopause and is trapped in rain to return to the surface of the earth. This nitrogen cycle is critically important to life on Earth because it plays a dominant role in regulating the stratospheric ozone concentration which, in turn, regulates the quantity and quality of ultraviolet radiation reaching the surface of the earth. It has been estimated that the natural flux of nitrogen oxides into the stratosphere lies somewhere between 2.6×10^8 and 12×10^8 $kg \cdot y^{-1}$ (Johnston, 1972).

Recently researchers have realized that nitrogen oxides found in the exhausts of supersonic transport airliners may partially deplete atmospheric ozone. Due to its large fuel consumption, a 500 member fleet of the 1970 design of the American SST would have introduced nitrogen oxides into the atmosphere at a rate three or four times as great as natural nitrogen oxide production. The exhausts of the British-French Concorde and Russian TU-144 SST engines introduce far less nitrogen oxides into the stratosphere, and a wide-body, subsonic airliner engine emits even less (Anderson, 1973; Jocelyn et al., 1973). In 1970, civil subsonic airliners emitted 0.95×10^7 kg of nitric oxide above the tropopause,

and military flights emitted 0.66 x 10^7 kg (Jocelyn et al., 1973). The operation of 500 Concorde-type airliners would be expected to emit approximately 3 x 10^8 kg·y^{-1} of nitric oxide, a doubling of the natural production (Jocelyn et al., 1973). The effect that the introduction of these massive amounts of nitrogen oxides would have on the ozone layer is still a matter of some speculation and the object of numerous investigations (e.g., Brewer, et al., 1973; Crutzen, 1972; Crutzen, 1974a; Crutzen, 1974b; Cutchis, 1974; Goldsmith et al., 1973; Grobecker et al., 1974; Hesstvedt, 1974; Johnston, 1971; McElroy et al., 1974; Wofsy and McElroy, 1974).

Because of the logarithmic relationship involved in ultraviolet absorption by ozone, an increase in the amount of nitrogen oxides in the stratosphere could result in a very large increase in the amount of ultraviolet radiation reaching the surface of the earth. The fraction of ultraviolet radiation passing through the ozone column, U/U_0, is calculated as follows:

$$U/U_0 = e^{-\alpha x}$$

where α is the absorption coefficient of ozone at a particular wavelength and x is the thickness of the column of ozone (Jagger, 1967). Johnston (1972) has calculated that a 5% decrease in the

atmospheric ozone concentration would result in a 26% increase in the amount of ultraviolet radiation at 298 nm reaching the earth; whereas a 50% decrease in ozone would result in a ten-fold increase in ultraviolet radiation at 298 nm. Bassett et al. (1974) have calculated that a 10% reduction in ozone at 40°N in spring would cause approximately a 55% increase in the intensity of the 300 nm ultraviolet radiation reaching the earth.

Halomethanes

More recently, concern has been expressed over the introduction of halomethanes (e.g. "Freons") into the stratosphere. At an altitude of approximately 30 kilometers these halomethanes absorb radiation of wavelengths less than 230 nm, yielding chlorine atoms and other photoproducts. The photochemical degradation products of the halomethanes can act as catalytic agents for the destruction of ozone by means of a catalytic series of reactions similar to the nitrogen oxides (Stolarski and Cicerone, 1974):

$$Cl + O_3 \longrightarrow ClO + O_2$$

$$O_3 + h\nu \longrightarrow O_2 + O$$

$$ClO + O \longrightarrow Cl + O_2$$

The near-exponential growth in consumption of chlorofluoromethanes as aerosol propellants and refrigerants has resulted in an annual worldwide production of nearly 10^9 kilograms (Hammond and Maugh II, 1974). The halomethanes are highly volatile, relatively insoluble in oceanic waters, and practically inert to chemical reaction, facts which indicate that nearly all the halomethanes which have been released to date are still resident in the atmosphere. This amounts to approximately 5×10^9 kilograms. Wilkniss et al. (1975) have observed a 67% increase of trichlorofluoromethane (e.g. "Freon-11") between 1971 and 1974 in the troposphere, an increase proportional to the increase in production of this halomethane during the same time period. If the models proposed for the interaction of ozone and the photochemical products of halomethanes are reasonably accurate, the maximum effects of the photoproducts would be felt ten to twenty years after the cessation of halomethane utilization (Cicerone, et al., 1974; Crutzen, 1974c; Molina and Rowland, 1974a; Wofsy et al., 1975). This is due to the essentially inert character of the halomethanes in the troposphere and the long period of time for transit through the tropopause into the stratosphere. By 1985, with continued production of halomethanes, the role that halomethane photoproducts play in ozone destruction may

may equal that of the nitrogen oxides (Cicerone et al., 1974).

Halomethanes other than those which are used as propellants or refrigerants that are being introduced into the stratosphere are carbon tetrachloride, methyl chloride (Molina and Rowland, 1974b), and bromine compounds, such as the methyl bromides being used as agricultural fumigates (Hammond, 1975). Nitrogen oxides created during nuclear weapons testing and chlorine released from the ammonium perchlorate used as an oxidizing agent for space shuttle rocket engines are further anthropogenic sources of stratospheric pollution (Hammond and Maugh II, 1974).

Behavioral Response

Organisms can conceivably use heat and visible light as cues to avoid ultraviolet overexposure. It has been pointed out (Porter, 1967; Smith, 1974) that some have developed protective coverings (e.g., hair, feathers, pigmented peritoneums, etc.) which could prevent excessive ultraviolet exposure to sensitive tissues. In man, melanin may be the main absorber of ultraviolet radiation in the skin, although approximately 16% of incident 300 nm ultraviolet radiation may penetrate to the dermis of light-skinned Caucasians (Daniels, Jr., 1969).

Most insects see ultraviolet radiation, and their behavioral responses reflect this capability in food-source identification, navigation and sex discrimination (Envionmental Studies Board, 1973; Goldsmith, 1973). Clark and Kimeldorf (1970, 1971) have demonstrated that UV-B radiation will induce tentacle retraction in the sea anemone. Non-exposed, as well as exposed, tentacles were observed to retract following irradiation of the animal. Lees and Carter (1972) observed a covering reaction when sea urchins were exposed to 360 nm ultraviolet radiation. Exposure of the rough-skinned newt to UV-A radiation elicits an avoidance behavioral response (Kimeldorf and Fontanini, 1974). The response appears to depend upon photoreceptor stimulation, since ophthalmectomized newts do not show this response. Even marine organisms one meter below the surface of the ocean may be exposed to approximately 50% of the ultraviolet radiation incident on the surface (Environmental Studies Board, 1973). Jerlov (1950) has stated that in clear oceans the "active region, where photochemical processes can be carried on, extends as far down as 20 meters." Among terrestrial plant life, agricultural plants are especially sensitive to ultraviolet radiation (Environmental Studies Board, 1973).

If the intensity of ultraviolet radiation reaching the surface of the earth is altered, the behavioral cues of a multitude of organisms may become altered and severe damage could result. If plants and animals do not have sufficient capacity to tolerate an increased intensity of ultraviolet radiation, ecological problems could arise (Caldwell, 1972).

The Ecological Niche of Toads

Amphibians play a unique role in nature by acting as a direct, biological link between terrestrial and aquatic ecosystems. Also, the adult anurans appear to possess a remarkable appetite for arthropods, especially insects. In one account (Kirkland, 1904) the economic status of the insects consumed by toads was calculated and it was found that 62% of the ingested insects were harmful to crops and 11% were beneficial (27% were either unidentified or classified as neutral). Smith and Bragg (1949) have found that, on the average, an adult toad's stomach contains between six and twenty different species of arthropods. They made the following observation:

> The possible effects of toads, especially in grasslands, on ecological succession should not be overlooked. Since it is widely recognized that arthropods, especially insects, are important contributors to seral stages, it seems to follow that any animal whose food consists largely of insects and ground-dwelling

> arachnids could make a difference not only in time relations in succession but also, in extreme cases, even influence its direction.

In order to maintain a viable population of amphibian adults, sufficient larvae must complete the metamorphic process. As Cole (1954) has stated:

> If it is to survive, every species must possess reproductive capacities sufficient to replace the existing species population by the time this population has disappeared.

Turner (1962) cited a study in which the frog population decreased to approximately three percent of its original size within three years. This marked decrease was attributed to insufficient larval survival. Another study (Savage, 1952) also noted that larval mortality was the primary regulator of the anuran populations under investigation:

> Thus the mortality rate, as well as the final percentage surviving, influences the ecological role of an animal population in its community (Calef, 1973).

An intricate balance exists in the ecological niche occupied by amphibians, and small shifts in the survival rates, or the introduction of malformed individuals into the ecosystem, resulting from UV-B damage could markedly alter this balance.

Related Suggestions for Future Investigations

Of relevant interest with respect to UV-B exposure would be the development and maturation of amphibian gonadal and neural tissue. Studies have shown that the development and maintenance of gonadal tissue is affected by the intensity and wavelength of the radiation incident on the organism. Oishi and Lauber (1973) have concluded that the most important factor controlling the photosexual response in male quails is the wavelength of the incident light. Their results may be due to the selective filtration of the light spectrum by the tissues covering the extraretinal photoreceptor. Wurtman and Weisel (1969) found that both male and female rats develop larger gonads when exposed to artificial white light enriched with UV-A and UV-B radiation than those animals deprived of the ultraviolet enrichment. In a study cited by Thorington et al. (1971), using similar radiation sources, hamsters exposed to the ultraviolet enriched source had more normal gonadal development than those animals deprived of the ultraviolet enrichment. Marshall and Bowden (1936) noted that maintenance of gonadal activity in ferrets is affected by 365 nm radiation. They reported that the ferrets came into

estrus early, and for an abnormally long time, after cessation of the exposure. The effect on gonad development of rearing tadpoles under conditions with enhanced UV-B radiation ought to be investigated.

A study by Davis (1944) in which chick embryos demonstrated that the folding process in neural tube formation is affected when the embryo is irradiated with UV-B radiation. During the neurula stage of amphibian development the primordial nerve cells are located on the upper surface of the embryo in a region with maximum exposure to solar ultraviolet radiation and could constitute a vulnerable target. Neural development in amphibians reared under conditions of enhanced UV-B radiation ought to be investigated.

A pilot study using a small number of tadpoles indicated that the larvae of the boreal toad may be able to detect UV-B radiation and, when given a choice, learn within 4.5-7.5 hours of continuous exposure to avoid the radiation (Worrest and Kimeldorf, unpublished). A study should be pursued which is designed to determine: (1) whether the organism under investigation can detect UV-B radiation, (2) if so, the site of the receptors, whether ocular and/or extraocular, and (3) whether successful protective behavioral responses are elicited by the radiation.

SUMMARY AND CONCLUSIONS

Hair, feathers, and pigmented skin usually can be expected to screen mature vertebrate organisms from the detrimental effects of ultraviolet radiation. On the otherhand, early developmental stages and neonates may be vulnerable because of their size and less developed ultraviolet screens. Moreover, the rapidly proliferating and differentiating tissue mass of the individual during development represents a particularly radiosensitive period in the life cycle of the vertebrate organism.

When compared with the number of published reports regarding the effects of ionizing radiation on the embryological development of vertebrates, there are relatively few reports concerned with the effects of ultraviolet radiation on vertebrate development. The energy spectrum of the radiation utilized in ultraviolet studies has been broad, ranging from the UV-C region on the short wavelength side, up through the UV-A region on the long wavelength end of the ultraviolet spectrum. There has been a deficiency of published reports regarding the effects on vertebrate development of radiation in the UV-B region (290-315 nm).

The effects of an increased exposure to radiation in the 290-315 nm region has recently become a topic of worldwide interest. Researchers have become aware that man-made pollutants, such as nitrogen oxides found in SST exhausts or halomethanes dispersed as propellants in aerosol sprays or as refrigerants, may directly or indirectly cause a partial depletion of stratospheric ozone. Although the atmospheric ozone layer is only approximately one-third of a centimeter thick at STP, it is the principal absorber of solar radiation in the ultraviolet range from 220 nm to 320 nm. Because the absorption of ultraviolet radiation is exponential in nature, a small decrease in the thickness of the ozone layer would result in a relatively large increase in the global solar flux of radiation between 290 nm and 315 nm.

The present series of studies was designed to determine the effects of UV-B radiation (290-315 nm) on temporal developmental patterns, systemic development and viability prior to metamorphic climax for boreal toad (Bufo boreas boreas) tadpoles. Over 3700 fertilized eggs were used in the studies.

The eggs were exposed to radiation from either "white-light" fluorescent lamps (Vita-Lite, Duro-Test Corporation, North Bergen, NJ) or "white-light" fluorescent lamps plus

fluorescent sunlamps (Westinghouse-FS40). Several radiation exposure schedules were utilized (chronic or varied, daily exposure schedules) and, in all but one study, filters were utilized - Kodacel cut-off filters to transmit wavelengths longer than 290 nm and Mylar cut-off filters to transmit wavelengths longer than 315 nm.

Without added filters, the eggs exposed either chronically or cyclically to the fluorescent sunlamps (which emit radiation with wavelengths shorter than 280 nm) survived less than 42 hours from time of oviposition. Other studies with enhanced exposure to radiation in the range from 290 nm to 315 nm resulted in significant departures from normal temporal developmental patterns, systemic development and viability. Exposure of the developing tadpoles to eleven or more Sunburn Units per day of ultraviolet radiation (see Appendix C) resulted in the development of tadpole populations with lordotic posture, hyperplasia in the presumptive cornea and other dorsal epithelial tissue, increased pigmentation in the presumptive cornea, desquamation of the dorsal surface, and increased mortality. Exposure of the tadpoles to 4.4 Sunburn Units per day resulted in a significant lengthening of the time periods required to achieve metamorphic climax.

Of three groups of eggs exposed to similar radiation sources and to similar daily radiant exposures (11.0 $SU \cdot d^{-1}$) at similar peak radiant exposure rates (1.1 $SU \cdot h^{-1}$), but differing lengths of exposure to longer wavelengths following UV-B insult, there was a direct relationship between the length of daily exposure to photoreactivating radiation following UV-B exposure and both the percent survival and the mean elapsed time until death if prior to metamorphic climax.

If abnormalities are induced in individual organisms in nature by solar ultraviolet radiation, it is likely that these individuals would be unable to compete successfully and would be eliminated. Similarly, even delay of development may interfere with the ability to compete. Thus, induction of developmental abnormalities or delay of metamorphosis are indirect means of killing, but become a significant problem only when the number of organisms affected becomes significantly large.

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APPENDICES

APPENDIX A

Analysis of the natural well water (Oregon State University Oak Creek Fisheries Laboratory, Corvallis, Oregon) and the synthetic pond water (amphibian Ringer's solution diluted to one-tenth standard concentration) used in the present series of experiments. The analysis was conducted by the Laboratory Services Branch of the National Environmental Research Center, Pacific Northwest Environmental Research Laboratory, Corvallis, Oregon on 18 May 1974.

Test	Well Water	Synthetic pond water
pH	7.8	6.9
Total inorganic carbon	24.0 mg/l	2.0 mg/l
Alkalinity	106.0 mg/l	15.0 mg/l
Bicarbonate alkalinity	106.0 mg/l	15.0 mg/l
Total hardness	90.0 mg/l	32.0 mg/l
Calcium	28.0 mg/l	6.6 mg/l
Magnesium	6.4 mg/l	0.2 mg/l
Sodium	9.0 mg/l	270.0 mg/l
Potassium	1.0 mg/l	8.2 mg/l
Chloride	4.0 mg/l	384.0 mg/l
Total Copper	5.0 µg/l	31.0 µg/l

APPENDIX B

Kodacel Transmission Characteristics

The transmission characteristics of Kodacel-TA401:0.005" in the region of interest (290-315 nm) significantly decrease with exposure to fluorescent sunlamps (Figure 5). The percent transmittance of previously unexposed five mil (0.13 mm) Kodacel at 310 nm is 77% and at 290 nm is less than 1% (relative to air) as measured with a Shimadzu Spectrophotometer:MPS-50L over a spectral range of 210-370 nm (Figure B1). After 24 hours of continuous exposure to the flux of four Westinghouse-FS40 sunlamps at a distance of 36 centimeters, the filter transmittance at 310 nm had fallen to 56%. The transmittancies in the range of 300-320 nm had decreased to 0.72, relative to unexposed Kodacel. After an additional 24 hours of exposure the transmittancies declined to about 0.71, and by the tenth day of continuous exposure the transmittancies were still more than 80% of those found after 24 hours (Figure B1). The spectral balance in the region of interest was not significantly altered by solarization, the transmission characteristics of the entire range being affected fairly equally (Figure B2). Further studies utilizing six FS40 sunlamps as the solarizing source yielded a similar stabilization of transmission characteristics after a 24-hour period of exposure.

Figure B1. Transmission spectra of Kodacel-TA401:0.005" solarized for various numbers of hours, as indicated on spectrogram. Four FS40 sunlamps used as solarization source. Exposure rate without filter: three Sunburn Units per hour as measured with a UV-B Meter.

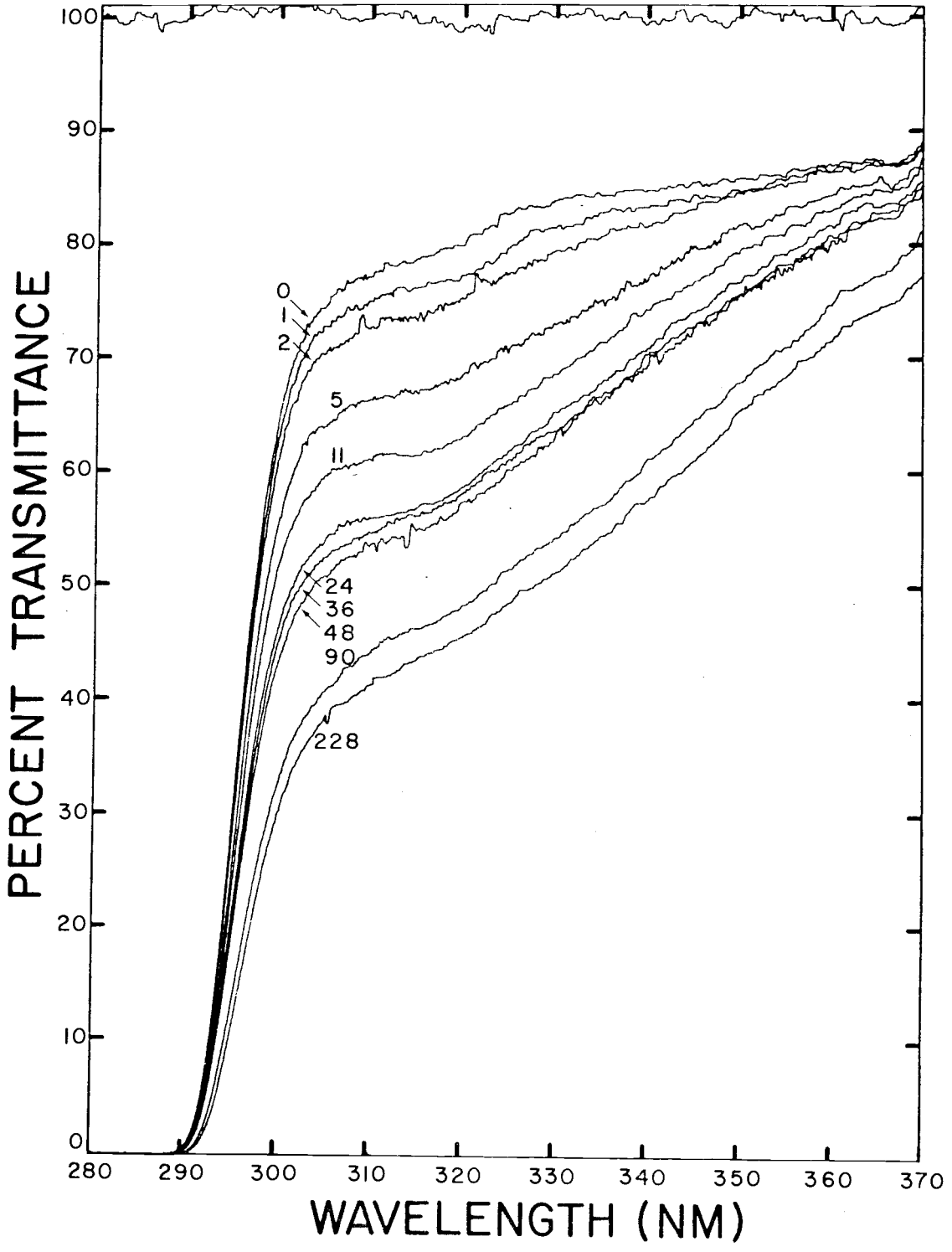
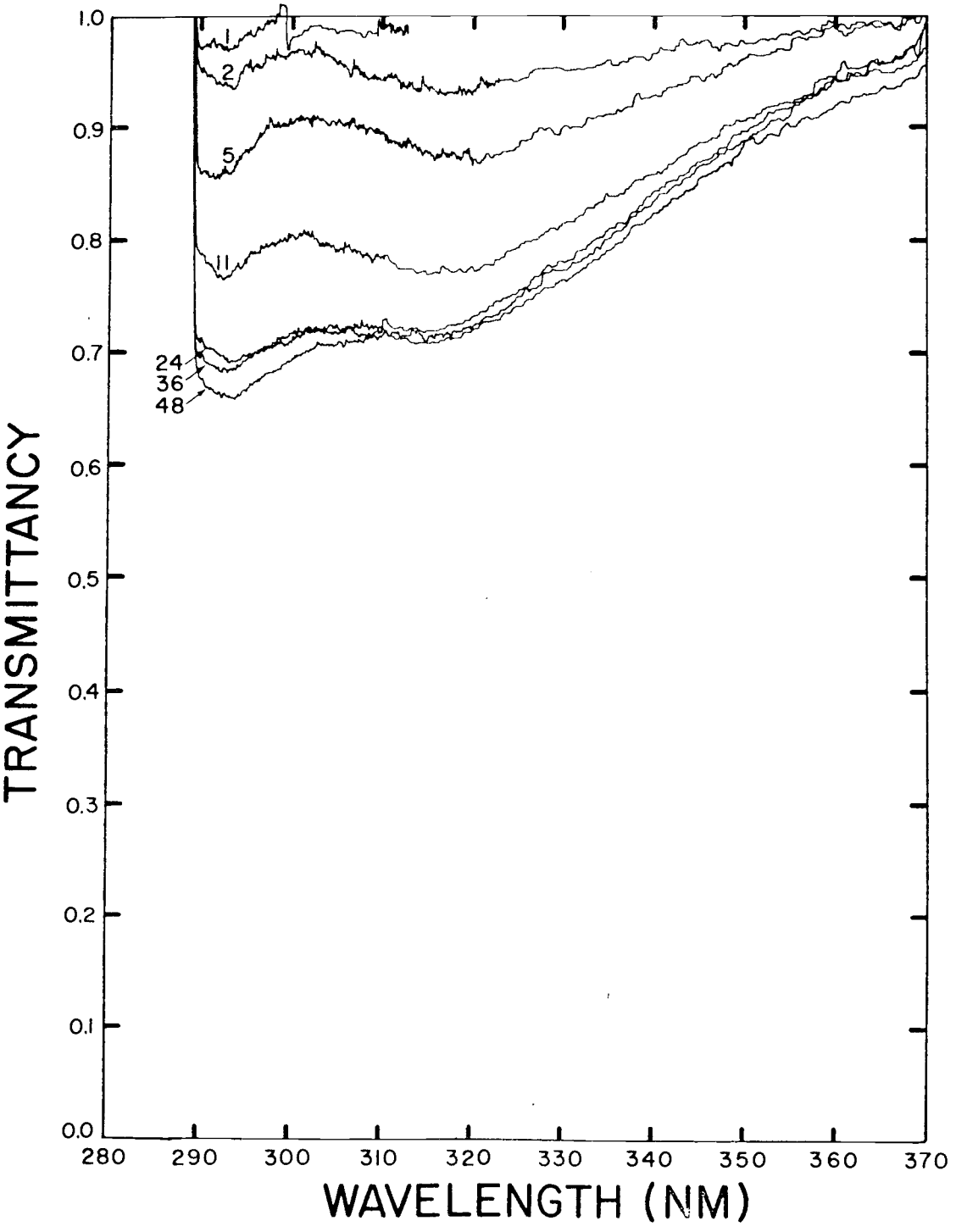

PERCENT TRANSMITTANCE
WAVELENGTH (NM)
100
90
80
70
60
50
40
30
20
10
0
280
290
300
310
320
330
340
350
360
370
0
1
2
5
11
24
36
48
90
228

Figure B2. Ratios of percent transmission of solarized Kodacel-TA401:0.005" to nonsolarized Kodacel. Length of solarization, in hours, is indicated on spectrogram. Four FS40 sunlamps used as solarization source. Exposure rate without filter: three Sunburn Units per hour as measured with a UV-B Meter.


TRANSMITTANCY
WAVELENGTH (NM)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
280
290
300
310
320
330
340
350
360
370
1
2
5
11
24
36
48

It was concluded that the transmission characteristics of the Kodacel filters could be considered relatively stable after the initial 24-hour solarization effect.

APPENDIX C

Dosimetry

UV-B Meter

The UV-B Meter (Robertson, 1969) was designed to have a spectral response similar in wavelength dependence to that for the production of human skin erythema (a maximum responsiveness to terrestrial sunlight of approximately 295 nm). The magnesium-phosphate phosphor of the UV-B Meter fulfills this requirement satisfactorily for those wavelengths found in the global solar spectrum, for it has a peak response at 300 nm and one percent of this sensitivity at 331 nm. The phosphor is bonded in a thin layer beneath an ultraviolet transmitting, light absorbing "black" glass (Schott UG11), and positioned above a narrow-pass, green filter (Corning 4010) which allows part of the phosphor fluorescence to pass through to a phototube. The function of the green filter is to absorb any ultraviolet or infrared radiation which passes through the light absorbing filter.

The phototube, located beneath the phosphor and filters, is activated by light emitted by the phosphor due to a fluorescent conversion of wavelengths of less than 320 nm. The amount of photocurrent generated is proportional to the number of effective photons absorbed by the phosphor.

The sensor package can be attached to recording devices which enable the operator to obtain either cumulative radiant exposures over half-hour time segments, or radiant exposure rates.

The Sunburn Unit

The Sunburn Unit (SU) is the unit of radiant exposure contrived for the response of the UV-B Meter to the effective wavelengths of radiation. Exposure to UV-B radiation equivalent to one Sunburn Unit will produce a minimally perceptable erythema in normal, light-skinned Caucasians. The peak radiant exposure rate during a clear June day in Corvallis, Oregon (44° 40' N) is approximately 2.9 $SU \cdot h^{-1}$, with a total radiant exposure of approximately 19 Sunburn Units. More than 50% (10.2 SU) of the total radiant exposure occurs within $\pm$ 2 hours of solar noon in Corvallis (Dr. D. S. Nachtwey, personal communication).

APPENDIX D

Analysis of Food as Supplied by the Ralston Purina Company

Purina Commercial Rabbit Chow Checkers
Ralston Purina Company
St. Louis, Mo.

crude protein not less than	15.5%
crude fat not less than	1.5%
crude fiber not less than	28.0%

contents

alfalfa meal
ground yellow corn
ground grain sorghums
wheat middlings
soybean meal
cottonseed meal
cane molasses
methionine hydroxy analogue calcium
defluorinated phosphate
iodized salt
iron oxide
manganous oxide
copper oxide
cobalt carbonate
zinc oxide

APPENDIX E

Histology

Fixative:

1. Bouin's fluid:

saturated aqueous picric acid	75 ml
40% formaldehyde	25 ml
glacial acetic acid	5 ml

Washing:

1. Modified Lenoir's fluid: — 1 h

distilled water	50 ml
95% ethanol	50 ml
ammonium acetate	10 g

Dehydration:

1. Ethanol series (15%, 40%, 75%, 95%, 100% I, 100% II) — 1 h each
2. Xylene I, xylene II — 1 h each

Infiltration:

1. Paraplast-plus and xylene — 25 min
2. Paraplast-plus (vacuum) — 25 min
3. Paraplast-plus — 25 min
4. Paraplast-plus — 25 min
5. Paraplast-plus — 25 min

Embed:

1. Paraplast

Mounting and Staining:

Mallory's Triple Stain (Gray, 1964):

First staining solution
1% acid fuchsin
Differentiating and mordanting solution
1% phosphotungstic acid

Mallory's Triple Stain (cont.)

Second staining solution

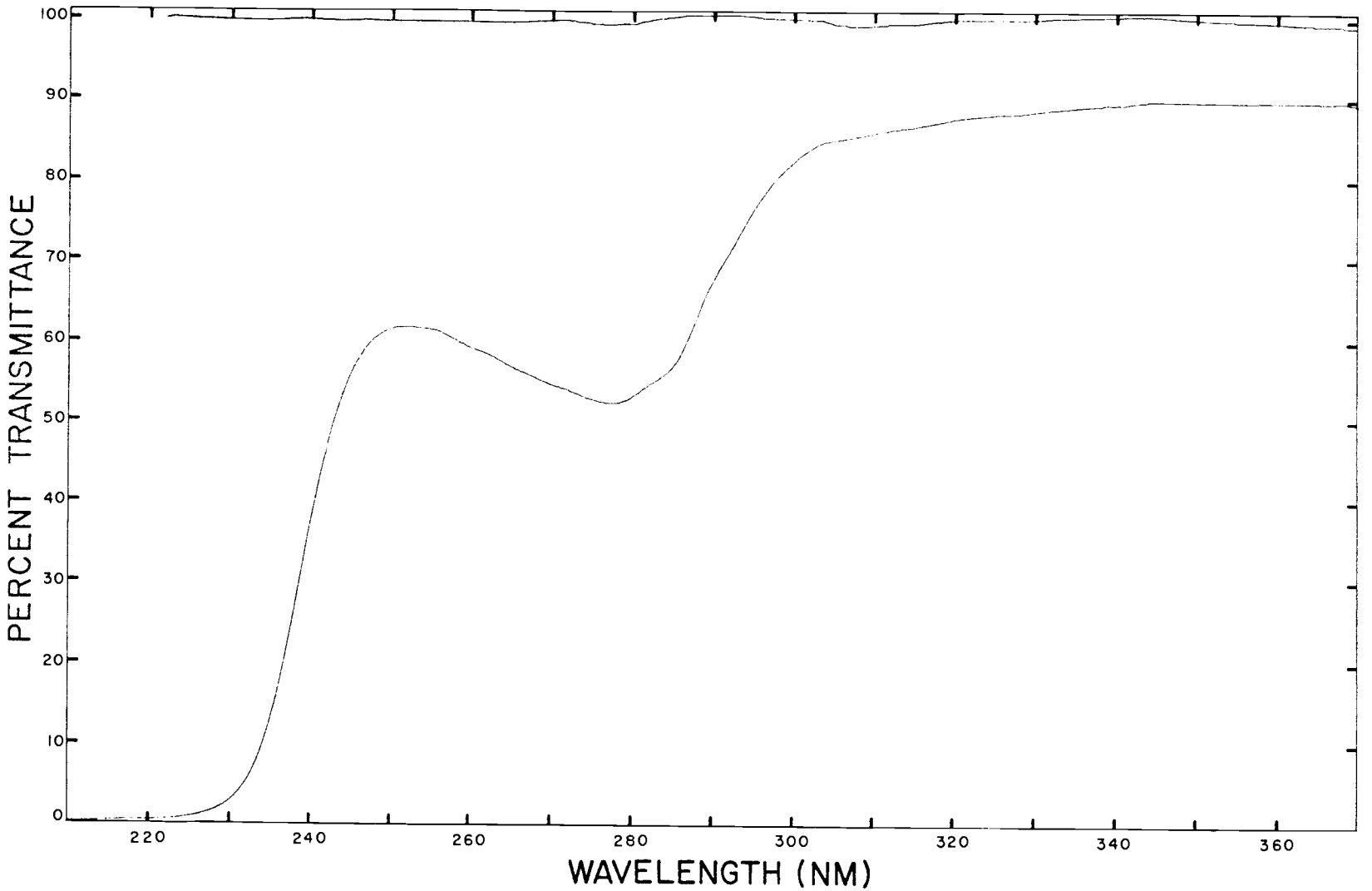
distilled water	100 ml
aniline blue	0.5 g
orange G	2 g
oxalic acid	2 g

1.	Cut serial sections.	
2.	Albumenize slides and place ribbons on slide with wet brushes.	
3.	Flood slides and place on slide warmer (43°C) overnight.	
4.	Warm slides over low flame until paraffin melts.	
5.	Xylene I	2-3 min
6.	Xylene II	2-3 min
7.	Absolute ethanol I	2-3 min
8.	Absolute ethanol II	2-3 min
9.	95% ethanol	2-3 min
10.	Distilled water	2-3 min
11.	First staining solution	2-3 min
12.	Rinse slides thoroughly in water.	
13.	Transfer to phosphotungstic acid.	2 min
14.	Give slides very quick rinse in water.	
15.	Transfer to second staining solution.	15 min
16.	Wash in water until no more color comes away.	
17.	95% ethanol	3 dips
18.	Absolute ethanol	5 dips
19.	Xylene I	2-3 min
20.	Xylene II	2-3 min or longer
21.	Mount.	

APPENDIX F

Transmission Spectrum of Jelly from the Egg-string of the Boreal Toad

Figure F1. Transmission spectrum of the albuminous coating from the egg-string of the boreal toad as measured with a Shimadzu Spectrophotometer:MPS-50L (one centimeter cell). It is typical of transmission spectra for proteins, illustrating peak absorption in the UV-B region at 280 nm (Compare with Fig. 17).


PERCENT TRANSMITTANCE
100
90
80
70
60
50
40
30
20
10
0
220
240
260
280
300
320
340
360
WAVELENGTH (NM)

APPENDIX G

Itemized Listing of the Days Required in Study #4 to Achieve the Two End-points: (1) Metamorphic Climax and (2) Death, if prior to Metamorphic Climax

Table G1. Results of radiation conditions for Study #4. Sources of radiation: Vita-Lite fluorescent lamps and Westinghouse-FS40 fluorescent sunlamps. One-half of the tadpoles were cultured under Mylar filters and the other half under Kodacel filters.

Group	Filter	Daily radiant exposure (SU)	End-point*	Days elapsed to achieve end-point	Mean elapsed time±st.error	Percent survival
1	Mylar	0.8	MC	45, 47, 47, 47, 47, 49, 49, 49, 52, 52, 52, 52, 52, 52, 54, 54, 54, 54, 54, 56, 56, 56, 56, 59, 59, 59, 59, 59, 59, 66, 66, 70, 70	54.8±1.1	97%
1	Mylar	0.8	D	35	35	-
1	Kodacel	23.8	MC	-	-	0%
1	Kodacel	23.8	D	19, 26, 26, 26, 28, 28, 28, 28, 28, 28, 28, 28, 28, 31, 31, 31, 31, 31, 32, 32, 32, 32, 32, 32, 33, 33, 33, 33, 33, 33, 35, 35, 35, 35, 37	30.6±0.6	-
2	Mylar	0.5	MC	45, 45, 45, 47, 49, 49, 49, 52, 52, 54, 54, 54, 54, 56, 56, 56, 59, 59, 59, 61, 61, 61, 61, 61, 61, 63, 63, 63, 63, 63, 66, 68, 68, 70	57.4±1.2	100%
2	Mylar	0.5	D	-	-	-
2	Kodacel	15.4	MC	-	-	0%
2	Kodacel	15.4	D	36, 38, 39, 39, 39, 39, 45, 45, 45, 47, 47, 49, 49, 52, 52, 52, 52, 52, 52, 52, 52, 54, 54, 54, 54, 54, 56, 59, 59, 59, 61, 63, 63, 66	50.9±1.3	-
3	Mylar	0.4	MC	38, 39, 42, 45, 45, 45, 45, 45, 47, 47, 47, 47, 49, 49, 49, 49, 52, 52, 52, 52, 54, 54, 54, 54, 57, 57, 66, 66, 66, 66, 68, 71, 73, 75	53.4±1.7	97%
3	Mylar	0.4	D	62	62	-
3	Kodacel	11.0	MC	-	-	0%
3	Kodacel	11.0	D	32, 33, 33, 33, 33, 35, 35, 36, 38, 38, 38, 38, 38, 39, 39, 39, 42, 42, 42, 42, 42, 42, 42, 45, 45, 45, 45, 45, 47, 47, 47, 49, 52, 52	40.9±0.9	-
4	Mylar	0.4	MC	38, 42, 45, 45, 45, 45, 47, 47, 49, 49, 52, 52, 52, 52, 52, 52, 52, 54, 54, 54, 54, 56, 56, 61, 63, 63, 66, 66, 66, 70, 73	53.9±1.4	94%
4	Mylar	0.4	D	19,24	21.5±2.5	-
4	Kodacel	11.0	MC	47, 49, 52, 52, 54, 56	51.7±1.3	17%
4	Kodacel	11.0	D	28, 36, 40, 40, 45, 49, 49, 49, 49, 52, 52, 52, 54, 56, 59, 59, 59, 59, 61, 63, 63, 63, 63, 66, 66, 68, 68, 71, 72	55.6±2.0	-
5	Mylar	0.4	MC	39, 40, 42, 42, 42, 42, 45, 47, 47, 49, 49, 49, 52, 52, 52, 54, 54, 54, 56, 56, 56, 56, 56, 57, 59, 59, 59, 61, 61, 61, 61, 61, 61, 66, 68, 70	53.8±1.3	100%
5	Mylar	0.4	D	-	-	-
5	Kodacel	11.0	MC	52, 56, 56, 56, 57, 63, 63, 66, 68, 68, 77, 87, 89	66.0±3.3	41%
5	Kodacel	11.0	D	40, 49, 52, 54, 57, 57, 59, 61, 61, 61, 61, 61, 63, 66, 66, 68, 77, 87, 89	62.6±2.7	-
6	Mylar	0.2	MC	39, 39, 40, 40, 42, 45, 45, 49, 49, 52, 52, 52, 52, 52, 54, 54, 54, 54, 54, 56, 56, 56, 56, 59, 59, 61, 61, 61, 63, 63, 63, 66, 73, 73	54.2±1.5	97%
6	Mylar	0.2	D	38	38	-
6	Kodacel	4.4	MC	47, 47, 49, 49, 49, 49, 49, 52, 52, 52, 52, 52, 52, 52, 54, 56, 56, 56, 59, 61, 61, 61, 63, 66, 68, 68, 70, 80, 84, 89	58.5±2.0	86%
6	Kodacel	4.4	D	59, 67, 67, 67, 84	68.8±4.1	-

* MC = metamorphic climax

D = death prior to achieving metamorphic climax