INCREASE IN LIFE-SPAN DUE TO PROLONGED PREREPRODUCTIVE STAGE IN THE ROTIFER, ASPLANCHNA BRIGHTWELLI, GOSSE

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ABSTRACT

INCREASE IN LIFE-SPAN
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In this study, the effects of dl-alpha-tocopherol and of the elimination of the photoperiod were examined for their effect on the life-span of the rotifer Asplanchna brightwelli. Both procedures resulted in a significant increase in the mean life-span. Subsequent analysis showed that this increase in mean life-span was due to a lengthening of the prereproductive stage. Irradiation experiments with ultraviolet light also showed that the prereproductive stage is probably the most sensitive stage of rotifer development.

The concentration of alpha-tocopherol which resulted in the longest mean life-span was determined. Introduction of alpha-tocopherol at this optimum concentration had the most effect when present during the prereproductive stage. An increase in offspring size in the alpha-tocopherol group was also observed. The actual uptake of the alpha-tocopherol was seen to be through dietary intake via the

Paramecia food source.

Total restriction of photoperiod, extended through several rotifer generations, produced an increase in life-span. As in the alpha-tocopherol experiments, a prolongation of the prereproductive stage accounted for the overall increase in mean life-span.

In further experiments, various dosages of whole body irradiation were applied to the rotifers. No stimulatory effect, in which the mean life-span increased, was observed at any radiation dose. However, alpha-tocopherol was seen to act as an ultraviolet protective agent. The prereproductive period was the most radiation sensitive stage, as measured by shortening of mean life-span.

This study shows that various treatments which increase the prereproductive period all result in an increase in the overall mean life-span of the rotifer Asplanchna brightwelli. Thus, the prereproductive period is the most labile stage of the life-span, and the stage in which length of life in this organism can be significantly altered.

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INTRODUCTION

Aging and senescence are inherent characteristics which are prevalent in most organisms. As indicated by Lamb (1977), there are many theories and hypotheses which try to explain aging. Though there are many biological and biochemical differences, the most basic mechanisms of aging may be quite similar in most organisms. Two fundamental factors to be considered in any aging theory are genetic make-up and the environmental parameters.

In the research that will be reported here, a study of aging was undertaken using the rotifer Asplanchna brightwelli, Gosse. There were many advantages in the use of this species, including its asexual form of reproduction and its short life-span. A. brightwelli is ovoviviparous and reproduces by diploid parthenogenesis (Birky 1967, Pennak 1978). Asexual reproduction through amictic females ensures genetic uniformity and minimizes the influence of genetic variability from one generation to the next. Sexual reproduction can occur under the influence of dietary alpha-tocopherol which induces the formation of males (Gilbert and Thompson 1968). Microscopic analysis can be used to determine the birth of male rotifers through the presence of the male reproductive structures.

A. brightwelli is a short lived rotifer with a life-span of around 6 days, making it an ideal species to work with for longitudinal life-span studies. The same individual may be studied continuously throughout its short life. Both life-span and reproduction patterns can be altered through enyironmental manipulations such as temperature and diet (Verdone-Smith and Enesco 1982). A. brightwelli is larger than rotifers of other genera and is thus easy to observe (Birky 1969) but small enough to be easily stored and handled. Environmental prameters can also be well controlled.

In this study, the presence of alpha-tocopherol (Vitamin E) and the elimination of photoperiod were examined for their effect on the life-span and reproduction of A. brightwelli. Experiments were also carried out to determine the effect of ultraviolet irradiation on rotifers both with and without vitamin E. The sensitivity of various stages of development to the U.V. radiation and alpha-tocopherol was also compared. Further experiments were done to check on food ingestion as well as to determine whether vitamin E acts through the environment or through dietary uptake by the rotifers. Stringent measures were taken to ensure consistency in the amount of food, pH levels, length of light, and temperature.

Environmental Variables

Alpha-Tocopherol

The scavenging ability and the singlet oxygen quenching abilities of vitamin E have been well established (Matsushita et al. 1978, Mc Cay et al. 1978). The antioxidant activity of alpha-tocopherol is through its hydroxyl functions and by metabolites of alpha-tocopherol (Boguth 1969). Aside from the antioxidant abilities, vitamin E has also been shown to influence mammalian physiology in several ways. Vitamin E has been shown to be an immunopotentiation agent (Tengerdy et al. 1973, Yasunage et al. 1982). Increased resistance to bacterial infection (Tengerdy et al. 1978) and enhancement of helper T-cell activity also was shown to occur through the dietary supplementation of vitamin E (Tanaka et al. 1979) in the mammalian system.

Alpha-tocopherol has been shown to control both sexuality and polymorphism in the rotifer Asplanchna (Gilbert and Thompson 1968, Gilbert 1971). The significant increase in body size mediated by alpha-tocopherol is most likely due to the effect of both cytoplasmic growth and nuclear division (Gilbert 1973). A dose as low as 0.2 ng of d-alpha-tocopherol per female was enough for the outgrowth of the body wall for Asplanchna sieboldi, clone 10C3-1-5 (Gilbert and Birky 1971). The different

morphotypes induced by alpha-tocopherol for A. sieboldi, are discussed by Gilbert (1974).

Sexual reproduction in Asplanchna is initiated by the production of mictic female and male offspring through dietary alpha-tocopherol. The lowest effective alpha and beta-tocopherol concentration to induce mictic females in A. brightwelli was from 14 - 70 ng/ml (Gilbert and Thompson 1968).

The addition of vitamin E resulted in an significant increase of life-span in the rotifer Philodina (Enesco and Verdone-Smith 1980). Similar increases were seen for the nematode Turbatrix (Kahn and Enesco 1981) and for the Drosophila (Miquel et al., 1973). Such increases in the life-span may be due to the antioxidant effects of vitamin E which stabilizes the cellular membrane as well as preventing any free radical attacks upon the DNA.

The interaction of vitamin E with other antioxidants has been shown to have a synergistic effect in rat and human mitochondria as a superoxide scavenger (Lippman 1981). Vitamin E pretreatment partially prevented the fall in the retinal superoxide dismutase activity in newborn kittens exposed to normobaric hyperoxia (Bougle et al. 1982). In this way, it has been shown that vitamin E can also work in association with other antioxidants. Thus the introduction of vitamin E may enhance and may work

with the natural antioxidants which exist in the organism.

Membrane breakdown within the cell may well reflect the aging process. Lysosomal membrane damages from free radical reactions may result in leakage of hydrolytic enzymes. Subsequent damages to the cellular machinery and to the genetic materials would only be to the detriment of the organism. Membrane stabilizing effects were seen when antioxidants were administered to Drosophila (Hochschild 1971). Moreover, alpha-tocopherol was shown to bring about the stabilization of the microsomal membrane in rats, thus protecting them from free radical attacks (Mc Cay et al. 1971).

Being a biological antioxidant, vitamin E should be endowed with radioprotective abilities. As expected, tumor growth retardation was seen with irradiated animals that were pretreated with vitamin E (Kagerud 1981). Ultraviolet light-induced erythema was also inhibited by the use of vitamin E (Roshchupkin, Pistsov, and Potapenko 1979). Protection from radiation therapy side effects in patients were also seen (Black and Chan 1975) and a decrease in erythrocyte fragility was found after whole-body irradiation with dietary Vitamin E supplementation (Hoffer and Roy 1975). Aside from the radioprotective ability, inhibition of carcinogenesis has also been associated with vitamin E (Haber and Wissler 1962) and to

other antioxidants (Wattehberg 1978).

Ultraviolet Radiation

In some experiments involving insects and mice, low doses of ionizing radiation have been shown to cause an increase in life-span (Lamb 1977). Ultraviolet radiation is not an ionizing radiation; its energy from 4.13 to 155 electron volts is just below that of X-ray radiation (Gofman 1981), but U.V. damage to the DNA has been well documented (Harm 1980, Hart et al. 1977, Southerland et al. 1980). Thus the effects of ultraviolet radiation, though lower in energy than X-ray but higher in energy than for the visible range, are of interest in this study. Since DNA repair is correlated with life-span (Hart and Setlow 1974, Hart et al. 1979), and since ultraviolet radiation may induce repair (Cleaver 1978, Dell'Orco and Whittle 1981), this adds a further dimension to studies by the effect of ultraviolet irradiation on the life-span of the rotifers.

The use of alpha-tocopherol has been shown to be a positive factor in the inhibition of erythema which was produced by U.V. irradiation (Roshchupkin et al. 1979). The addition of vitamin E prevented malonaldehyde formation in the rat liver lysosomes during U.V. irradiation (Torinuka et al 1980). In this respect, alpha-tocopherol may have some form of protective effects similar to those

reported for X-ray irradiation (Hoffer and Roy 1975).

Light and Dark

One common environmental stimulus is light. In three rotifer species, positive phototactic reactions have been seen for a range of the spectrum including the U.V. range (Menzl and Roth 1972). Such phototactic reaction is probable due to the cervical eyespots of the rotifers (Pennak 1978).

Variations in the photoperiods are known to cause a number of changes to different organisms. Physiological degradation in the eyes of a salamander larvae, Tryphlotrition spelaeus, was shown to occur when maintained in darkness for 215 or 279 days (Besharse and Brandon 1976). Goss (1976) showed that photoperiod variations affect the antler growth in the sika deer, Cervus nippon. Biochemical differences have also been found to exist due to the variation in the photoperiod of rodents (Ottenweller and Hedge 1982, Vaughan et al. 1982, Yellon et al. 1982).

Clement and Pourriot (1972, 1974) have shown that in the rotifer Notommata copeus, mictic females may be induced by a long photoperiod. The parental age did not have any significant influence on the rate of mictic-female production (Pourriot and Clement 1975). The rate of production of mictic female may depend on the turnover rate of the photopigment (Clement and Pourriot 1976). With

the start of sexual production induced by an extended photoperiod, the effect of light may have many and varied effects upon an organism.

In the terrestrial isopod Porcellio dilatatus, reproduction was stopped with a decrease in the photoperiod and a long photoperiod hastened the occurence of parturial molts (Mocquard et al. 1978). The influence of photoperiod in the reproductive pattern can also be seen in the crayfish, Orconectes (Aiken 1969, Rice and Armitage 1974).

Longevity in the Drosophila was found to be a function of circadian, light/dark cycle (Pittendrigh and Minis 1972). By varying the light/dark cycle, Drosophila were shown to live the longest on a 12 hour light/12 hour dark cycle. Constant light throughout their life shortened the life-span of the Drosophila. In adult Drosophila grown under permanent darkness, Allemand et al. (1973) showed that life-span could be increased. The exact reason behind this phenomenon is yet unclear. It is known that virgin females live longer, but eggs were present in Drosophila grown under permanent darkness and copulation is preferentially in the light (Grossfield 1970). The darkness may prevent flight thereby reducing metabolism, but for Drosophila a reduction in metabolism does not seem to increase longevity (David et al. 1971). In the present study, the question being asked is whether light/dark

conditions alter the longevity or reproductive pattern in A. brightwelli.

Objectives of This Study

With the use of the short-lived rotifer A. brightwelli, an in vivo study of aging was undertaken, with the manipulation of environmental variables. All alterations in longevity and reproductive patterns were detected and analyzed.

A study on the effect of alpha-tocopherol was carried out in the first set of experiments to determine what concentrations of vitamin E would influence the mean life-span. Ultraviolet irradiation experiments were also run in conjunction with the alpha-tocopherol experiments as to see whether any protective effects of alpha-tocopherol could be detected after U.V. irradiation. The rotifers were also irradiated at different stages of development to see whether they have any period of development which is most sensitive to U.V. radiation.

Another series of experiments were performed to determine the influence of light/dark conditions. A. brightwelli was grown under continuous light or continuous darkness, to see if its life-span or reproductive pattern could be altered in any way.

Environmental conditions of temperature and diet are

known to affect the life-span of A. brightwelli (Verdone-Smith and Enesco 1982). Therefore, stringent controls were carried out so that at any given experiment there was only one environmental factor which was different from the control being used. The temperature, pH and light conditions were always measured and controlled to maintain consistency. The availability of food was also adjusted to be of equal amount for the control and experimental groups.

The results of each experiment, which should reflect a single environmental manipulation, were also compared with those from experiments in series involving other variables. Thus a single environmental manipulation could be compared with two environmental manipulations to determine correlations. The experimental results reported here and the possible explanations for the data will be discussed in the light of previous research.

MATERIALS AND METHODS

Organism

The organism used in this study was the short lived rotifer species, Asplanchna brightwelli, Clone 4B61. The rotifers were originally obtained from Dr. John J. Gilbert, Dartmouth College, Hanover, New Hampshire.

Culture Methods

Paramecium caudatum, the food source of the rotifers, were cultured in a Cerophyll infusion medium (Bridger 1970). The method, outlined below, is a modified version of that used by Verdone-Smith (1981).

A 4-liter Erlenmeyer flask containing 1900 ml of distilled water, 0.12 g $CaCO_3$ and 14.0 ml of stock buffer solution (See Appendix I) was heated to boiling. Then, 1.5 g of Cerophyll (Ward's Natural Science Establishment, Rochester, New York), suspended in 56 ml of distilled water, were added to the mixture followed by 30 ml of distilled water rinse, and the entire suspension was boiled for 10 minutes. The boiled solution was cooled in an ice bath to room temperature, and filtered through Whatman #4 filter paper. The filtered solution was autoclaved for 20 minutes.

After autoclaving, the solution was cooled to room

temperature under running tap water. After cooling, a loopful of E. coli, (Type K12, originally obtained from Dr. E. Newman, Concordia University) was directly inoculated into the solution using sterile technique, to serve as food source for the Paramecia. The solution was incubated at 38°C for 48 hours, without shaking, and was cooled down to room temperature. After cooling, the pH was measured with a pH meter (Concordia University technical center); the ideal range was 6.5 - 7.0. A 0.05 N NaOH solution was used to adjust the pH, but this was usually unnecessary.

A 50-ml culture of Paramecium caudatum (originally from Boreal Laboratories Ltd., Mississauga, Ontario) was added to the solution, and left at room temperature for 72 hours before use.

Transfer and Feeding of Stock Cultures

Every fifth day, 20 rotifers of varied age were transfered into a 250-ml Erlenmeyer flask containing 150 ml of the prepared medium in it. Six flasks, labelled "Stock Culture", were always maintained at room temperature.

Maintenance of Paramecia and E. coli

At least 300 ml of the prepared Paramecium medium were kept aside to maintain Paramecia growth for new medium

preparation, which was made every 10 days. The E. coli was grown on dextrose agar (Difco Laboratories, Detroit, Michigan) and was refrigerated until needed. New E. coli plates were prepared every month to maintain the bacteria.

Sterility

All glassware, culture dishes and any pipettes used in the experiments were sterilized for 20 minutes by autoclaving or were bought sterile from the companies involved. Sterile techniques were maintained throughout the experiment. Furthermore the E. coli, used as the food source for the Paramecia, were always present in order to exclude other bacteria species by interspecific competition. The medium was occasionally checked, during and after preparation, for contamination using dextrose agar plates (Difco) at 38°C and at room temperature. Bacterial contamination was very rare.

Glassware

All glassware used in the experiment was carefully cleaned and then left in chromic acid (See Appendix II) for at least 24 hours. After the chromic acid wash, all of the glassware were extensively rinsed to remove any dichromate ions and autoclaved for 20 minutes. The glassware was reautoclaved prior to use if left for more than three weeks.

Experimental Methods

New Paramecium medium, stock buffer and E. coli cultures were prepared and used to begin any experiment. The stock culture to be used was fed with new Paramecia 24 hours prior to the beginning of any new experiment, to ensure that every experiment had well fed rotifers to begin with.

To start the experiment, rotifers from the stock culture were placed in a Petri dish and examined under a dissecting microscope. Adult rotifers were individually segregated into each of the wells of a 24-welled tissue culture dish (No. 76-063-05; Flow Laboratories, Mc Lean Virginia). Approximately 2.5 ml of Paramecium medium were placed with each individual rotifer. Transfer of the rotifers was carried out through the use of a micropipette on a 5 microliter setting (Finnipipette Ky, Helsinki, Finland).

Each of the wells (chambers) was checked every three hours for offspring. Only new born rotifers were separated and used for a given experiment, since their age was known. Thus the age accuracy for the new born rotifers was within 3 hours.

Paramecia Counts

During all major experiments, the number of Paramecia in the wells (the wells chosen at random) were counted

twice daily at 7:00 A.M. and 7:00 P.M. to determine the Paramecia count. Approximately 100 microliters of Paramecium medium were placed on a 60 x 15 mm Petri dish with 2 mm grids (No. 3160; Costar, Cambridge, Massachusetts) and 2 microliters of 0.1 mg/ml neutral red solution were added and mixed. The neutral red concentration was high enough to kill the Paramecia and to stain them thereby allowing quick counts to be taken.

Size Measurements

The rotifers were washed twice in distilled water, to remove any Paramecium medium, and placed in 0.1 mg/ml neutral red solution for 5 minutes. The rotifers were still alive but were sufficiently immobilized to take size measuments.

Size measurements were made on the Zeiss Ultraphot II Phase Contrast Microscope (Zeiss Corporation, West Germany) using a calibrated ocular micrometer. A 6X ocular micrometer with the Ph 2/16X objective, for a final magnification of 96X, was used to measure the body size of the rotifers.

Photomicrographs

Photomicrographs were taken with the Zeiss-Ultraphot II Phase Contrast Microscope with the Zeiss Ikon automatic photomicrographic camera attachment (Zeiss Corporation),

Kodak Panatomic-X B & W; ASA 32, and Kodacolor II color films; ASA 100, were used. For the photomicrographs, a 50% ethanol solution for 3 minutes was used to fix the rotifers. Death and the ensuing immobilization caused a slight enlargment in the size of the rotifers.

Temperature

The temperature was maintained at 19 $\pm$ 0.5^{o}C for all of the experiments in an incubator. Any greater variation in temperature, due to incubator breakdown, resulted in the immediate termination of that given experiment. This temperature was chosen since it gave the longest mean life-span in a preliminary experiment and since the room temperature, at the time of most of the major experiments, was between 19 - 20^{o}C.

Life-span and Determination of Death

Life-span data were collected four times a day for each of the chambers. The viability of the rotifer, as well as the presence of offspring were examined. Any offspring present were counted and removed from the chamber. With each examination, 1.0 ml of medium was removed and an equal amount of the appropriate fresh medium was added to assure a constant fresh food source.

Since the mean life-span was determined in a pilot experiment to be around 5 - 6 days, the fifth day was

considered to be the beginning of senescence. When the beginning of senescence was observed for any culture dish, that respective culture dish was observed every 3 hours; this gave a better estimate of the life-span of the rotifers. A gradual slowing of movement was generally the first sign of loss in viability. The rotifer was considered dead when there were no movement in its cilia or when it was in a state of deterioration.

Neutral Red Experiments

Using sterile water, a 1 mg/ml solution of neutral red (lot No. 021387; C.I. No. 50040, Aldrich Chemical, Milwaukee, Wisconsin) was prepared and labelled "Stock Neutral Red". Paramecium medium was prepared with various neutral red concentrations to obtain an optimal non-toxic level. A range of neutral red concentration between 0.1 and 1.6 microgram/ml were tried. The 0.75 microgram/ml concentration was found to give sufficient color to identify rotifers without any influence on life-span or reproduction. This concentration was used in all subsequent experiments. The dye was introduced into the Paramecium medium approximately 24 hours prior to its use to feed the individually segregated rotifers.

Ingestion of the dyed Paramecia by rotifers was detected through the gut content, seen as a dark red stain, whithin the rotifers. Newborn Asplanchna are known not to

eat for several hours (Birky 1964), thus ingestion experiments were carried out with rotifers at least 12 hours old. The assimilation of the stained Paramecia was seen through the loss of coloration in the gut of the rotifers; this was complete within 12 hours after ingestion of the stained Paramecia.

Alpha-Tocopherol Experiments

Solubilization of Alpha-tocopherol

The dl-alpha-tocopherol (Lot 19C-0439), obtained from Sigma Chemical Company, St. Louis, was solubilized by the use of Tween 80 (Sigma Chemical) as indicated by Enesco and Verdone-Smith (1980), with some modifications. The actual solubilization procedure was initially carried out at 80°C. One gram each of alpha-tocopherol and Tween 80 was stirred as 98 ml of distilled water, heated to 80°C, was slowly added. The solution was mixed for three hours under total darkness. To prevent any oxidation, nitrogen gas was bubbled into the mixture for 10 minutes and the solution was refrigerated and stored in total darkness. The storage of dissolved vitamin E was maintained only for one week. For any major experiments, new stock vitamin E was prepared and used. Using Tween 80 solubilized alpha-tocopherol, the concentration of alpha-tocopherol which would induce the longest mean life-span of rotifers was determined.

Because Tween 80 may influence rotifer life-span and synergism between Tween 80 and alpha-tocopherol may be present, the use of ethanol as a solvent for alpha-tocopherol was investigated. The exact molar concentration, determined in the alpha-tocopherol/Tween 80 experiment for the longest mean life-span was used for the solubilization with ethanol. Exactly 2.1535 g of alpha-tocopherol was mixed with 50.0 ml of 99% ethanol at room temperature. Absolute ethanol was not used due to benzene impurities required to manufacture absolute ethanol. The fact that alpha-tocopherol dissolved rapidly, usually in 1 - 3 minutes, and the lack of heat limited oxidation of alpha-tocopherol to a minimum. Ethanol was deemed to be the best alternative for solubilization as it reduced the possibility of oxidation, and did not influence rotifer life-span. For further experiments, ethanol was used as the solvent for alpha-tocopherol.

Detection of Alpha-Tocopherol

Presence of alpha-tocopherol in the medium was measured with a scanning U.V. spectrophotometer (Model SP8-100; PYE Unicam, Cambridge, England). Samples of 10 ml were filtered through a 8.0 micrometer Millipore filter (Millipore corporation, Bedford, Massachusetts) under suction. Wavelengths from 260 - 360 nm were scanned to determine the concentration of alpha-tocopherol, which has

a U.V. absorption maximum of 294 nm in alcohol, from a standard curve. This method was the most rapid and cost efficient means to determine the presence and concentration of alpha-tocopherol in the medium.

Uptake of Alpha-tocopherol

The question of whether alpha-tocopherol was influencing the rotifer life-span by its presence in the medium alone (environmental effect) or whether it had to be ingested by the rotifers (dietary effect) was examined.

Paramecium medium was prepared in the usual way and alpha-tocopherol was added, allowing the Paramecia to grow in the presence of alpha-tocopherol. After 1 day, this medium was filtered through a 8.0 micrometer Millipore filter (Millipore Corporation) to half its volume. The Paramecia were then mixed with medium that did not contain any alpha-tocopherol or any Paramecia and was rinsed through the filter in a similar manner four times. Rotifers fed with this medium would obtain alpha-tocopherol only through the ingestion of the Paramecia which alone would contain the alpha-tocopherol component. The control group was prepared in the same manner except that the control group did not have any alpha-tocopherol.

Bacterial contamination was prevented by carrying out the procedure in a laminar flow hood (CCI, Kupsville, Pennsylvanian) using sterile techniques. The Paramecia

numbers of both experimental and control group were adjusted to the same value as was obtained in the alpha-tocopherol life-span experiments by the addition of medium without any alpha-tocopherol or more Paramecia.

Lifespan study

Media with the appropriate concentration of alpha-tocopherol and controls were prepared 20 minutes prior to the life-span study. Rotifers of known age were transferred to new experimental chambers, along with 2.5 ml of medium with various alpha-tocopherol concentrations or control media. After transfer, the culture dishes were kept in the dark at 19°C in an incubator.

Ultraviolet Exposure Experiments

The effects of various U.V. dosages upon the rotifers were studied. The U.V. light came from a 4W mercury light source (Atomic Laboratories Inc., Berkeley, California) with an output of 10 $J/m^2/sec$ at a distance of 15 cm and with a frequency of 253.7 nm. The actual dosage was determined by the total length of exposure. One control received a 4W incandescent light source for the same duration as the U.V. exposure while a second control group was untreated in any way.

Experimental Setup

Whatman qualitative #1 filter paper, 4.25 cm in diameter, was cut exactly in half and autoclaved for 15 minutes. Using sterile technique, the cut filter paper piece was placed inside a sterile 60 x 15mm Petri dish (No. 1007; Falcon, Oxnard, California). Approximately 200 microliters of sterile distilled water were applied to the filter paper.

Rotifers 12 hours of age were washed twice in sterile distilled water to remove Paramecia as well as to remove any medium. Around 30 rotifers were picked up with a micropipette, on a 40 microliter setting, and spread onto the prepared filter paper. U.V. light readily passes through a water layer less than 1 mm (Dr. R. M. Roy; personal communication). Since the water layer on the surface of the rotifers was calculated and measured to be less than 0.5 mm with the total volume of water used, the absorption of U.V. due to the water was not taken into consideration. The low water content further immobilized the rotifers during the experiment.

Life-Span Studies

Rotifers were placed on top of the filter paper, as indicated previously, in a totally dark room. One set was given U.V. light while another was given incandescent light for the same length of time. After the appropriate

exposure period, 10 ml of fresh Paramecium medium were applied to each of the groups. The rotifers were transferred into tissue culture wells and then placed in an incubator for 12 hours, before the first examination. Total darkness was required throughout, to prevent any photo-reactivation which might otherwise have occured.

Ultraviolet Light and Alpha-tocopherol

The effects of ultraviolet light upon rotifers with alpha-tocopherol were examined. Rotifers were treated in the same manner as the ultraviolet experiment except that the offspring used for this experiment were born and kept in a medium with 25 ug/ml of vitamin E until the U.V. irradiation. After the U.V. light exposure, the rotifers were placed back and grown with alpha-tocopherol in its environment.

Light and Dark Experiments

Experiments were performed to determine the effects of light and darkness on rotifer life-span. The light source consisted of four 60W incandescent light bulbs (Canadian General Electric) and four cool white fluorescent lamps (No. F48T12/CW/SHO: Westinghouse) approximately 45 cm away from the culture plates. Those rotifers which were maintained in total darkness were kept in three 250 Erlenmeyer flasks with black plastic coating,

covered with aluminum foil. The flasks also had an aluminum cap on top of the sponge stopper, which prevented entrance of light from the top but allowed exchange of gases.

Transfer and feeding of the rotifers grown in total darkness required great care. The flasks were swirled and 30.0 ml of medium, containing the rotifers grown in darkness, were transferred into another covered 250 ml Erlenmeyer flask which contained 150 ml of fresh medium via a macropipetter (Oxford 10 ml Macro-Set, St. Louis, Missouri). This procedure was carried out in a totally dark room. The control groups were grown and transferred in the same manner as those grown in total darkness except that the Erlenmeyer flasks had only the aluminum cap; flasks were not covered, and the transfer of the control groups were done in the presence of light.

To collect life-span data, the same procedure was used as indicated in "Experimental Methods", except that the rotifers were exposed to a maximum of only 36 minutes of light daily.

Continuous Dark/Alpha-tocopherol

Alpha-tocopherol supplementation to the continuous dark reared rotifers was tested to see if there are any synergistic effects upon its life-span. Rotifers were prepared and treated as in the continuous dark experiment

except that new born rotifers were added into Paramecium medium in which alpha-tocopherol had been dissolved.

Statistical Analysis

A one-way analysis of variance (ANOVA), followed by a post hoc Tukey test were carried out using the formula described by Brunning and Kinz (1977) and Sokal and Rohlf (1981). Regression analyses were carried out as indicated by Sokal and Rohlf (1981). Life tables were constructed for all studies according to Krebs (1978) and are presented in Appendix III.

From the data obtained in the one-way ANOVA test, a one-tailed or two-tailed T-test can also be done as described by Brunning and Kinz (1977). The T-test was performed when the p value was between 0.05 and 0.10 in the one-way ANOVA test. Under normal conditions, such a p range from the one-way ANOVA test will show up as nonsignificant in the post hoc Tukey test. Thus the T-test was performed to see whether a less stringent post hoc test can show any significance for any of the groups that were analysed.

To facilitate the statistical analysis, computer programs were custom written with the aid of an IBM PC computer in the BASIC language, Version D1.10, with the help and assistance of Mr. Hiroyuki Sawada (personal communication). All of the BASIC programs are presented

in Appendix IV. A sample data input and program output is also shown after each of its respective program.

RESULTS

The inital step in each series of experiments was to establish the normal life-span and fecundity of A. brightwelli; standard control. In some cases involving a certain treatment, a second set of controls were needed to separate the effect of this treatment process from the variable being tested.

A reproductive profile was constructed by dividing the life-span of the rotifer into three developmental periods; prereproductive, reproductive and postreproductive time. Any experimental treatment could thus be evaluated for its influence on any or all of the different stages of the life cycle.

Neutral Red

Neutral red was introduced to the Paramecium medium where it was ingested first by the Paramecia and then in turn by the rotifer. This procedure made the rotifer more visible through the accumulation of the dye in its gut and its food ingestion could be checked.

Longevity

The life-span of the rotifers grown in several concentrations of neutral red was compared to the life-

span of untreated control rotifers. The results are shown in table 1. The data indicate that longevity was not affected by neutral red in the concentrations range of 0.1 to 1.6 ug/ml. A one-way analysis of variance showed that there were no significant differences between any groups. ($(F\ 4, 235) = 0.746$, $p>0.5$). Thus, neutral red did not have any effect upon the life-span of the rotifers.

TABLE 1

MEAN LIFE-SPAN FOR A. BRIGHTWELLI AT VARIOUS NEUTRAL RED CONCENTRATIONS (N = 48)

Neutral Red Concentration (microgram/ml)	Mean Life-span (days ± S.E.)
0.0 (control)	5.38 ± 0.12
0.1	5.14 ± 0.13
0.5	5.30 ± 0.15
0.75	5.27 ± 0.10
1.6	5.46 ± 0.17

Fecundity

The reproductive profile of A. brightwelli can be seen in table 2. The influence of various concentrations of neutral red on the reproductive profile of A. brightwelli is compared with control values. The average prereproductive time does not differ between the

TABLE 2

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI AT VARIOUS NEUTRAL RED CONCENTRATIONS
(N = 48)

Neutral Red (ug/ml)	Ave. Prereprod. Time (days) ± S.E.	Ave. Reprod. Time (days) ± S.E.	Ave. Postrep. Time (days) ± S.E.	Ave. No. Offspring per rotifer
0.0 *	2.31 ± 0.04	3.08 ± 0.12	0.19 ± 0.10	9
0.1	2.24 ± 0.06	2.89 ± 0.12	0.15 ± 0.01	8
0.5	2.21 ± 0.06	3.08 ± 0.16	0.10 ± 0.04	9
0.75	2.20 ± 0.04	3.11 ± 0.13	0.15 ± 0.04	9
1.6	2.23 ± 0,08	3.79 ± 0.15	0.10 ± 0.04	10

* Control

groups ((F 4, 235) = 0.578, p>0.5). In contrast, the length of the reproductive period was found to be significantly different among groups with the ANOVA test ((F 4, 235) = 6.615, p<0.001). The post hoc Tukey test revealed that at the concentration of 1.6 ug/ml neutral red, the reproductive time was significantly longer as compared to the control or to any other dye concentration (alpha = 0.01). However, the average offspring number per rotifer did not differ between groups ((F 4, 235) = 2.071, 0.10>p>0.05), and the post hoc Tukey test (alpha = 0.05) also showed that at the concentrations used, there was no significant effect of neutral red on the number of offspring. However, when a less stringent post hoc two-tailed T-test was applied to these data, it showed that at the concentration of 1.6 ug/ml, the average offspring number was higher than at the concentration of 0.1 ug/ml (alpha = 0.01, T = 2.617). To optimize visibility of the rotifers and to avoid any adverse effect of the dye, a neutral red concentration of 0.75 ug/ml was selected as most appropriate for this study.

Paramecia Ingestion Through Neutral Red

The gastric gland and the cornal ciliated area of the head of the rotifers frequently took up the stain and were lightly colored. Nevertheless, dye uptake was primarily through the ingestion of the Paramecia, which

readily took up the dye through diffusion.

Pilot experiments were carried out to determine what density of neutral red-stained Paramecia should be fed to the rotifers so as to see them readily by the dye spot in their gut. Paramecia were exposed to 0.75 ug/ml neutral red for 5 hours and then fed at different densities (measured by number of Paramecia per ml) to rotifers. The data in Table 3 show that 94% of the roifers fed 206 $\pm$ 11 Paramecia/ml became stained in the 5 hour test period. Therefore a density greater than 206 $\pm$ 11 stained Paramecia/ml was used in subsequent experiments so that the rotifers would have the noticable dark red spot in the gut for clear microscopic visualization.

If any of the rotifers in the experiment were transfered to a regular paramecia medium or to a 0.75 ug/ml neutral red distilled water solution, the dark spot in the gut was no longer visible after 12 hours. Although the results do not quantify the number of Paramecia ingested, they do show that neutral red is an indicator that the Paramecia have been ingested by the rotifers. This method could be used to show whether a certain experimental condition would prevent or reduce the ingestion of the Paramecia by the presence or absence of the dark coloration within the gut.

TABLE 3

THE EFFECT OF VARIOUS DENSITIES OF NEUTRAL RED STAINED PARAMECIA IN GUT COLORATION OF 12 HOUR OLD *A. BRIGHTWELLI* AFTER 5 HOURS

Density of Paramecia (Paramecia/ml) ± S.E. (n = 6 counts).	No. of Rotifer With Dark Spot in Gut (n = 192).	% Rotifers With Dark Spot in Gut
0	0	0.0
53 ± 3	30	15.6
118 ± 6	122	63.5
206 ± 11	181	94.3
2450 ± 15	190	99.0

Alpha-tocopherol Experiments

Optimum Alpha-tocopherol Determination

The experiments performed in this section were designed to determine how various concentrations of alpha-tocopherol would influence life-span and fecundity. Since vitamin E is not water soluble, an equal concentration of Tween 80 was used as an agent to solubolize the vitamin E.

Longevity

Longevity data are presented in table 4 for rotifers cultured in various concentrations of Tween 80 ranging from 5 to 100 ug/ml of Tween 80. One-way analysis of variance showed that there were no significant differences in life-span between the control group or those cultured in Tween 80 at any concentration ($(F\ 5, 138) = 0.441$, $p>0.75$).

The influence of vitamin E on the life-span of rotifers was examined in the next series of experiments. Rotifers were cultured in vitamin E solubilized in Tween 80 at concentrations ranging from 5 - 100 ug/ml. Data on the life-span of these groups of vitamin E/Tween 80 cultured rotifers as compared to control rotifers is presented in table 5. The data show that rotifers cultured in 25 ug/ml vitamin E/Tween 80 have a significantly longer life-span than any other group. A one-way analysis of variance

TABLE 4

THE EFFECT OF TWEEN 80 ON THE LIFE-SPAN OF A. BRIGHTWELLI (N = 24)

Concentration of Tween 80 (microgram/ml)	Maximum Longevity (Days)	Average Life-span ± S.E. (Days)
0	6.5	5.46 ± 0.13
5	6.0	5.42 ± 0.09
10	6.5	5.40 ± 0.13
25	6.0	5.29 ± 0.08
50	6.5	5.42 ± 0.13
100	6.5	5.54 ± 0.15

- No significant difference between any group

indicated that there were significant differences among the mean life-span of these groups ((F 5, 138) = 9.589, $p<0.001$). The results of the post hoc Tukey test, shown in table 6, indicates that 25 ug/ml of vitamin E/Tween 80 significantly increased the mean life-span of the rotifers (6.38 ± 0.12 days) compared with that of the regular control (5.46 ± 0.13 days) or of its respective Tween control (5.29 ± 0.08 days). As verified in an earlier section, the presence of Tween 80 did not have any effect

upon the life-span at any of the concentrations used. Thus the increase in the mean life-span can be attributed only to the vitamin E component.

TABLE 5

THE EFFECT OF VITAMIN E ON THE LIFE-SPAN OF *A. BRIGHTWELLI* (N = 24)

Concentration of Vitamin E/Tween (microgram/ml)	Maximum Longevity (Days)	Average Life-span ± S.E.M. (Days)
0	6.5	5.46 ± 0.13
5	7.0	5.67 ± 0.15
10	7.0	5.77 ± 0.13
25	7.5	6.38 ± 0.12
50	7.0	5.29 ± 0.16
100	6.5	5.22 ± 0.12

TABLE 6

RESULTS OF THE POST HOC TUKEY TEST USED TO EVALUATE SIGNIFICANCE BETWEEN MEAN LIFE-SPAN OF VITAMIN E/TWEEN GROUP WITH CONTROLS

Vitamin E/Tween Concentration (microgram/ml)	F Value		Control	Tween 80 Control
5	(F 2, 29) = 1.095,	p>0.2	NSD	NSD
10	(F 2, 29) = 2.393,	0.2>p>0.1	NSD	NSD
25	(F 2, 29) = 26.781,	p<0.001	*	*
50	(F 2, 29) = 0.386,	p>0.2	NSD	NSD
100	(F 2, 29) = 1.403,	p>0.2	NSD	NSD

NSD - no significant difference

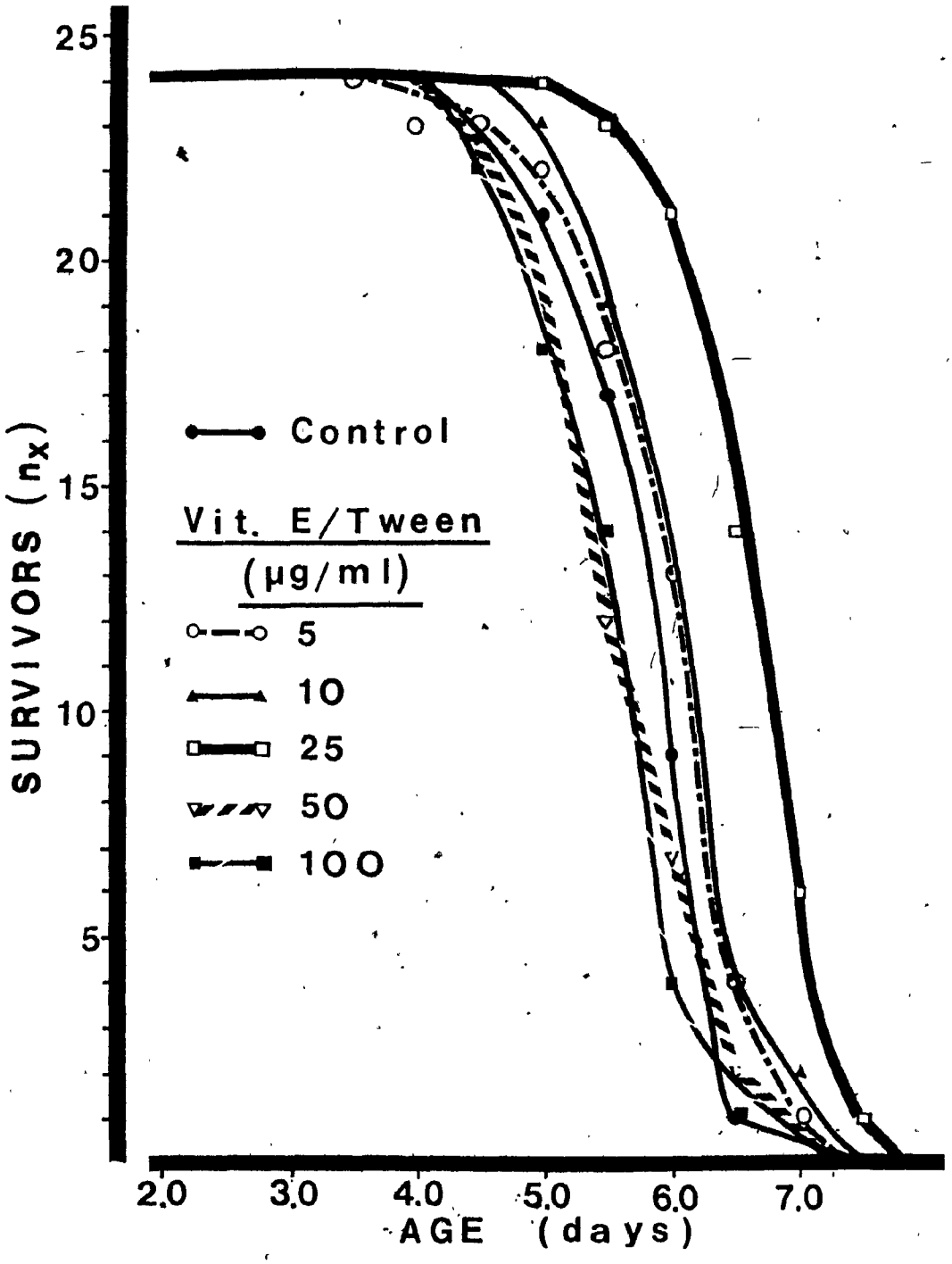
* alpha = 0.01

A series of figures, Figures 1-4, allows a graphic presentation emphasizing various relationships in the life-span data. As noted previously, only when rotifers are exposed to a concentration of 25 ug/ml of vitamin E is there a significant increase in the life-span.

Figure 1 shows the survival curves of the vitamin E group. The group of rotifers treated with 25 ug/ml of vitamin E has the curve which lies farthest to the right, indicative of the longest life-span. It is important to note that the entire life-span is influenced.

To examine the relationship between the various concentrations of vitamin E and to consider the effect of the Tween 80 solubilizing agent, life-span in days is plotted against concentration of both vitamin E and Tween 80 in Figure 2. This presentation emphasizes that Tween 80 has very little effect on life-span. It further emphasizes that the effect of vitamin E on life-span occurs in a very limited concentration range. The fact that 5 and 10 ug/ml vitamin E give a slight increase in life-span may indicate that there is a regular dose response curve peaking at 25 ug/ml, over a very limited range of concentrations. In taking the mean life-span values from 0 - 25 ug/ml, a linear response (Mean life-span in days = 0.04 * Concentration of vitamin E in ug/ml + 5.46; $r^2 = 0.99$) is seen.

FIGURE 1. Survivorship curves of
A. brightwelli exposed to different vitamin E/Tween
concentrations as compared to control. (n = 24)


25
20
15
10
5
SURVIVORS (n_x)
Control
Vit. E/Tween
(µg/ml)
5
10
25
50
100
2.0
3.0
4.0
5.0
6.0
7.0
AGE (days)

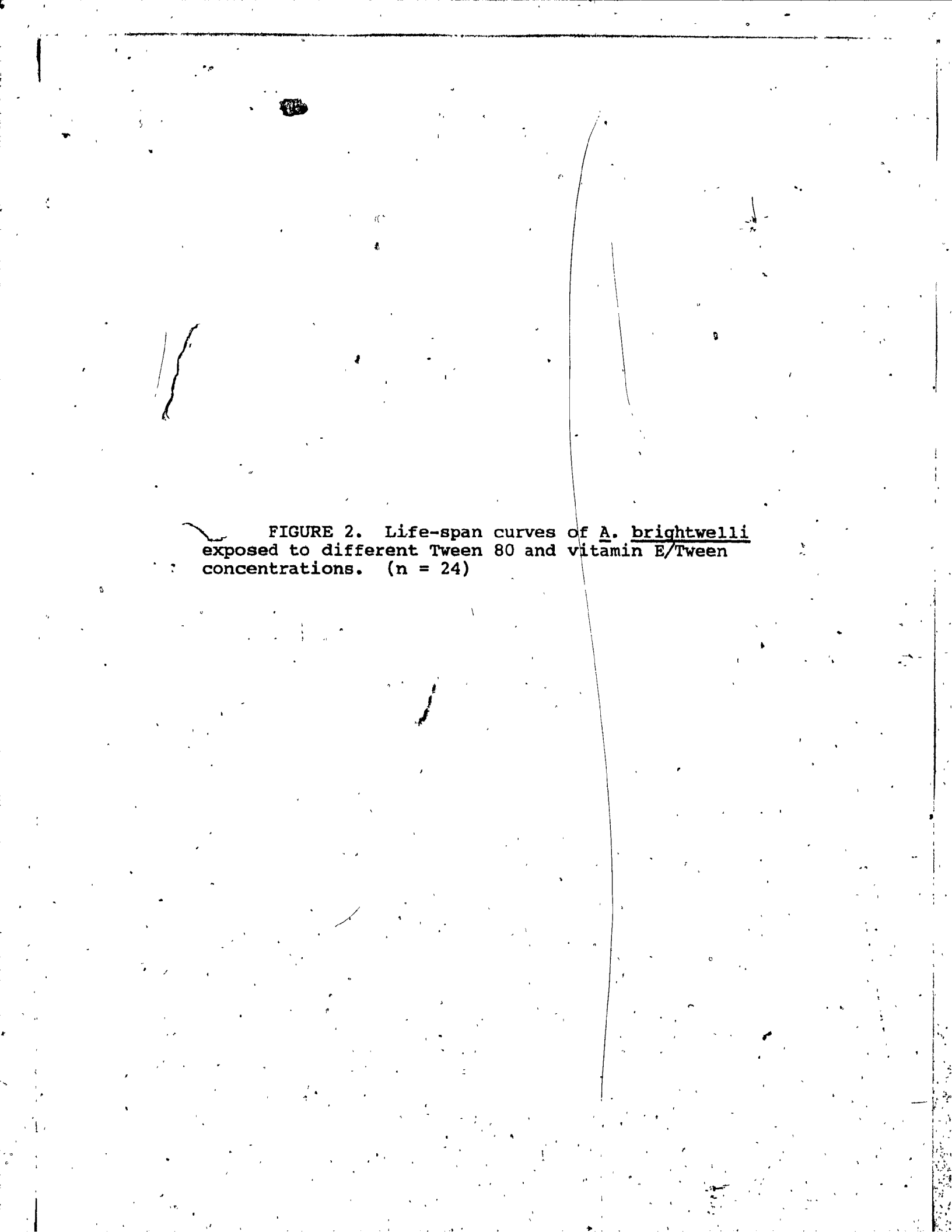
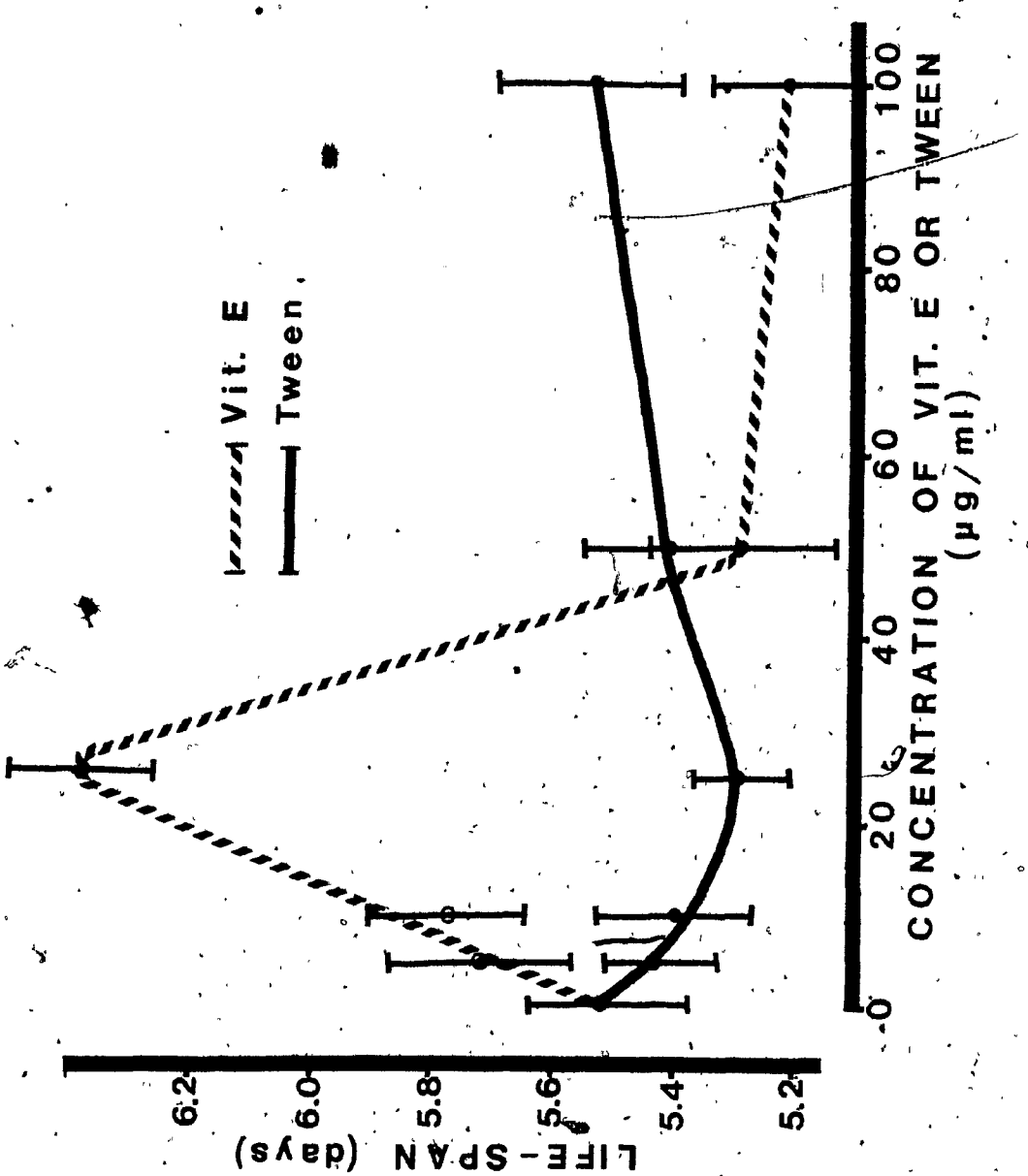


FIGURE 2. Life-span curves of A. brightwelli exposed to different Tween 80 and vitamin E/Tween concentrations. (n = 24)


Vit. E
Tween
LIFE-SPAN (days)
6.2
6.0
5.8
5.6
5.4
5.2
0
20
40
60
80
100
CONCENTRATION OF VIT. E OR TWEEN (µg/ml)

The histogram in Figure 3 clearly shows that maximum life-span is attained at 25 ug/ml of vitamin E and that the expected life-span at this concentration is equally high. Thus rotifers born at this concentration can expect to live for the longest time period. The expected life-span for this histogram was obtained from the lifetables in appendix III.

Figure 4 shows the age specific death rate of the rotifers from 0 - 100 ug/ml of vitamin E. Note that the vitamin E curve is consistently to the right of its respective Tween control group at concentrations of 5, 10 and 25 ug/ml. However, from the 50 ug/ml concentration on, the overlap of the curves indicates that both vitamin E and Tween curves have their maximum death period during the same time. At higher concentration of 50 or 100 ug/ml of vitamin E, the life-span was no different from that of the control and the age specific death rate was no different from the control rate. Vitamin E was seen to influence life-span only at the 25 ug/ml concentration.

The reproductive profile, showing the prereproductive, reproductive and postreproductive time under the varied vitamin E concentrations can be seen in table 7 along with their respective controls. The one-way analysis of variance and the post hoc Tukey test were performed between each experimental and its respective

FIGURE 3. Life-span histograms of *A. brightwelli* exposed to different vitamin E/Tween concentrations. (n = 24)

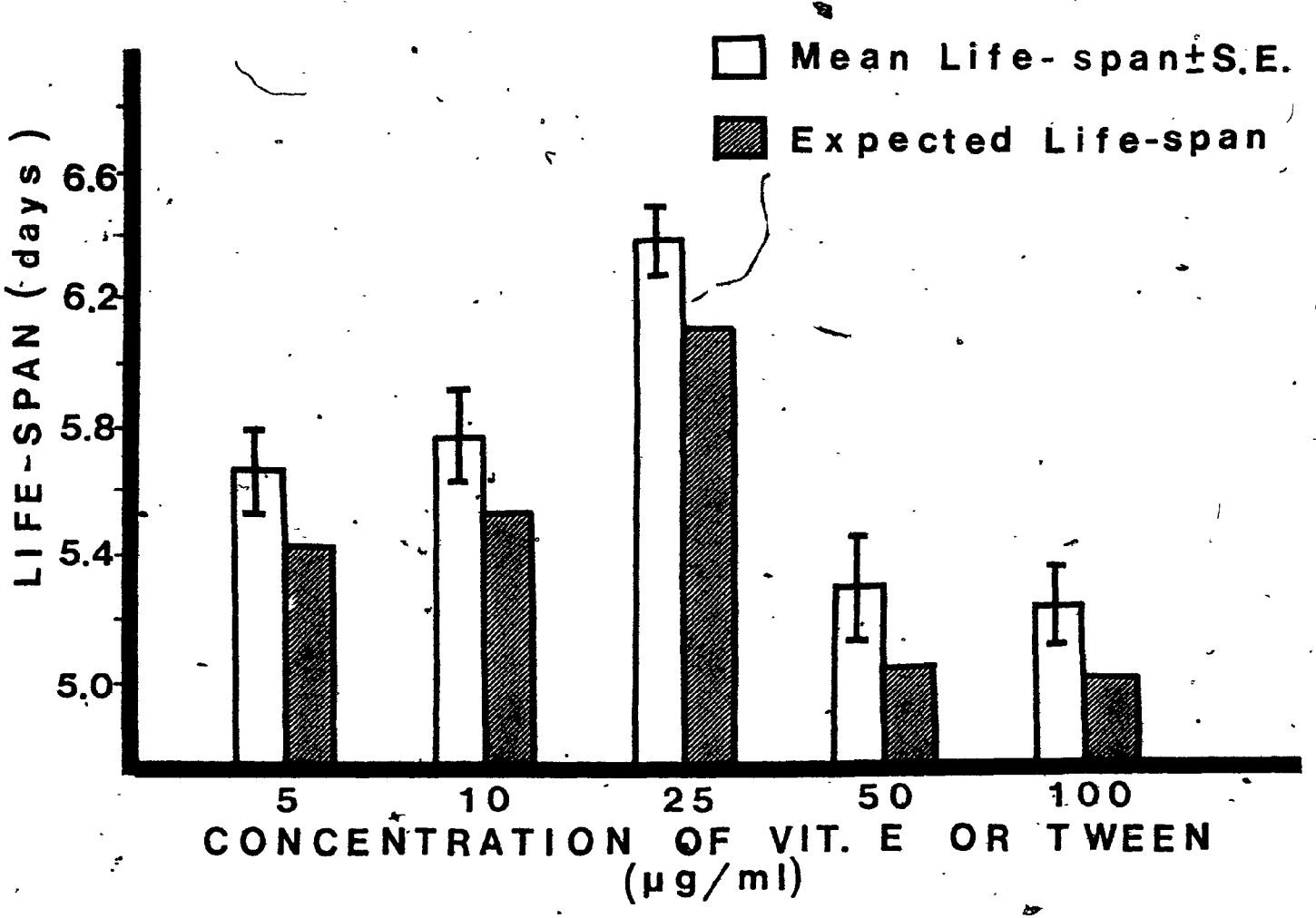

Mean Life-span±S.E.
Expected Life-span
LIFE-SPAN (days)
6.6
6.2
5.8
5.4
5.0
5
10
25
50
100
CONCENTRATION OF VIT. E OR TWEEN
(µg/ml)

FIGURE 4. The age specific death rates (dx) for A. brightwelli exposed to different vitamin E/ Tween concentrations as compared with Tween controls. (n = 24)

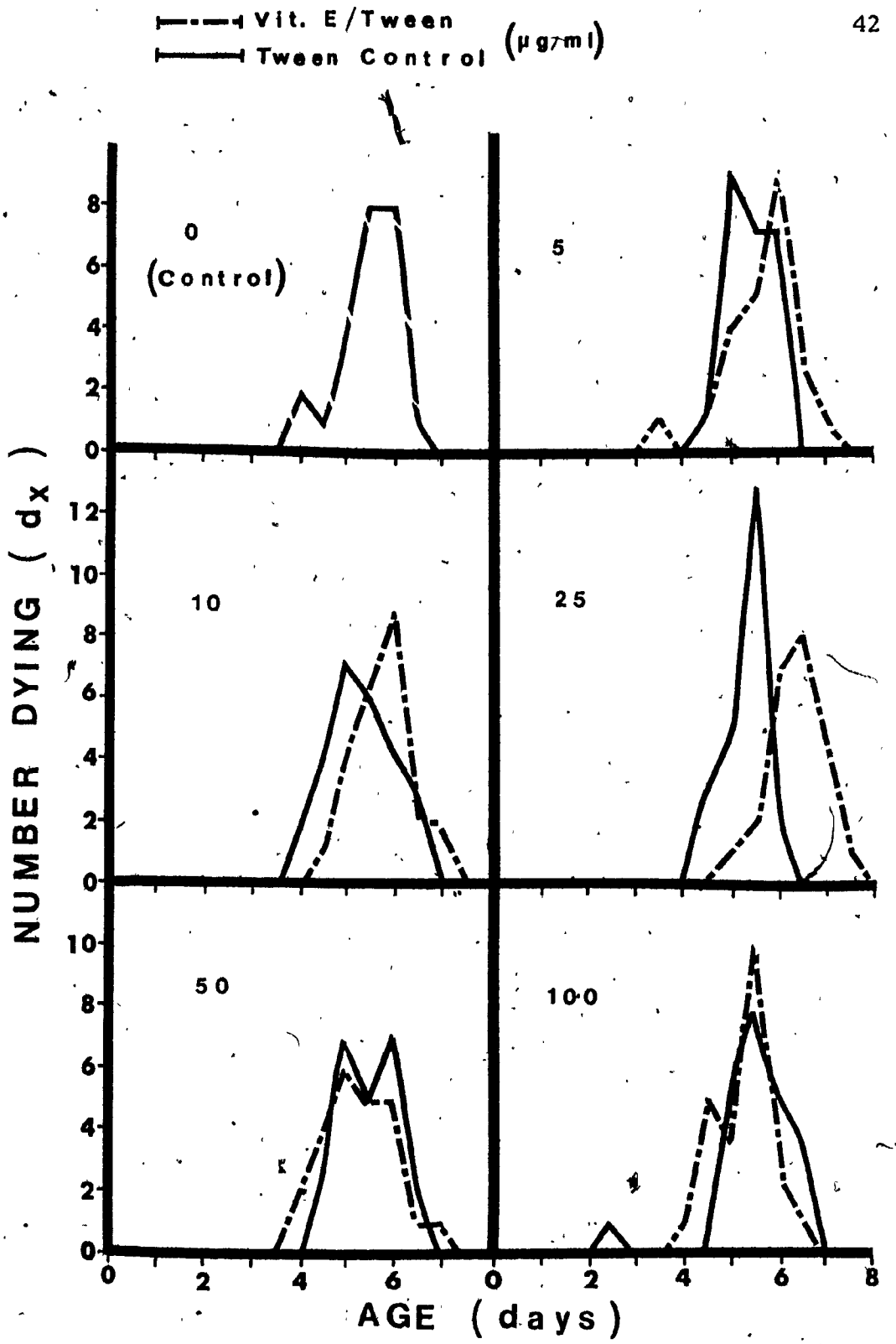

Vit. E/Tween
Tween Control
(µg/ml)
0
(Control)
5
10
25
50
100
NUMBER DYING (dx)
AGE (days)

TABLE 7

REPRODUCTIVE PROFILE OF *A. BRIGHTWELLI* EXPOSED TO DIFFERENT VITAMIN E/TWEEN CONCENTRATIONS

Concentration (ug/ml)	Ave. Prereprod. Time ± S.E. (days)	Ave. Reproductive Time ± S.E. (days)	Ave. Postreprod. Time ± S.E. (days)	Ave. Offspring Number/ Rotifer
0	2.31 ± 0.05	3.00 ± 0.15	0.15 ± 0.06	9
5 Vit. E/Tween	2.48 ± 0.06	3.04 ± 0.12	0.15 ± 0.06	6
5 Control	2.46 ± 0.05	2.90 ± 0.01	0.06 ± 0.03	8
10 Vit. E/Tween	2.54 ± 0.06	3.10 ± 0.13	0.13 ± 0.06	7
10 Control	2.38 ± 0.07	2.94 ± 0.11	0.10 ± 0.05	8
25 Vit. E/Tween	2.67 ± 0.05	3.35 ± 0.15	0.33 ± 0.08	9
25 Control	2.46 ± 0.05	2.73 ± 0.09	0.10 ± 0.04	8
50 Vit. E/Tween	2.67 ± 0.06	2.25 ± 0.14	0.38 ± 0.11	6
50 Control	2.46 ± 0.05	2.92 ± 0.10	0.15 ± 0.05	9
100 Vit. E/Tween	2.65 ± 0.06	2.46 ± 0.13	0.15 ± 0.06	6
100 Control	2.33 ± 0.12	2.90 ± 0.19	0.08 ± 0.04	9

control and the results are presented in tables 8A through 8D. In all cases, there were no significant differences between the untreated control and the Tween control. The life profile, showing the reproductive period in relation to the entire life-span is shown in Figure 5.

A significant increase in the length of the prereproductive period, as seen by the post hoc Tukey test in table 8A, was found at vitamin E/Tween concentration of 25, 50 and 100 ug/ml. The prereproductive and reproductive time periods are plotted against the various vitamin E and Tween concentration values in Figure 6. Both the vitamin E/Tween and the Tween caused an increase in the mean prereproductive time but this leveled off to plateau at 2.67 days for the vitamin E and 2.46 days for the Tween group. The length of the prereproductive period was significantly longer only at vitamin E concentrations of 25, 50 and 100 ug/ml (alpha = 0.01; regular control, alpha = 0.05; respective Tween control). Since the Tween control and the regular control were not significantly different, only the vitamin E component was responsible for the increase in the length of prereproductive period of the rotifer.

The length of the reproductive time for rotifers exposed to 25 ug/ml of vitamin E (3.35 $\pm$ 0.15 days) was not significantly different from that of the regular

FIGURE 5. Life profiles of A. brightwelli exposed to different Tween 80 and vitamin E/Tween concentrations. (n = 24)

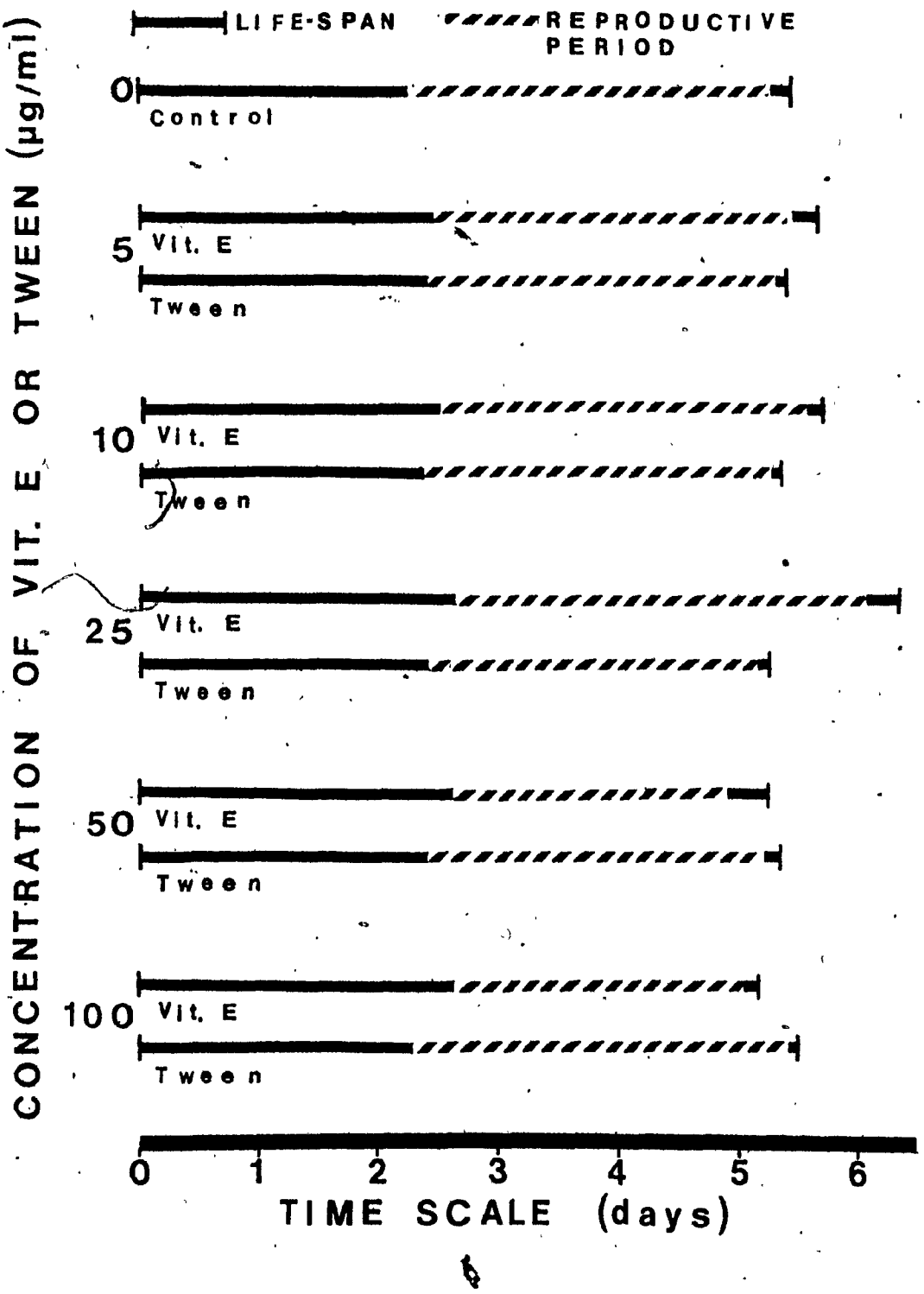

LIFE-SPAN
REPRODUCTIVE PERIOD
0
Control
5
Vit. E
Tween
10
Vit. E
Tween
25
Vit. E
Tween
50
Vit. E
Tween
100
Vit. E
Tween
CONCENTRATION OF VIT. E OR TWEEN (µg/ml)
0 1 2 3 4 5 6
TIME SCALE (days)

Figure 6. Mean prereproductive and reproductive time period curves under different Tween 80 and Vitamin E/Tween concentrations.

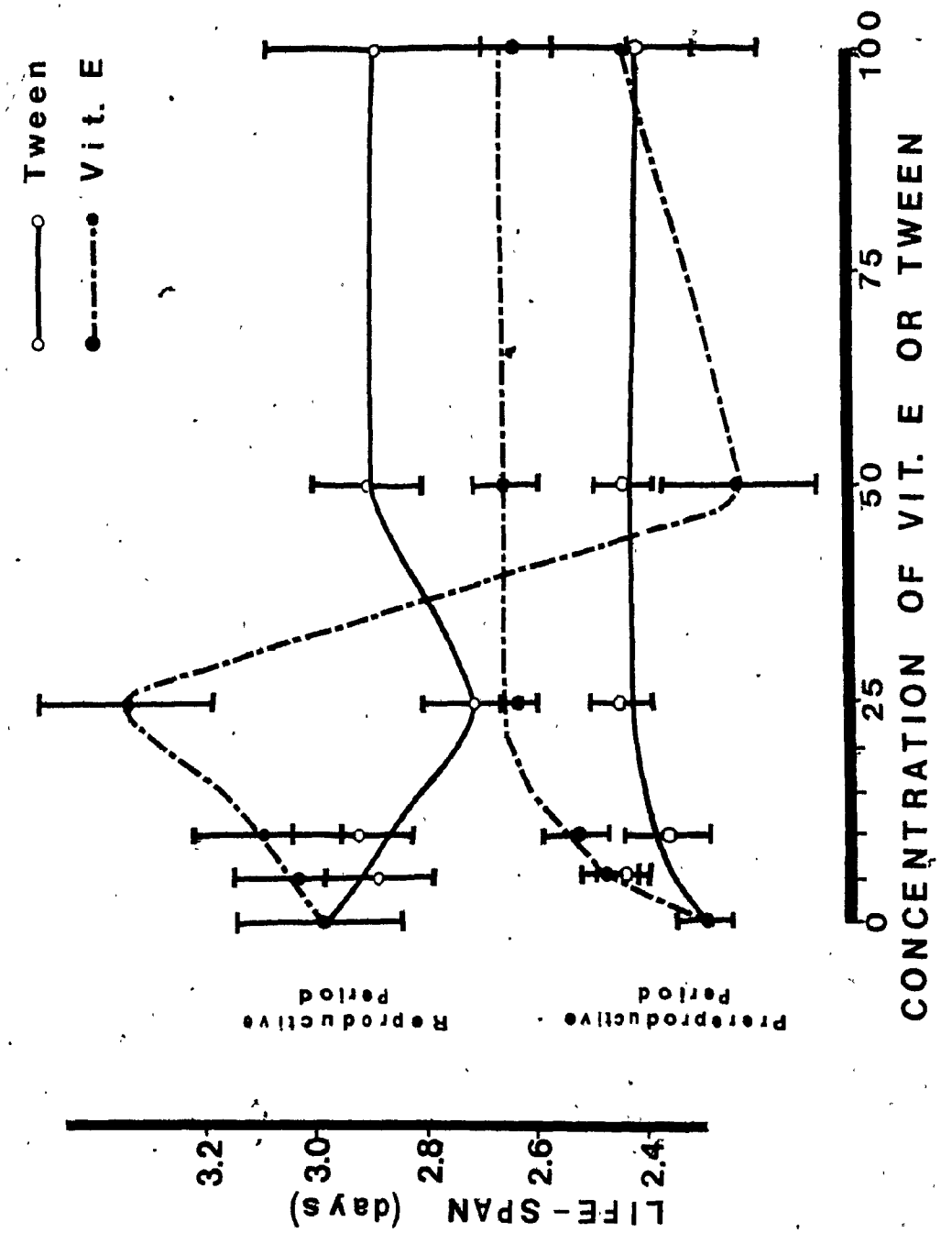

Tween
Vit. E
CONCENTRATION OF VIT. E OR TWEEN
0
25
50
75
100
Preproductive period
Reproductive period
LIFE-SPAN (days)
3.2
3.0
2.8
2.6
2.4

TABLE 8A

RESULTS OF THE POST HOC TUKEY TEST USED TO EVALUATE SIGNIFICANCE BETWEEN LENGTH OF THE VITAMIN E/TWEEN PREREPRODUCTIVE PERIOD AND THAT OF CONTROLS

Vitamin E/Tween Concentration (microgram/ml)	F values	Control	Tween 80 Control
5	(F 2, 69) = 2.966, $0.10>p>0.05$	NSD	NSD
10	(F 2, 69) = 3.880, $0.05>p>0.025$	NSD	NSD
25	(F 2, 69) = 10.095, $p<0.001$	*	**
50	(F 2, 69) = 11.169, $p<0.001$	*	**
100	(F 2, 69) = 5.510, $0.01>p>0.005$	*	**

NSD - no significant difference

* alpha = 0.01

** alpha = 0.05

control group (3.00 ± 0.15 days). However, the reproductive period for the Tween control (2.73 ± 0.09 days) was significantly shorter than in the vitamin E treated or in regular control rotifers (alpha = 0.01). This effect can be seen in table 8B and graphically shown in Figure 6. At 50 ug/ml of vitamin E with mean reproductive time of 2.25 ± 0.14 days, the experimental group had a significantly lower reproductive period than either the regular control (3.00 ± 0.15 days) or to its Tween control (2.92 ± 0.10 days, alpha = 0.01). The other concentrations studied did not significantly alter the length of the reproductive period.

The length of the postreproductive period for rotifers reared at various vitamin E/Tween concentration is shown in table 8C. The postreproductive period of rotifers treated with 25 ug/ml vitamin E group (0.22 ± 0.08 days) was significantly longer than that of its Tween control (0.10 ± 0.04 days, alpha = 0.01). No other concentrations produced any significant differences as evaluated with the post hoc Tukey test. The rotifers exposed to 50 ug/ml of vitamin E seemed to have a higher postreproductive time (0.38 ± 0.11 days), but the difference was not significant using the post hoc Tukey test. However, the use of a less stringent post hoc two-tailed T-test indicates that postreproductive duration

TABLE 8B

RESULTS OF THE POST HOC TUKEY TEST USED TO EVALUATE SIGNIFICANCE BETWEEN LENGTH OF THE VITAMIN E/TWEEN REPRODUCTIVE PERIOD AND THAT OF CONTROLS

Vitamin E/Tween Concentration (microgram/ml)	F values	Control	Tween 80 Control
5	(F 2, 69) = 0.367, p>0.75	NSD	NSD
10	(F 2, 69) = 0.403, p>0.5	NSD	NSD
25	(F 2, 69) = 5.566, 0.01>p>0.005	NSD	*
50	(F 2, 69) = 9.380, p<0.001	*	*
100	(F 2, 69) = 3.185, 0.05>p>0.025	NSD	NSD

NSD - no significant difference

* alpha = 0.01

TABLE 8C

RESULTS OF THE POST HOC TUKEY TEST USED TO EVALUATE SIGNIFICANCE BETWEEN LENGTH OF THE VITAMIN E/TWEEN POSTREPRODUCTIVE PERIOD AND THAT OF CONTROLS

Vitamin E/Tween Concentration (microgram/ml)	F values	Control	Tween 80 Control
5	(F 2, 69) = 0.827, 0.50>p>0.25	NSD	NSD
10	(F 2, 69) = 0.123, p>0.75	NSD	NSD
25	(F 2, 69) = 3.494, 0.05>p>0.025	NSD	**
50	(F 2, 69) = 3.358, 0.05>p>0.025	NSD	NSD
100	(F 2, 69) = 0.750, p>0.50	NSD	NSD

NSD - no significant difference

** alpha = 0.05

in the 50 ug/ml vitamin group is higher than either of the controls (alpha = 0.05, T = 2.00).

The data thus far indicate that the increase in the mean life-span of the rotifers was primarily due to the significant increase in the prereproductive stage.

Number of Offspring

Aside from the 25 ug/ml of vitamin E/Tween group ((F 2, 69) = 0.546, p>0.50) with mean of 9 offspring per rotifer, the average mean number of offspring per rotifer for all other vitamin E groups was found to be statistically lower than in the regular control or than in the respective Tween control group (see table 8D). Thus the rotifers exposed to 25 ug/ml of vitamin E were the only group which did not have a significantly lower number of mean offspring per rotifer.

Paramecia Ingestion

Paramecia numbers were counted to determine their availability to the rotifers. The ingestion of Paramecia was also checked through the use of neutral red as indicated previously. These measures were necessary since dietary restriction on the rotifers can increase the life-span and influence the reproductive profile of A. brightwelli. Paramecia counts were compared in cultures exposed to the optimal vitamin E concentration of 25 ug/ml,

TABLE 8D

SIGNIFICANCE BETWEEN THE OFFSPRING NUMBER/ROTIFER WITH CONTROLS USING THE POST HOC TUKEY TEST

Vitamin E/Tween Concentration (ug/ml)		Control	Tween 80 Control
5	(F 2, 69) = 10.174, p<0.001	*	**
10	(F 2, 69) = 9.936, p<0.001	*	*
25	(F 2, 69) = 0.546, p>0.50	NSD	NSD
50	(F 2, 69) = 12.719, p<0.001	*	*
100	(F 2, 69) = 10.687, p<0.001	*	*

NSD - no significant difference

* alpha = 0.01

** alpha = 0.05

in Tween cultures and in control culture. Comparisons were also made for different age groups to make sure that sufficient and equal amounts of food were available for the rotifers (see table 9A). Since ingestion of the Paramecia can be detected by the use of neutral red (see table 9B), this acts as a further control to show that the Paramecia that are available are being eaten. This control is needed to eliminate the possibility that the vitamin E may actually be an agent which interferes with the ingestion of the Paramecia thereby creating a situation similar to dietary restriction.

Data for the Paramecia counts taken from culture wells of rotifers at different ages are shown in table 9A. Paramecia counts were not significantly different for any age group. This establishes that a constant supply of food was available throughout the life-span. The rotifers were replenished every 6 hours with fresh Paramecia.

The data on percent Paramecia ingestion in table 9B show that the rotifers were eating the food source to an equal extent in all treatment groups. Therefore at any given time period, the amount of Paramecia was the same and they were being eaten to an equal extent by the rotifers. Therefore dietary restriction can be ruled out as a factor which caused the increase in the mean life-span or influenced the reproductive pattern of A. brightwelli.

TABLE 9A

PARAMECIA COUNT WITH 25 UG/ML OF VITAMIN E/TWEEN (n = 6)

Age (days)	Paramecia Levels ± S.E.M. (No./ml) Control	Tween Control	Vitamin E/ Tween
0 - 1	1696 ± 48	1804 ± 79	1830 ± 40
1 - 2	1836 ± 63	1834 ± 97	1831 ± 110
2 - 3	1646 ± 76	1678 ± 90	1798 ± 128
3 - 4	1788 ± 95	1974 ± 104	2126 ± 196
4 - 5	2001 ± 117	2176 ± 69	2145 ± 155
5 - 6	1819 ± 79	1916 ± 82	1974 ± 81
6 - 7	2100 ± 32	2045 ± 62	2211 ± 79
7 - 8	2023 ± 88	1976 ± 85	2025 ± 48

No significant difference between any group

TABLE 9B

INGESTION OF PARAMECIA BY A. BRIGHTWELLI AT THREE DIFFERENT AGES REARED WITH 25 UG/ML OF VITAMIN E/TWEEN AFTER FIVE HOURS (n = 48)

Age (days)	% Rotifers With Dark Spot in Gut Control	Tween Control	Vitamin E/Tween
0.5	95.8	95.8	97.9
2.5	97.9	95.8	97.9
4.5	97.9	97.9	100.0

Solubilization of Vitamin E in Ethanol

An alternative solvent was introduced during the course of these experiments in an attempt to lessen the oxidation of vitamin E as well as to show that the effects of vitamin E were not caused by a synergistic reaction with the Tween 80. In this series of experiments, vitamin E was dissolved in ethanol rather than in Tween 80. The effect of ethanol solubilized vitamin E was then evaluated on the same parameters examined above; life-span, reproductive profile and fecundity of A. brightwelli. The results of these experiments are shown in table 10, which compares the effects of Tween solubilized and ethanol solubilized vitamin E.

The vitamin E dissolved in ethanol produced very similar biological results to the vitamin E/Tween group at the same concentration of 25 ug/ml. The mean life-span of 6.81 $\pm$ 0.11 days for the vitamin E/ethanol group was slightly longer than the mean life-span of 6.38 $\pm$ 0.12 days of the vitamin E/Tween group. This difference was not significant using the post hoc Tukey test. However, with the less stringent post hoc two-tailed T-test, a significant difference was seen (alpha = 0.05, T = 2.00). The survivorship curve in Figure 7 illustrates the difference between the vitamin E dissolved in Tween 80 or in ethanol. In either case the entire curve is shifted to

TABLE 10

THE EFFECT OF VITAMIN E DISSOLVED IN ETHANOL FOR A. BRIGHTWELLI
(n = 24)

	Regular Control	Ethanol Control (29 ul/ml)	Vitamin E/ Tween (25 ug/ml)	Vitamin/E ethanol (25 ug/ml
Ave. Life-span ± S.E. (days)	5.46 ± 0.13	5.63 ± 0.17	6.38 ± 0.12 *	6.81 ± 0.10 *
Ave. Prerep. Time ± S.E. (days)	2.31 ± 0.05	2.48 ± 0.06	2.67 ± 0.06 *R	3.06 ± 0.09 *A
Ave. Reprod. Time ± S.E. (days)	3.00 ± 0.15	2.96 ± 0.14	3.35 ± 0.14	3.50 ± 0.12 **
Ave. Postrep. Time ± S.E. (days)	0.14 ± 0.06	0.10 ± 0.04	0.33 ± 0.08	0.19 ± 0.07
Ave. Offspring No./Rotifer	9	9	9	9

* alpha = 0.01 from both controls

*R alpha = 0.01 from regular control

*A alpha = 0.01 from both controls and vitamin E/Tween

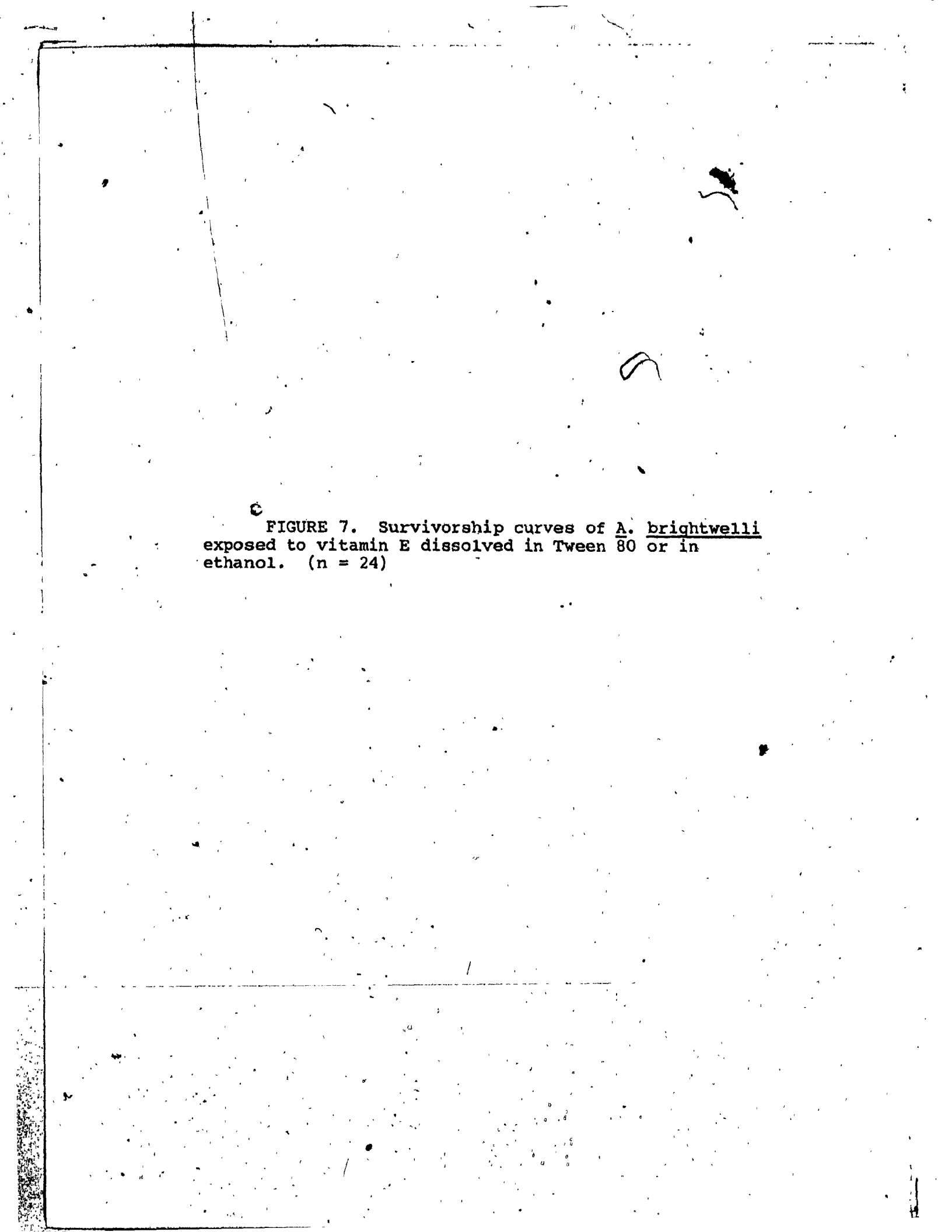
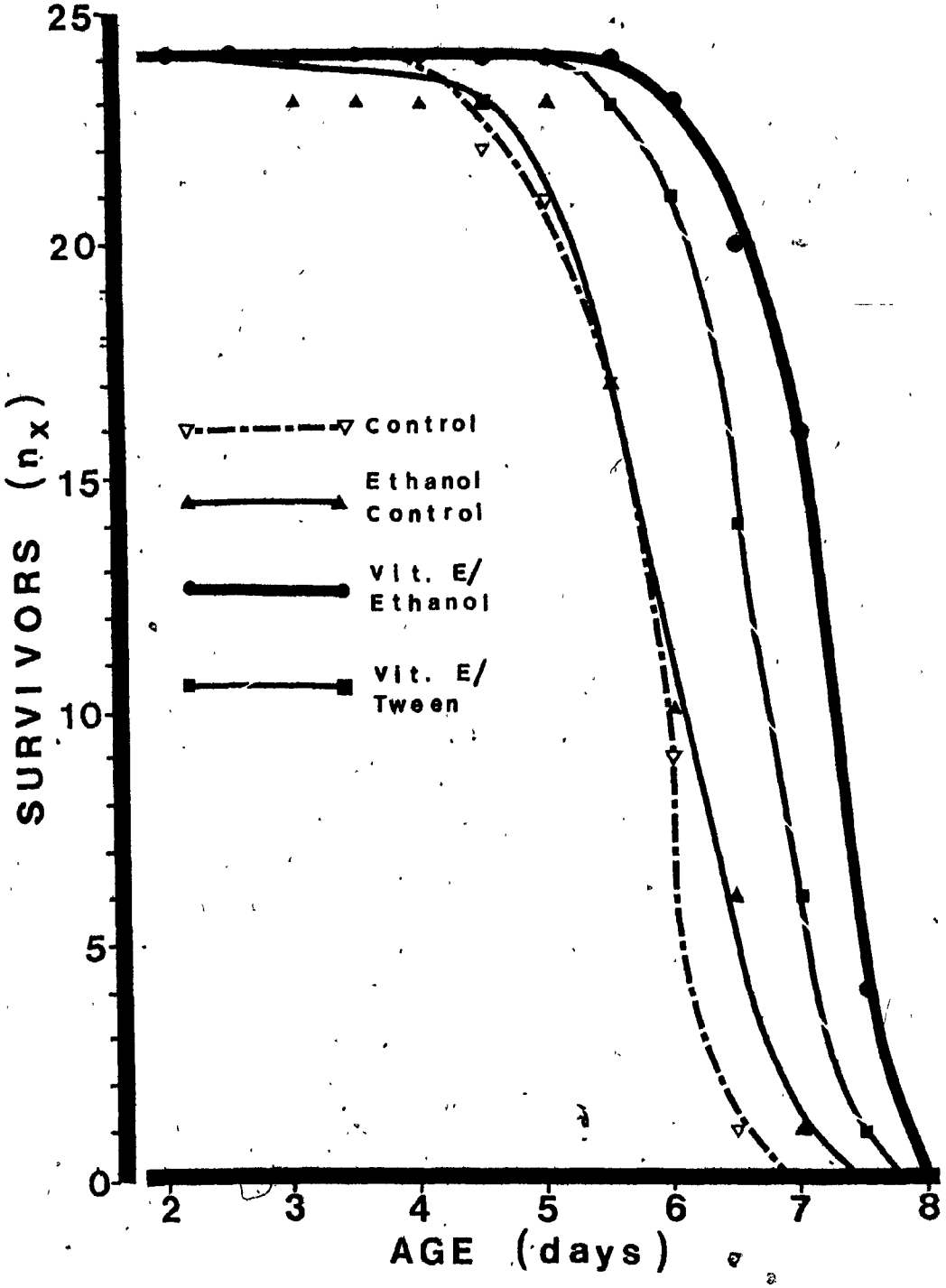


FIGURE 7. Survivorship curves of A. brightwelli exposed to vitamin E dissolved in Tween 80 or in ethanol. (n = 24)

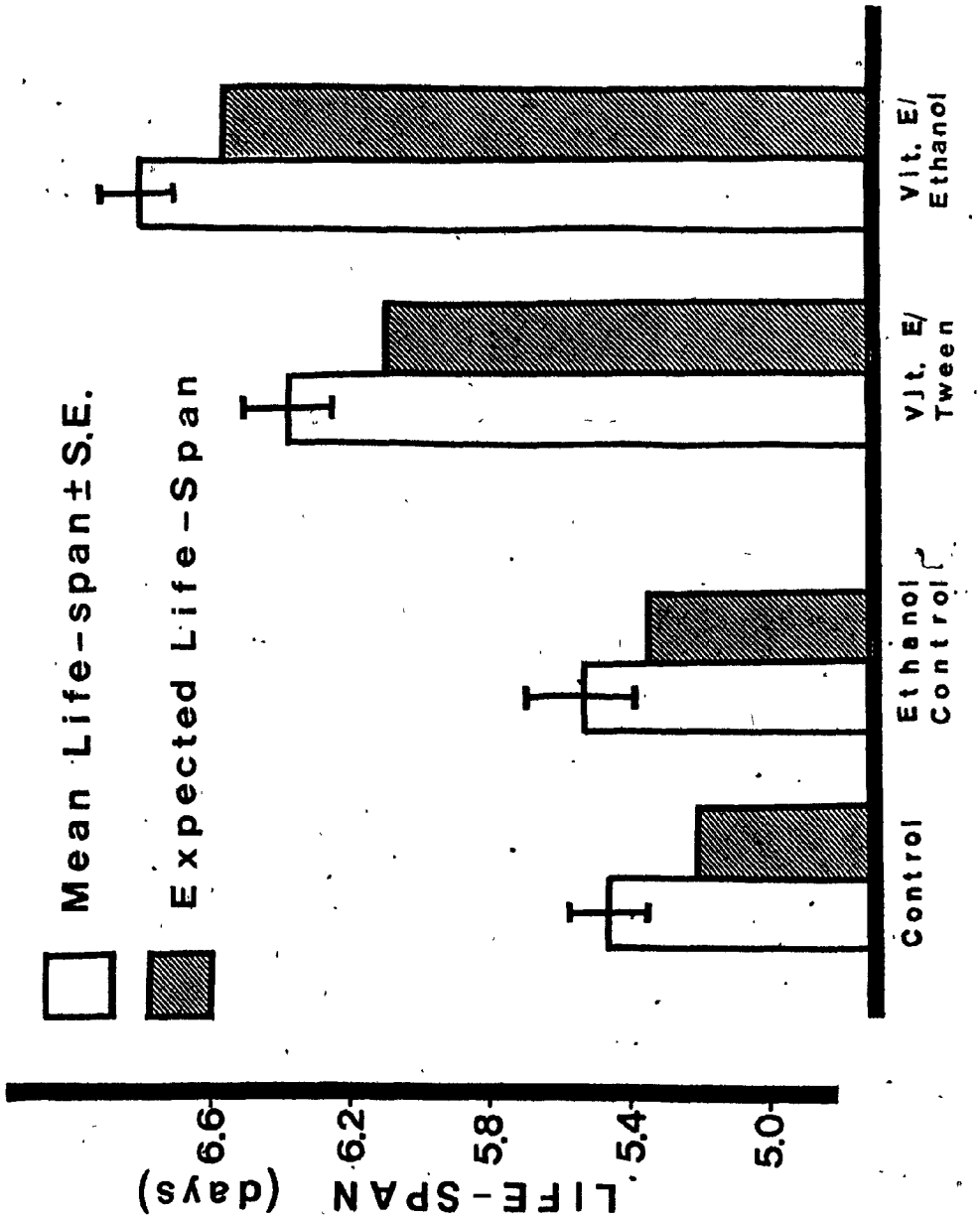

25
20
15
10
5
0
SURVIVORS (n_x)
Control
Ethanol Control
Vit. E/ Ethanol
Vit. E/ Tween
2
3
4
5
6
7
8
AGE (days)

the right, which indicates that the entire life-span is affected.

The histogram in Figure 8 compares the mean and expected life-span of the vitamin E dissolved in either Tween or ethanol. Note how the vitamin E/ethanol group has a expected life-span of almost 0.5 days longer than for the vitamin E/Tween group.

The effect of vitamin E/Tween and vitamin E/ethanol was next compared with respect to reproductive parameters. A one-way analysis of variance indicated that the length of the prereproductive period was significantly different between the groups ($(F\ 3, 92) = 23.829$, $p<0.001$). Subsequent analysis using the post hoc Tukey test revealed that the length of the prereproductive stage for the vitamin E/ethanol group (3.06 ± 0.09 days) was significantly longer than for either the regular control (2.31 ± 0.05 days) or the ethanol control (2.48 ± 0.06 days); it was significantly longer than in the vitamin E/Tween group as well (2.67 ± 0.06 days, alpha = 0.01). The reproductive period for the vitamin E/ethanol group (3.5 ± 0.12 days) was significantly larger than for the ethanol control (2.96 ± 0.14 days, alpha = 0.05) but not different from the regular control (3.00 ± 0.15 days). The vitamin E/Tween group showed a similar pattern. Its reproductive period was significantly longer than that of

FIGURE 8: Life-span histograms of A. brightwelli exposed to vitamin E dissolved in Tween 80 or in ethanol. (n = 24)

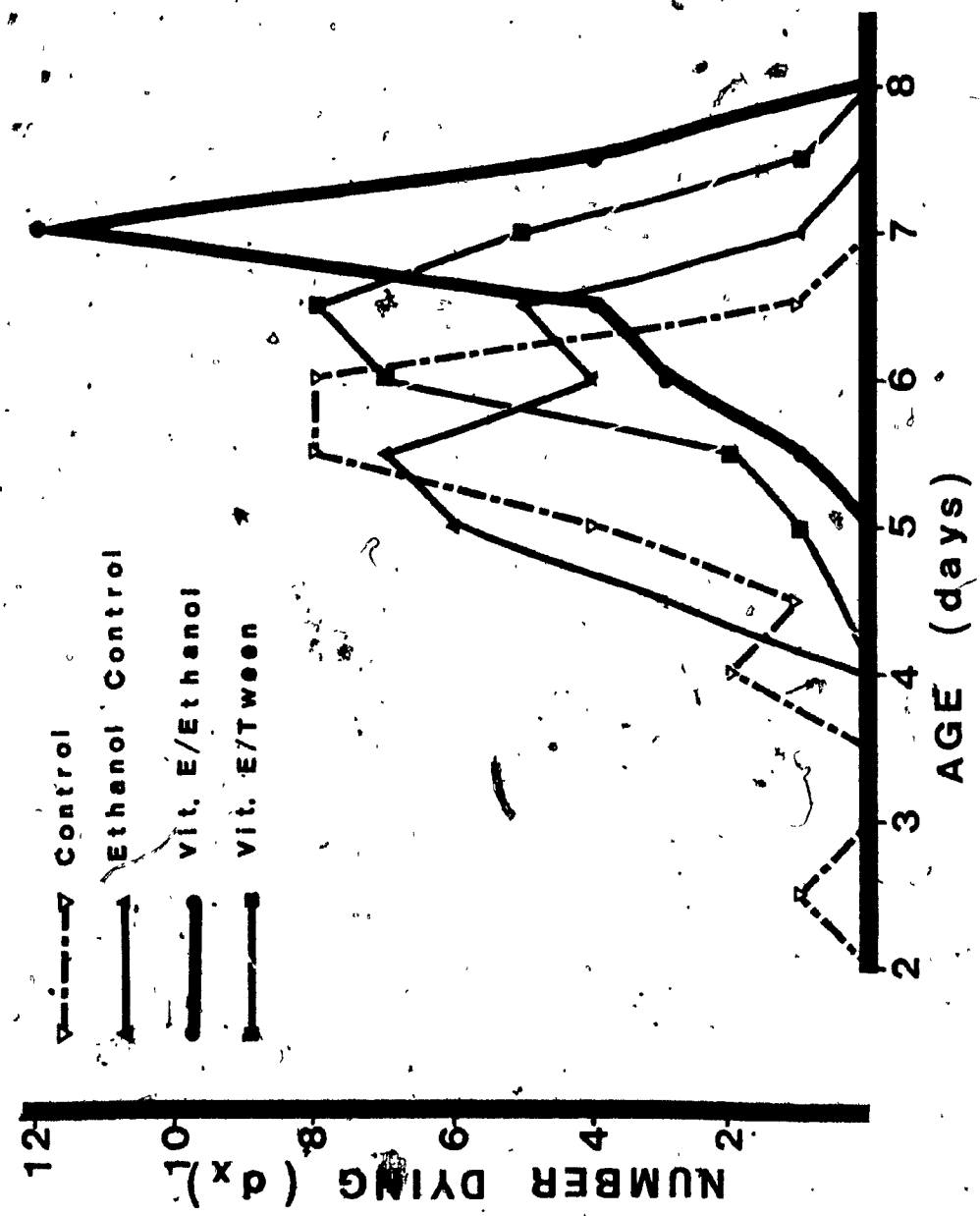

LIFE-SPAN (days)
6.6
6.2
5.8
5.4
5.0
Mean Life-span±S.E.
Expected Life-Span
Control
Ethanol Control
Vit. E/ Tween
Vit. E/ Ethanol

its Tween control but no different from that of the regular control (see table 8B).

The age specific death rate for for rotifers exposed to vitamin E solubolized in ethanol or Tween 80 can be seen in Figure 9. The two vitamin E groups have a later time of death than the two controls, which overlap each other. The overlap of the two control groups shows that that their time of death was not affected by the ethanol. It is of interest to note that though both vitamin E groups had a later peak death period than in the controls, the vitamin E/ethanol group had the longest maximum life-span along with the latest peak death period of any group, extending to age 7.0 days. The vitamin E/ethanol group was statistically similar in all aspects in its life profile to the vitamin E/Tween group. As in the previous case, an increase in the prereproductive period was responsible for the increase in mean life-span.

The results thus show that the enhanced effect of vitamin E on life-span was due purely to the effects of vitamin E itself and not due to the solvent used or interaction with the vitamin E and the solvent. The significant increase in the prereproductive time in the vitamin E/ethanol group and its longest mean life-span indicates that the ethanol is preferable as a solubilizing agent. The ethanol alone has no such effect. The action

FIGURE 9. The age specific death rates (dx) for A. brightwelli exposed to vitamin E dissolved in Tween 80 or in ethanol. (n = 24)


NUMBER DYING (d_x)
12
10
8
6
4
2
AGE (days)
2
3
4
5
6
7
8
Control
Ethanol Control
Vit. E/Ethanol
Vit. E/Tween

of vitamin E is similar regardless of the solvent used.

Vitamin E Introduction At Different Age

The experiment in this section was performed to determine the life-span effects of vitamin E when introduced at different age period of the rotifers.

The data in table 11 show that prolongation of life-span occurs when vitamin E is introduced up until age 2 1/2 days (post hoc Tukey test). The prereproductive stage for the rotifers ends at around 2 1/2 days. The data

TABLE 11

THE LONGEVITY EFFECT OF 25 UG/ML OF VITAMIN E/ETHANOL ADDED AT DIFFERENT AGE: DAYS (n = 24)

Age (days)	Control	Ethanol Control	Vitamin E/Ethanol
1/2	5.41 ± 0.13	5.38 ± 0.14	6.83 ± 0.12 *
1 1/2	5.36 ± 0.16	5.32 ± 0.17	6.87 ± 0.10 *
2 1/2	5.35 ± 0.14	5.27 ± 0.14	6.32 ± 0.13 **
3 1/2	5.62 ± 0.16	5.49 ± 0.13	5.97 ± 0.17
4 1/2	5.47 ± 0.09	5.14 ± 0.14	5.63 ± 0.09
5 1/2	5.37 ± 0.18	5.32 ± 0.16	5.58 ± 0.10
6 1/2	5.34 ± 0.16	5.39 ± 0.19	5.72 ± 0.11

* Significant at alpha = 0.01

** Significant at alpha = 0.05

therefore shows that introduction of vitamin E must occur during the prereproductive stage in order to significantly extend the life-span of the rotifers.

Size of Offspring

In examining the offspring during the vitamin E experiment, an increase in the overall offspring size was noticed at all of the vitamin E concentrations used. Since the optimal concentration with respect to life-span has been determined to be 25 ug/ml of vitamin E, this concentration was used to quantify the increase in offspring size. Table 12A shows the body length of A. brightwelli and table 12B shows body width with and without vitamin E present. These data are shown in Figure 10 by both the length and the width of control and vitamin E treated rotifers from age 0.5 days to 6.5 days. The size of the offspring at birth for the Vitamin E group appeared to be larger than for the controls but sufficient offspring numbers could not be obtained for significant measurements.

The length of the vitamin E treated rotifer was significantly longer at age 1/2, 1 1/2 and 4 1/2 days of age and they were significantly wider at age 1/2, 2 1/2 and 3 1/2 days (post hoc Tukey test analysis). At age 6 1/2, the senescent period, there were no significant difference between the controls and the experimental groups

TABLE 12A

THE EFFECT OF 25 ug/ml OF VITAMIN E/ETHANOL ON BODY LENGTH OF A. BRIGHTWELLI. (n = 20, UNLESS OTHERWISE INDICATED)

Age (days)	Control ± S.E. (um)	Ethanol Control ± S.E. (um)	Vitamin E ± S.E. (um)
1/2	483.0 ± 8.8	490.0 ± 7.0	545.5 ± 12.0*
1 1/2	617.4 ± 10.1	609.6 ± 12.0	658.0 ± 7.0**
2 1/2	676.5 ± 4.7	673.8 ± 4.0	695.5 ± 11.5
3 1/2	690.0 ± 10.1	712.8 ± 6.8	718.5 ± 10.2
4 1/2	746.5 ± 3.7	751.3 ± 4.6	770.0 ± 6.2*
5 1/2	780.6 ± 5.7	781.3 ± 5.1	786.5 ± 6.8
6 1/2 (n=7)	811.4 ± 8.1	817.5 ± 6.0	827.9 ± 5.9

* Significantly larger at alpha = 0.01

** Significantly larger at alpha = 0.05

TABLE 12B

THE EFFECT OF 25ug/ml OF VITAMIN E/ETHANOL ON BODY WIDTH OF A. BRIGHTWELLI. (n = 20, UNLESS OTHERWISE INDICATED)

Age (days)	Control ± S.E. (um)	Ethanol Control ± S.E. (um)	Vitamin E ± S.E. (um)
1/2	309.5 ± 6.7	312.5 ± 6.5	358.5 ± 8.2*
1 1/2	353.0 ± 7.9	365.0 ± 4.6	377.5 ± 6.9
2 1/2	380.8 ± 4.2	379.8 ± 3.5	393.3 ± 2.8**
3 1/2	392.0 ± 3.3	384.5 ± 4.9	412.5 ± 6.3**
4 1/2	415.0 ± 3.2	414.6 ± 4.1	406.0 ± 5.4
5 1/2	403.5 ± 6.5	400.5 ± 4.7	401.5 ± 4.3
6 1/2 (n=7)	426.4 ± 6.4	424.2 ± 5.6	439.6 ± 3.5

* Significantly larger at alpha = 0.01

** Significantly larger at alpha = 0.05

(one way ANOVA ($F_{2, 330} = 51.50$), $p>0.5$). As seen in Figure 10, there is a linear relationship between the age and the length or the width of both the control and the experimental group. Since the curves tapers off to a common length and a common width, this indicates that the rotifers have a limiting maximum size. Thus the vitamin E will affect the rate of enlargement but the maximum size

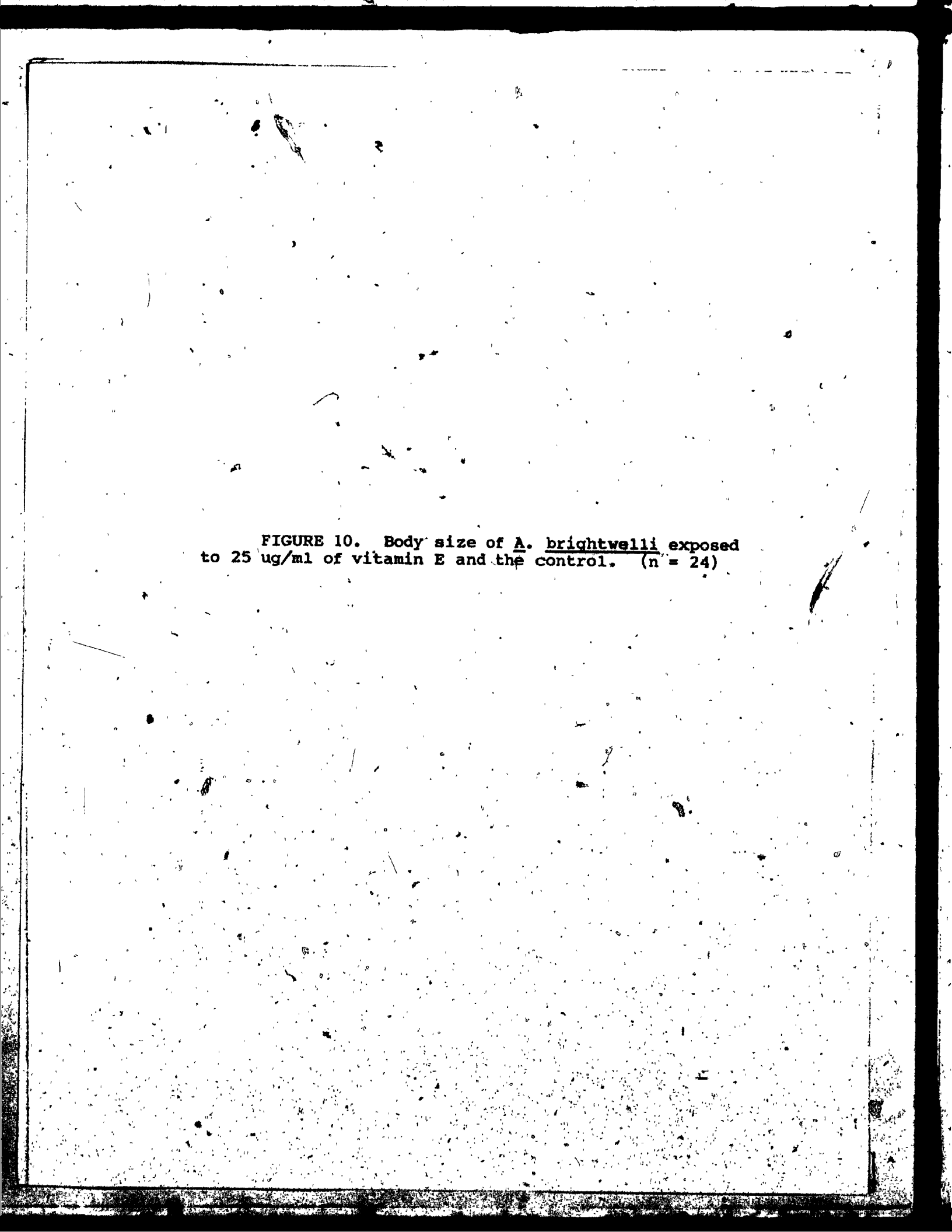
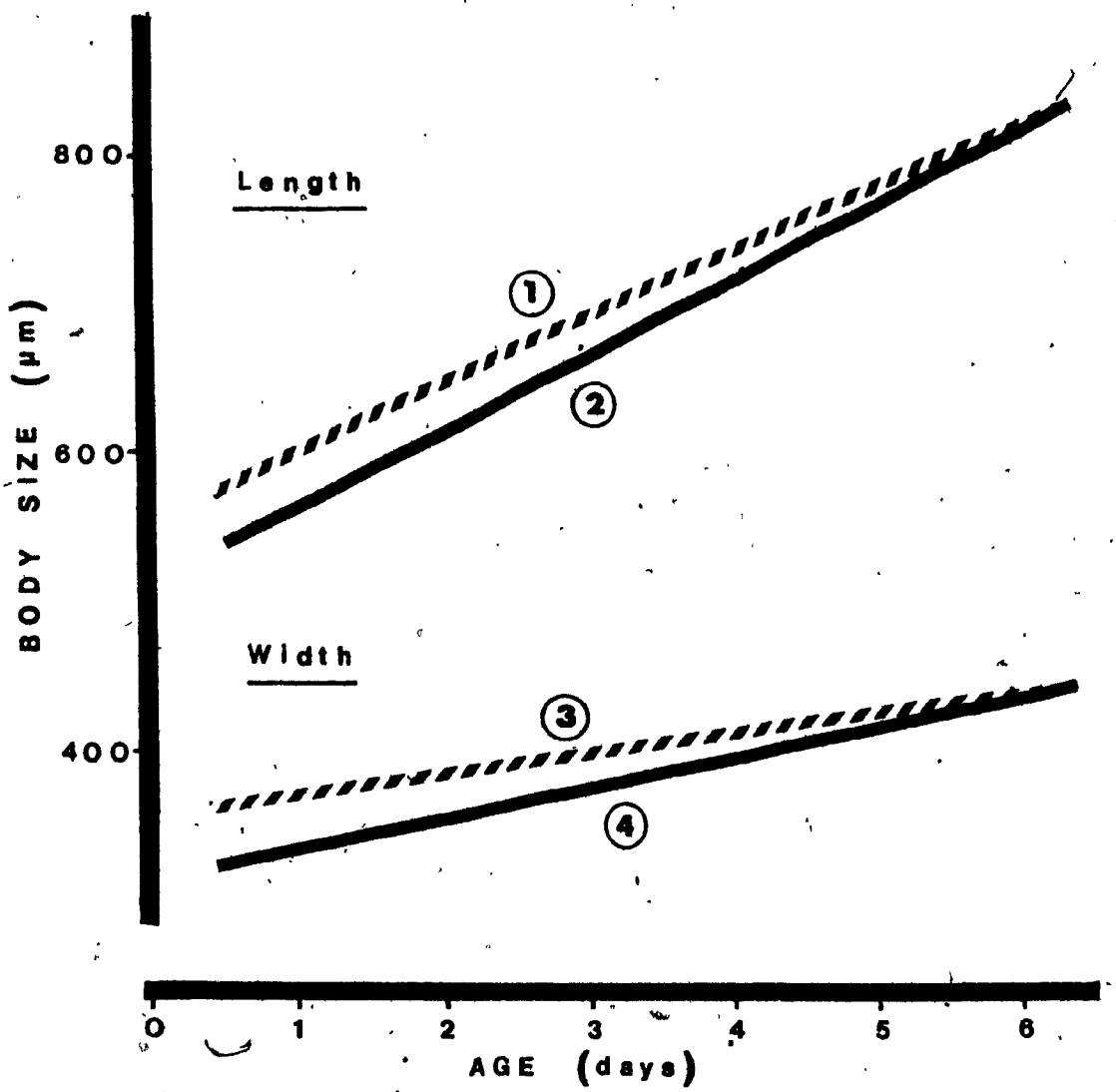


FIGURE 10. Body size of A. brightwelli exposed to 25 ug/ml of vitamin E and the control. (n = 24)



① = 42.77 age + 562.57 r = .95

② = 49.35 age + 513.78 r = .92

③ = 10.86 age + 360.39 r = .82

④ = 17.36 age + 322.13 r = .87

that may be obtained is not affected. Note that neither the regular control or the ethanol control showed any significant difference from each other. From this, it may be concluded that ethanol was not the factor which was involved in the enlargement of the body size.

Dietary or Environmental Uptake of Alpha-tocopherol

One problem which has yet to be solved was whether the vitamin E influenced the rotifers by means of dietary uptake or by environmental diffusion of vitamin E into the rotifers. The following experiment was carried out to differentiate between these possibilities. Paramecia was prepared with 40 ug/ml of vitamin E and the medium was washed away so that any vitamin E would be obtained only through dietary uptake of the Paramecia. The Paramecia were treated with 40 ug/ml of vitamin E, rather than 25 ug/ml, to compensate for any losses which might occur during the preparation of the Paramecia.

The effect of dietary vitamin E uptake via the Paramecia are presented in table 13. The mean life-span of the rotifers was the first parameter evaluated. One-way analysis of variance showed a significant difference in the mean life-span ($(F\ 2,\ 69) = 6.525\ p<0.005$). Subsequent analysis showed that the mean life-span of the group which ingested vitamin E via the Paramecia (6.15 ± 0.13 days), was significantly higher than for its ethanol control

TABLE 13

LIFE PROFILE RESULTS DUE TO THE DIETARY UPTAKE OF VITAMIN E THROUGH PARAMECIA FOR A. BRIGHTWELLI (n = 24)

	Regular Control	Ethanol Control (29 ul/ml)	Vitamin E From Paramecia
Ave. Life-span ± S.E. (days)	5.54 ± 0.11	5.56 ± 0.16	6.15 ± 0.13 *
Ave. Prereprod. Time ± S.E. (days)	2.31 ± 0.06	2.35 ± 0.05	2.85 ± 0.08 *
Ave. Reprod. Time ± S.E. (days)	3.08 ± 0.08	3.04 ± 0.13	3.10 ± 0.11
Ave. Postreprod. Time ± S.E. (days)	0.10 ± 0.06	0.10 ± 0.04	0.17 ± 0.06
Average Offspring No./rotifer	9	9	9

* alpha = 0.01 from controls

(5.56 ± 0.16 days) or the regular control (5.54 ± 0.11 days, alpha = 0.01)

Analysis of the developmental stages revealed that the differences in the length of the prereproductive period was highly significant with the ANOVA test ((F 2, 69) = 23.563, $p<0.001$). Subsequent post hoc Tukey test indicated that the length of the prereproductive stage for the dietary vitamin E group (2.85 ± 0.08 days) was significantly longer than for its ethanol control (2.35 ± 0.05 days).

All other data were not statistically significant and the results obtained were just like those in the other vitamin E experiments. The data again showed that it was the prolonged prereproductive stage of development which caused the increase in the mean life-span.

An increase in the size of the rotifers due to the dietary uptake of vitamin E was examined and is presented in table 14. The data show significant increases for both the length and width at 1/2 and 1 1/2 age group. The results indicate that the dietary component of vitamin E was sufficient to cause the increase in the body size. Note however that the environmental influence upon the rotifer body size by the environmental diffusion of vitamin E has not been determined in this experiment. Nevertheless, dietary ingestion of vitamin E was sufficient to have

resulted in an increase in the body as well as to have caused a prolonged prereproductive stage, which resulted in the greater mean life-span of the rotifers.

TABLE 14

THE EFFECT OF BODY SIZE DUE TO THE DIETARY UPTAKE OF VITAMIN E THROUGH PARAMECIA FOR A. BRIGHTWELLI (n = 20)

Age (days)	Regular Control (um)	Ethanol Control :29 ul/ml (um)	Vitamin E :25 ug/ml equivalence (um)
		Length	
1/2	492.0 ± 6.2	485.0 ± 8.2	558.5 ± 10.5 *
1 1/2	605.0 ± 8.5	615.0 ± 10.5	662.0 ± 8.7 **
		Width	
1/2	300.0 ± 8.2	305.0 ± 7.0	360.5 ± 8.0 *
1 1/2	360.0 ± 6.9	370.0 ± 5.5	380.0 ± 9.5

* alpha = 0.01

** alpha = 0.05

Ultraviolet Experiments

So far, vitamin E at 25 ug/ml has been shown to cause an increase in the life-span of A. brightwelli. This increase results from an increase in the prereproductive stage. The peak time of death has also been shown to occur at a later age with vitamin E than with the controls. The effect of U.V. irradiation on life-span was examined next to determine whether the prereproductive period was particularly critical with respect to total life-span.

Minimum U.V. Dose Determination

Rotifers 12 hours of age were exposed to U.V. dosages from 50 - 4800 J/m^2. Values for the mean life-span and maximum longevity for A. brightwelli at various U.V. doses, along with their respective controls, are presented in table 15A. In no case was there any significant difference between the regular control and the U.V. control. This showed that the treatment procedure required to irradiate the rotifers was not detrimental to the rotifers. A one-way analysis of variance followed by a post hoc Tukey test did show any significant difference between the U.V. control group at 200 J/m^2 (5.96 $\pm$ 0.16 days) and the U.V. control group at 600 J/m^2 (5.45 $\pm$ 0.19 days: $(F\ 7, 196) = 2.866$, $0.005>p>0.01$, at alpha = 0.05). However, the lack of any significant difference with the control group (5.69 $\pm$ 0.17 days) shows that the procedure required prior to U.V. irradiation was

not a serious factor to consider, as to alter the mean life-span, with respect to the regular control group.

TABLE 15A

MEAN LIFE-SPAN AND MAXIMUM LONGEVITY FOR A. BRIGHTWELLI AT VARIOUS U.V. DOSES AND THEIR RESPECTIVE CONTROLS. (n = 24, UNLESS OTHERWISE INDICATED)

U.V. Dose (J/m^2)	Mean Life-span ± S.E. (days)	MAXIMUM LONGEVITY (days)
Regular Control	5.69 ± 0.17	7.63
50	5.89 ± 0.14	6.75
50 Control	5.90 ± 0.11	7.88
150	5.31 ± 0.21	6.25
150 Control	5.58 ± 0.16	7.63
200	5.31 ± 0.14	6.38
200 Control	5.96 ± 0.16	7.38
300	4.73 ± 0.24	6.50
300 Control	5.38 ± 0.13	6.75
600	3.98 ± 0.09	5.63
600 Control	5.23 ± 0.13	6.38
1200 *	3.30 ± 0.12	4.63
1200 Control *	5.37 ± 0.11	7.25
4800 *	1.82 ± 0.07	3.13
4800 Control *	5.45 ± 0.19	7.38

* n = 30

Statistical analysis across the dosage groups in the U.V. dose determination experiment using the post hoc Tukey test (table 15B) shows that 200 J/m^2 was the initial dose which caused a significant decrease in life-span to 5.31 $\pm$ 0.14 days in comparison with its U.V. control which had a mean life-span of 5.98 $\pm$ 0.16 days. However, it was not different from the regular control with a mean life-span of 5.69 $\pm$ 0.17 days (alpha = 0.05). U.V. doses from 300 J/m^2 up all showed a significant decrease in life-span as seen in the significance values across U.V. dosage groups found in table 16A.

Figure 11 shows the survivorship curve for all the experimental U.V. group. As the dosage of U.V. increases, the survivorship curve goes to the left which indicates that the percentage of survivors decreases with higher U.V. doses. This decrease in the mean life-span can be seen on the longevity plot of Figure 12, which shows a logarithmic relationship between the mean life-span for A. brightwelli and the U.V. dose, where the mean life-span (days) = 9.712 - 2.080 log Dose (J/m^2); r^2 = 0.957.

Figure 13 shows the age specific death rate of the rotifers treated with the different U.V. irradiation doses, (the dx curve). The regular control showed a peak time of death between 5 - 6 1/2 days and all of the U.V. control groups had a death peak within the range of this regular

TABLE 15B

RESULTS OF THE POST HOC TUKEY TEST USED TO EVALUATE SIGNIFICANCE ACROSS DOSAGE GROUP IN U.V. DOSE DETERMINATION EXPERIMENT

Dose Group	F Value		Regular Control	Respective U.V. Control
50	(F 2, 81) = 0.720,	p>0.25	NSD	NSD
150	(F 2, 81) = 1.171,	p>0.25	NSD	NSD
200	(F 2, 81) = 4.220,	0.025>p>0.01	NSD	*
300	(F 2, 81) = 7.124,	0.005>p>0.001	*	**
600	(F 2, 81) = 42.963,	p<0.001	*	*
1200	(F 2, 81) = 96.280,	p<0.001	*	*
4800	(F 2, 81) = 217.580,	p<0.001	*	*

NSD - no significant difference

* alpha = 0.01

** alpha = 0.05

FIGURE 11. Survivorship curves of A. brightwelli irradiated with varied U.V. radiation on day 1/2. (n = 24, unless otherwise specified)

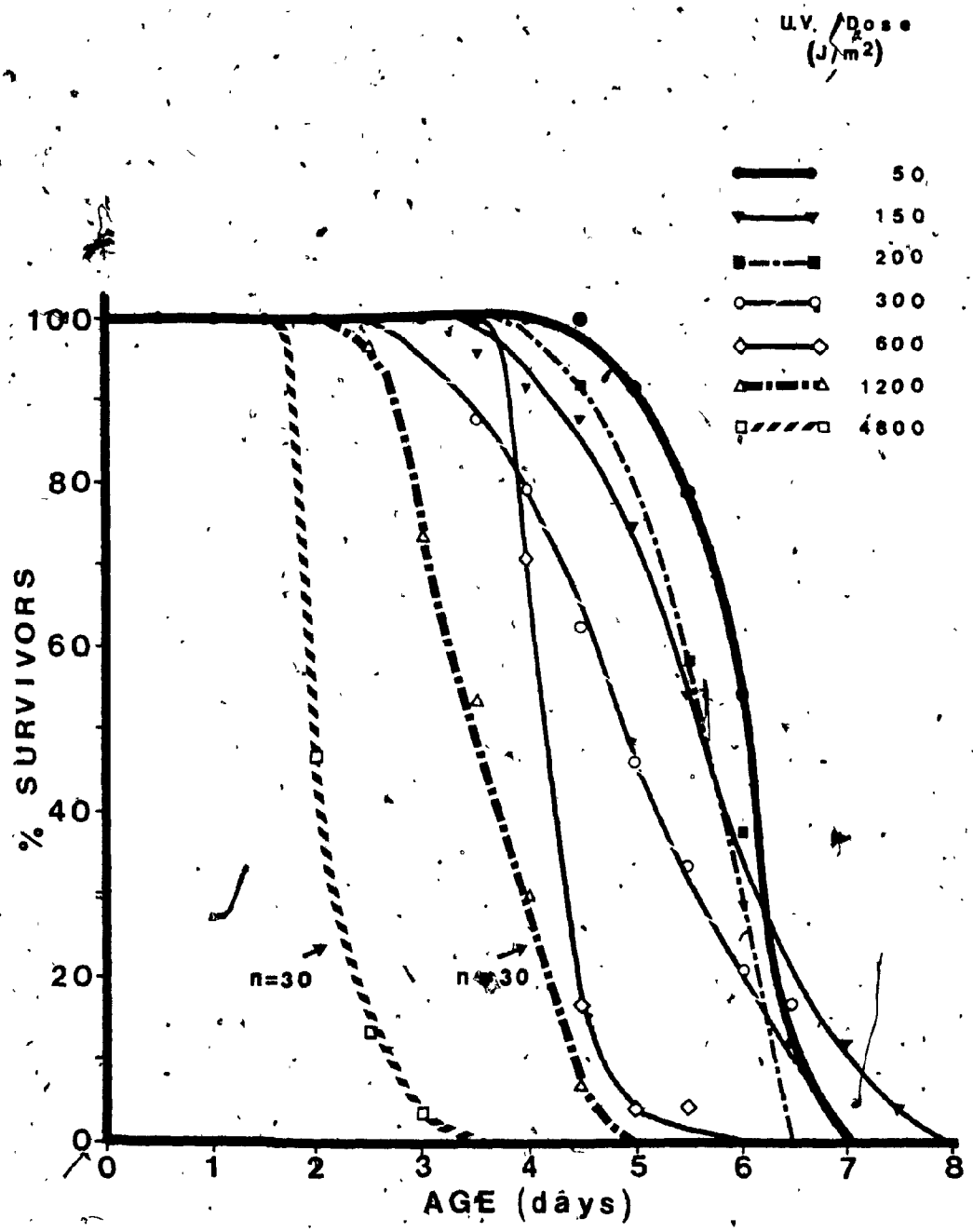
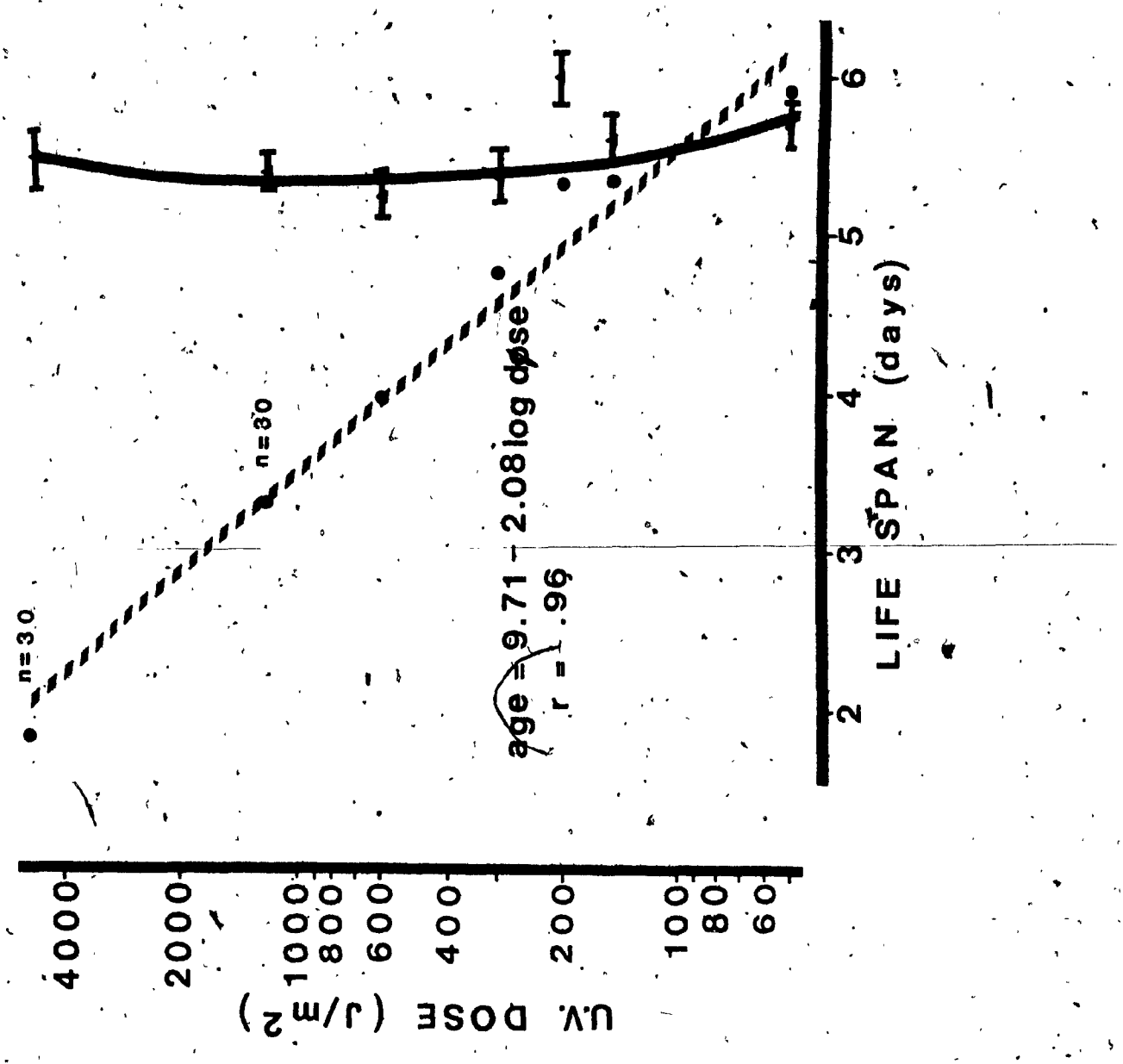

U.V. Dose
(J/m2)
50
150
200
300
600
1200
4800
100
80
60
40
20
0
% SURVIVORS
n=30
n=30
0
1
2
3
4
5
6
7
8
AGE (days)

FIGURE 12. Logarithmic longevity plot of *A. brightwelli* irradiated with varied U.V. radiation at age 1/2 days. (n = 24, unless otherwise specified)


LIFE SPAN (days)
2
3
4
5
6
U.V. DOSE (J/m²)
4000
2000
1000
800
600
400
200
100
80
60
n=30
n=80
age = 9.71 − 2.08 log dose
r = −.96

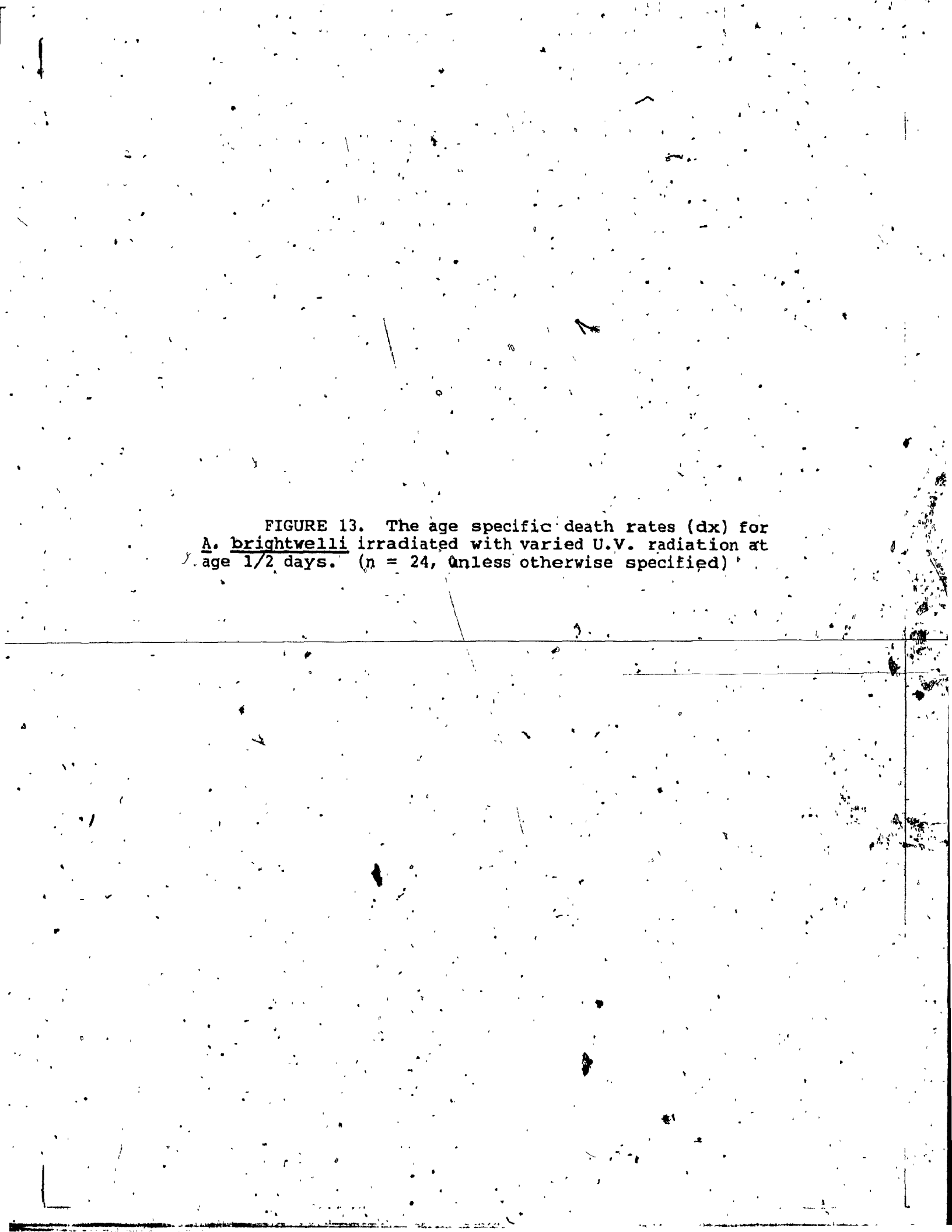
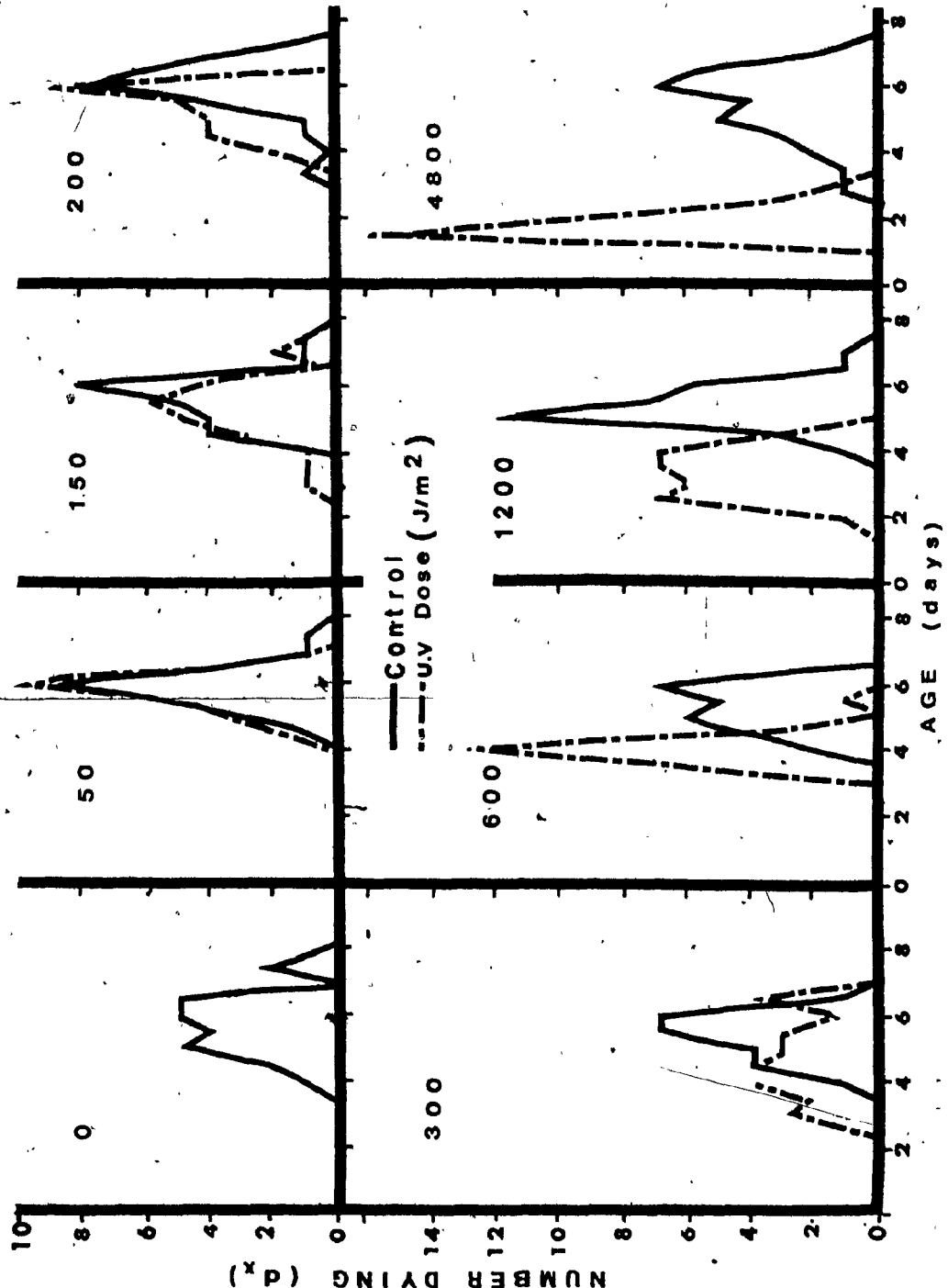


FIGURE 13. The age specific death rates (dx) for A. brightwelli irradiated with varied U.V. radiation at age 1/2 days. (n = 24, unless otherwise specified)


NUMBER DYING (d_x)
AGE (days)
Control
UV Dose (J/m²)
0
50
150
200
300
600
1200
4800

control. The overlap in the dx curves for the U.V. and its control, at 50 and 150 J/m^2 dose groups, show that rotifers were dying at the same time as the control at either of the two U.V. doses. Significant decrease in life-span was first seen at 200 J/m^2 as compared to its control group (from table 15B). The rotifers exposed at 200 J/m^2 showed a shift in the dx curve to the left which indicates that the majority of the rotifers began dying at an earlier time, but the peak death period at 6 days for the 200 J/m^2 or its U.V. control was not changed.

At 300 J/m^2, the U.V. group started dying at an earlier time with a wider range of death period. The peak death period of 4 - 4 1/2 and 6 1/2 days contrasted to 5 1/2 - 6 days for its U.V. control, shows that the death period for the U.V. exposed group was spread out over a greater time period. From the 600 J/m^2 dosages on, the irradiation caused a distinct shift in the dx curve indicating that the majority of the rotifers died at an earlier time period than did their respective controls.

A stimulatory effect, so as to increase the mean life-span, was not present at any of the U.V. doses that were used. Since the 300 J/m^2 was the first dose to cause a decrease in the mean life-span as compared both to the regular control and to its own respective control, this dose was considered to be the minimum dose required to

statistically reduce the life-span.

The reproductive profile of A. brightwelli at 300 J/m^2 is shown in table 16. The length of the reproductive period at this dose (2.08 ± 0.23 days) was significantly lower than the reproductive period for the regular control (3.21 ± 0.16 days) or for its own control group (2.88 ± 0.21 days). This may be due to the fact that the reproductive structures are one of the two organs in the rotifers which undergo cell division. Damage to the reproductive structure produced by the U.V. irradiation would be expected to cause a significant decrease in the reproductive period. Since only the life-span is being taken into consideration in the following experiment, the 300 J/m^2 dose was considered as the initial dosage required to significantly reduce the mean life-span of the rotifers.

U.V. Exposure at Different Age Groups

Rotifers of different ages were irradiated at 300 J/m^2. The results of this irradiation on mean life-span are presented in table 17. Note that exposure data at 2 1/2 days were not collected since this was the developmental period where reproduction normally starts. Thus the data would not have been applicable to either the prereproductive or to the reproductive period. The lack of sufficient rotifers at age 6 1/2 days prevented the senescent group from being used.

TABLE 16

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI AT 300 J/m^2 U.V. DOSE

	Ave. Prereprod. Time ± S.E. (days)	Ave. Reprod. Time ± S.E. (days)	Ave. Postrep. Time ± S.E. (days)	Ave. Offspring No Per rotifer
Regular Control	2.31 ± 0.06	3.21 ± 0.16	0.17 ± 0.07	8
U.V. Control	2.38 ± 0.11	2.88 ± 0.21	0.08 ± 0.05	7
Exposed Group	2.30 ± 0.11	2.08 ± 0.23**	0.24 ± 0.09	4*

* Significantly lower from either control, alpha = 0.01

** Significantly lower from either control, alpha = 0.05

One-way analysis of variance indicated that there was significant difference in the life-span of rotifers exposed to 300 J/m^2 at different stage of their life cycle ((F 5, 138) = 9.141, $p<0.001$). The post hoc Tukey test (see table 17) shows that the mean life-span of rotifers exposed at 1/2 days was 4.81 ± 0.19 days and the mean life-span of rotifers exposed at 1 1/2 days was 4.49 ± 0.22 days. The 1 1/2 day exposure resulted in a significantly lower life-span than for any other exposure time. Since both 1/2 day and 1 1/2 days falls within the

TABLE 17

MEAN LIFE-SPAN AND SIGNIFICANCE OF A. BRIGHTWELLI EXPOSED TO 300 J/m^2 U.V. RADIATION AT DIFFERENT AGE (n = 24)

Age at Exposure (days)	Mean Life-span (days)	Significance With The Post Hoc Tukey Test (alpha)
1/2	4.81 ± 0.19	0.01
1 1/2	4.94 ± 0.22	0.01
3 1/2	5.73 ± 0.13	NSD
4 1/2	5.65 ± 0.15	NSD
5 1/2	5.98 ± 0.15	NSD
Control	5.90 ± 0.12	-

NSD - no significant difference

prereproductive time period, the detrimental life decreasing effects of the U.V. were active only during the prereproductive stage and not at any other period of the rotifers life cycle. U.V. exposure at any other period did not lower the mean life-span.

U.V. Irradiation and Alpha-tocopherol on Life-span

In the previous section, 25 ug/ml of vitamin E was shown to increase the mean life-span while 300 J/m^2 of U.V. irradiation was the minimal dose to lower the life-span. An experiment involving both vitamin E and U.V. irradiation was performed to determine whether vitamin E can be used as an agent to protect against the effects of U.V. irradiation.

Table 18 shows the effect of U.V. irradiation upon rotifers which were treated with vitamin E. One-way analysis of variance showed a significant difference in the mean life-span ($(F\ 3,\ 92) = 24.026$, $p<0.001$). The post hoc Tukey test shows that the mean life-span of the vitamin E/U.V. irradiated group (6.04 ± 0.09 days) was significantly greater than the mean life-span of the regular control (5.54 ± 0.14 days) or the mean life-span of the U.V. control (5.02 ± 0.14 days, alpha = 0.05). The mean life-span for the vitamin E control group (6.35 ± 0.11 days) was not significantly higher than the vitamin E/U.V. group according to the post hoc Tukey test. However,

the use of a one-tailed directional T-test shows that the mean life-span of the vitmin E/U.V. group can be considered lower than the vitamin E control group (alpha = 0.05, T = 1.671) thereby showing that vitamin E cannot totally compensate for the detrimental effects of the U.V. irradiation. In any case, vitamin E may act as an ultraviolet protective agent and may counter the effects of the U.V. irradiation which normally decrease the mean life-span.

TABLE 18

U.V. IRRADIATION EFFECT ON LIFE-SPAN IN A. BRIGHTWELLI REARED WITH VITAMIN E AND THE SIGNIFICANCE LEVEL

	Mean Life-span ± S.E. (days)	Significance Level With the Post Hoc Tukey Test (alpha)
Regular Control	5.54 ± 0.14	-
Vitamin E Control	6.35 ± 0.11	-
U.V. Control	5.02 ± 0.14	-
Vitamin E/U.V.	6.04 ± 0.09	0.05 from Regular and U.V. Control

Light and Dark Experiments

Rotifers were reared for at least 20 generations under various light conditons. Although the visible light energy is not as strong as that from U.V. radiation, visible light is still a form of stimulus which might affect life-span or the reproductive profile of A. brightwelli.

Longevity

Table 19 shows the mean life-span and maximum longevity of the rotifers after being reared in continuous light, darkness, or in a 12 hour light/dark cycle. A one-way analysis of variance showed that there were significant differences between the mean life-spans of the

TABLE 19

MEAN LIFE-SPAN AND MAXIMUM LONGEVITY OF A. BRIGHTWELLI AFTER BEING REARED UNDER DIFFERENT LIGHT/DARK CONDITIONS

Experiment	Mean Life-span ± S.E. (days)	Max. Longevity (days)
Control	5.56 ± 0.18	7.13
Cont. Dark	6.56 ± 0.15*	7.75
Cont. Light	5.25 ± 0.16	7.00

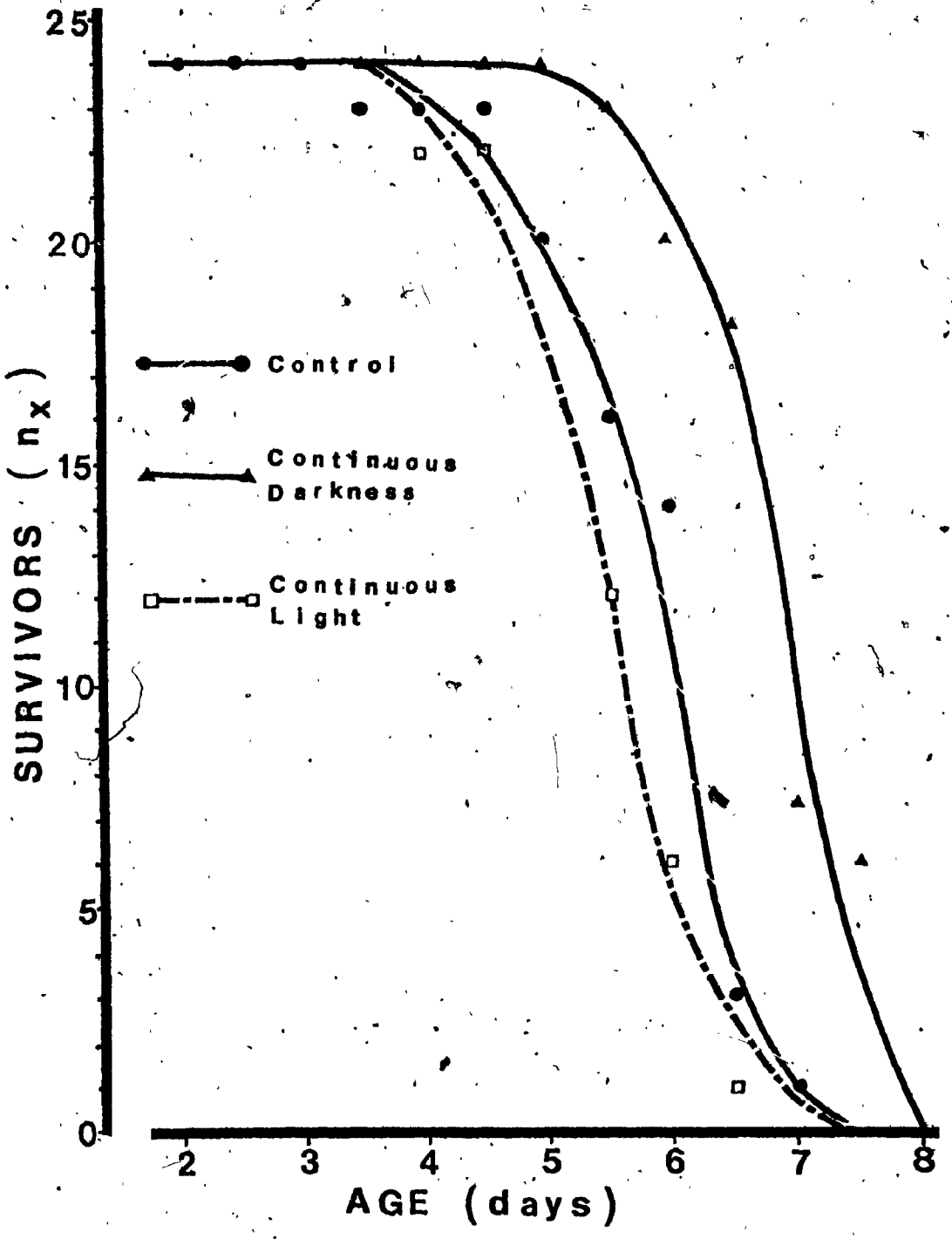
* alpha = 0.01

different groups ($(F\ 2, 69) = 17.971$, $p<0.001$). Subsequent analysis using the post hoc Tukey test revealed that the continuous dark group, with its mean life-span of 6.56 $\pm$ 0.15 days, had a significantly longer mean life-span than the control with its mean life-span of 5.56 $\pm$ 0.17 days or with the continuous light group with a mean life-span of 5.25 $\pm$ 0.16 days.

Figure 14 shows the survivorship curve of A. brightwelli after being reared under continuous light or continuous darkness. Note that the continuous darkness group has an average of one day longer mean life-span as compared to the control group. This longer time period was consistent throughout its life-span. The continuous darkness group was found not to be significant with any other group with the post hoc Tukey test. A histogram depicting the mean life-span of the rotifers reared under the varied light conditons can be seen in Figure 15. The data shows that rotifers grown for several generations of continuous darkness can expect to live for a longer time period than rotifers reared under the control culture conditions or those reared in continuous light.

In Figure 16, the age specific death rate is shown for the rotifers reared under the different light conditions. Note how the continuous darkness group has a peak death period of 6 1/2 days. This is a half day later

Figure 14. Survivorship curves of A. brightwelli after being reared under continuous light or darkness.


25
20
15
10
5
0
SURVIVORS (n_x)
Control
Continuous Darkness
Continuous Light
2
3
4
5
6
7
8
AGE (days)

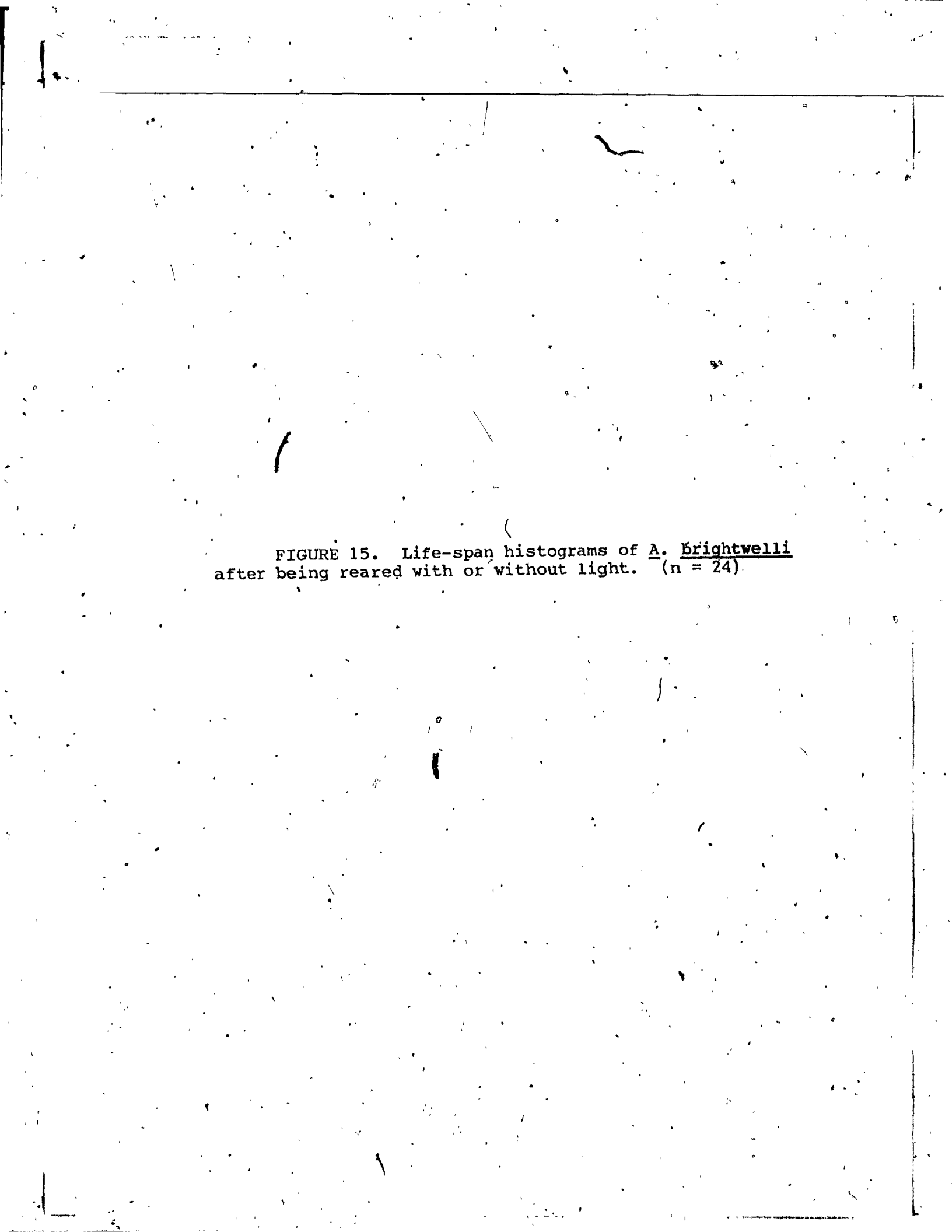
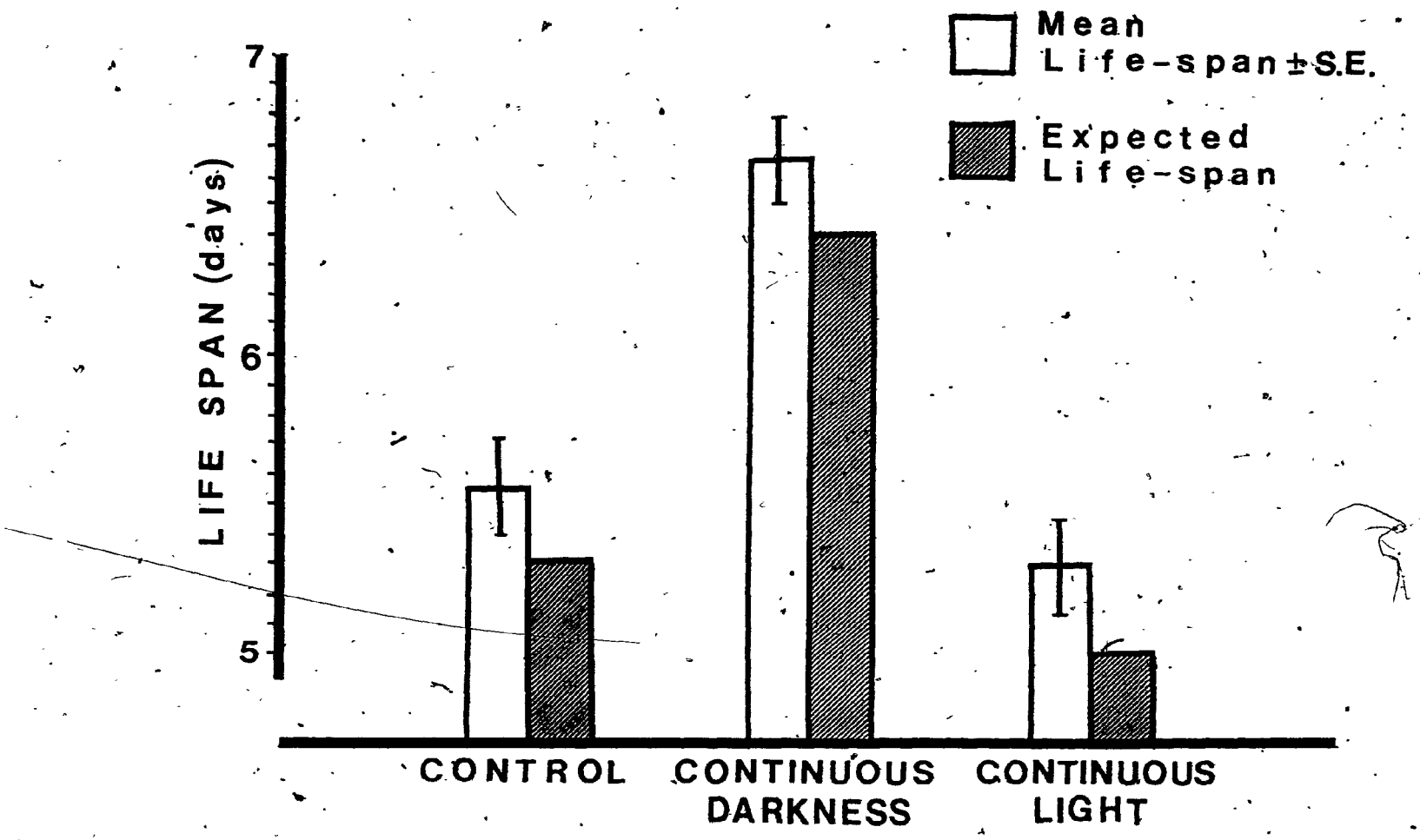


FIGURE 15. Life-span histograms of A. brightwelli after being reared with or without light. (n = 24)


Mean
Life-span ± S.E.
Expected
Life-span
LIFE SPAN (days)
7
6
5
CONTROL
CONTINUOUS
DARKNESS
CONTINUOUS
LIGHT

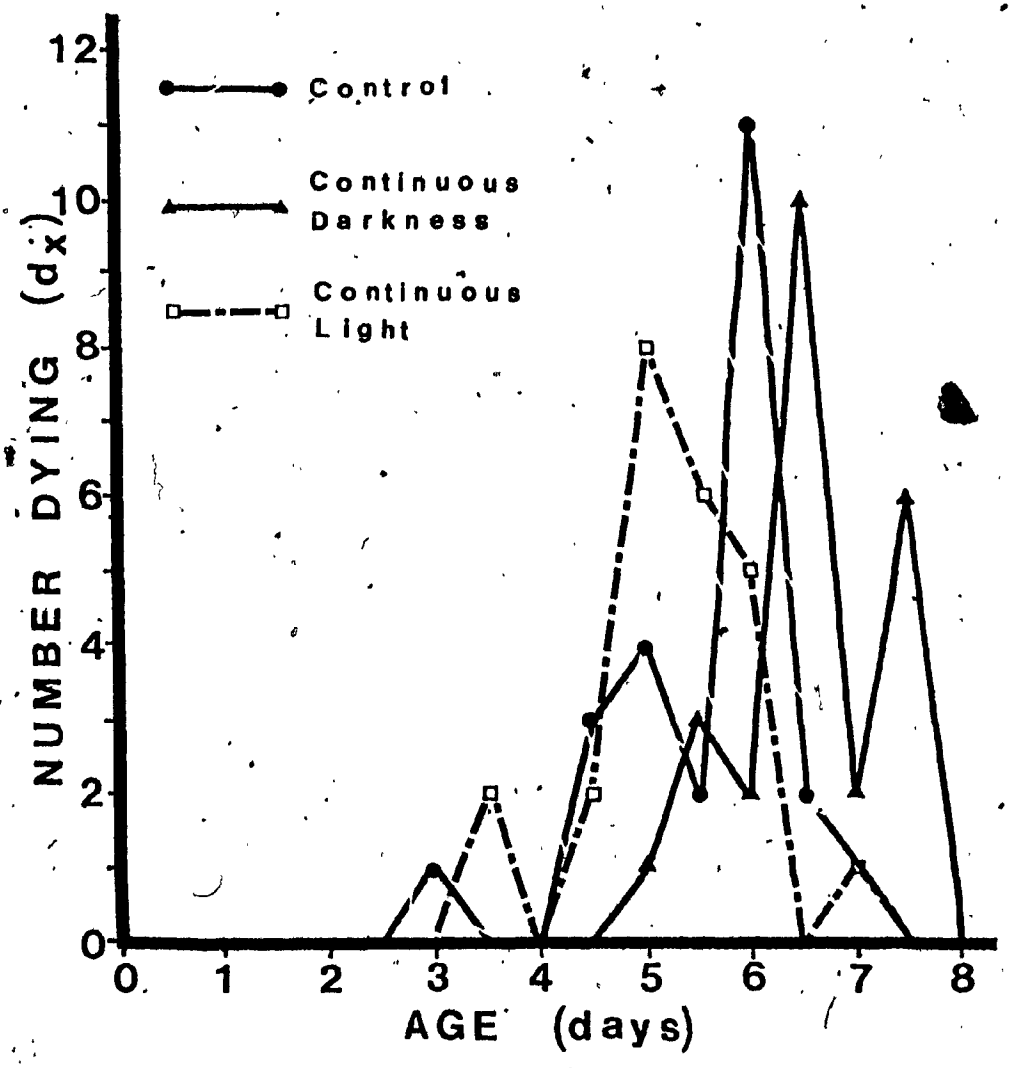


FIGURE 16. The age specific death rates (dx) for A. brightwelli after being reared under continuous light or darkness.

than the control group which had the greatest death number at 6 days of age. The continuous light group has a peak death at 5 days of age. This shows that relative to the control, the continuous darkness delayed death while continuous light caused death at an earlier period.

Fecundity

Table 20 shows the reproductive profile of A. brightwelli under the varied light/dark conditions. A way analysis of variance showed a significant difference for the length of the prereproductive period ($(F\ 2, 69) = 51.564$, $p<0.001$) but not for the reproductive period ($(F\ 2, 69) = 0.601$, $p>0.50$), postreproductive period ($(F\ 2, 68) = 0.678$, $p>0.50$) or for the average offspring number per rotifer ($(F\ 2, 69) = 2.209$, $p>0.10$). The post hoc Tukey test subsequently showed that the mean prereproductive period of 3.31 ± 0.08 days for the continuous dark group was significantly greater than the mean prereproductive time period of 2.15 ± 0.11 days for the control or the mean postreproductive time of 2.38 ± 0.05 days for the continuous light group (alpha = 0.01).

Ingestion of Paramecia

Ingestion of Paramecia was checked for rotifers reared under the different light conditions (table 21). In all cases the rotifers equally ingested the Paramecia.

TABLE 20

REPRODUCTIVE PROFILE OF A. BRIGHTWELLI AFTER BEING REARED UNDER DIFFERENT LIGHT/DARK CONDITIONS

Light Condition	Average Prereprod. Time ± S.E. (days)	Average Reprod. Time ± S.E. (days)	Average Postreprod. Time ± S.E. (days)	Ave. Offspring No. per Rotifer
Control	2.15 ± 0.11	2.94 ± 0.23	0.21 ± 0.08	6
Continuous Darkness	3.31 ± 0.08 *	2.88 ± 0.19	0.35 ± 0.13	6
Continuous Light	2.38 ± 0.05	2.65 ± 0.16	0.23 ± 0.06	7

* alpha = 0.01

TABLE 21

INGESTION OF PARAMECIA BY THREE DIFFERENT AGE OF A. BRIGHTWELLI THAT HAVE BEEN REARED UNDER VARIED LIGHT/DARK CONDITIONS (n = 48)

Age (days)	% Rotifers With Dark Spot In Gut: 12 hr Light/ Dark Control	Continuous Darkness	Continuous Light
0.5	91.7	91.7	93.8
2.5	95.8	95.8	97.9
4.5	97.9	100.0	91.7

Transfer of Continuous Dark Group to Light/Dark Cycle

Rotifers reared in darkness were transferred to a 12 hour light/dark cycle condition (as in control) for one generation (table 22). Analysis of the group data with the post hoc Tukey test indicates that the mean life-span of the continuous dark control was significantly longer (5.46 $\pm$ 0.11 days) than the 12 hr light/dark control (4.81 $\pm$ 0.19 days, alpha = 0.05). However, the transferred group had a significantly shorter life-span (4.25 $\pm$ 0.22 days, alpha = 0.01) than the continuous dark control. In addition, the survivorship curve (Figure 17) showed that the transferred group was the least viable of the three.

Analysis of the developmental stages (table 23) indicates that the prereproductive stage in the transferred group (2.44 $\pm$ 0.17 days) was no different than the 12 hour

TABLE 22

THE EFFECT OF TRANSFERRING CONTINUOUS DARK GROUP BACK TO 12 HR LIGHT/DARK CYCLE (n = 24)

Experiment	Mean Life-span ± S.E. (days)	Life Expectancy (days)
12 hr Light/Dark Control	4.81 ± 0.19	4.56
Continuous Dark Control	5.46 ± 0.11	5.21
Transferred (Dark to 12 hr Light/dark)	4.25 ± 0.22 *	4.00

* alpha = 0.01 from Continuous Dark Control

light/dark control (2.21 ± 0.19 days). The significant prolongation obtained in the prereproductive stage of the continuous dark control (2.96 ± 0.08 days, alpha = 0.05) therefore shows that the increased prereproductive stage was not a genetically fixed factor.

No difference in the postreproductive period was found ((F 2, 69) = 0.825, p>0.25). However, the transferred group showed a significant decrease in the reproductive period (1.46 ± 0.16 days) as compared with the 12 hour light/dark control (2.10 ± 0.24 days, alpha =

TABLE 23

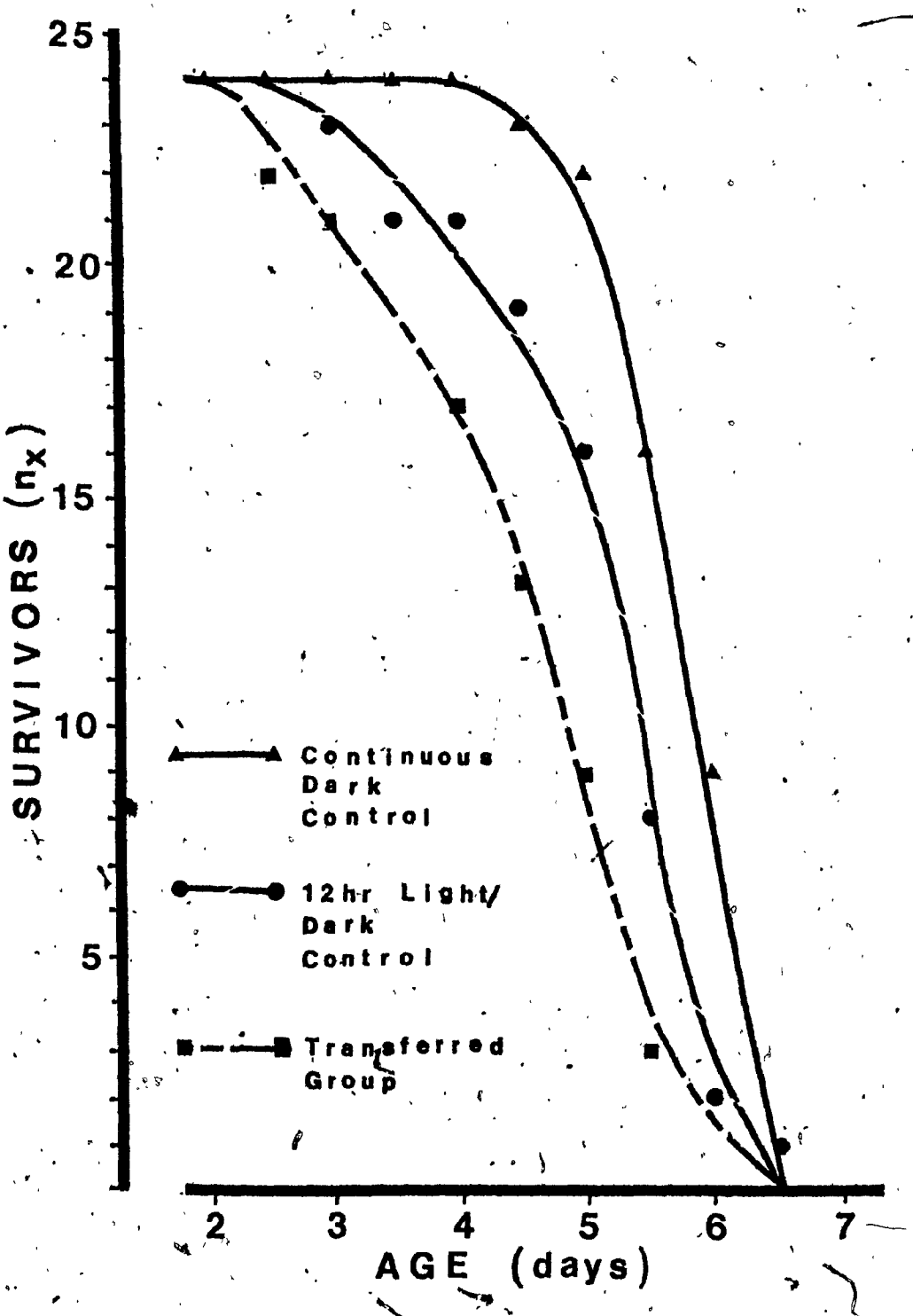
REPRODUCTIVE PROFILE OF A. BRIGHTWELLI REARED IN CONTINUOUS DARKNESS AND TRANSFERRED TO A 12 HOUR LIGHT/DARK CYCLE (n = 24)

	12 hr Light/ Dark Control	Continuous Dark Control	Transferred Group
Ave. Prereprod. Time ± S.E. (days)	2.21 ± 0.19	2.96 ± 0.08	2.44 ± 0.17
Ave. Reprod. Time ± S.E. (days)	2.10 ± 0.24	2.27 ± 0.10	1.46 ± 0.16 *
Ave. Postreprod. Time ± S.E. (days)	0.13 ± 0.05	0.19 ± 0.07	0.23 ± 0.06
Ave. No of Offspring/ Rotifer	6	7	4

* alpha = 0.01 from Continuous Dark Control

= 0.05 from 12 hr Light/Dark Control

Figure 17. Survivorship curves of A. brightwelli after being reared under continuous darkness and transferred to a 12 hr light/dark cycle with its controls.


25
20
15
10
5
SURVIVORS (n_x)
Continuous Dark Control
12hr Light/ Dark Control
Transferred Group
2
3
4
5
6
7
AGE (days)

0.05) or with the continuous dark control (2.27 ± 0.10 days, alpha = 0.01). The decrease in the reproductive period for the transferred group also resulted in the expected significant decrease in the average number of offspring per rotifer (see table 23).

The age specific death rate (dx, see Figure 18) shows that the rotifers were dying at an earlier time period than the controls. This would indicate that the rotifers which has been cultured in continuous darkness became sensitive to light at the reproductive stage so as to result in the lower mean offspring number per rotifer as well as in the earlier occurance of death.

Vitamin E On Continuous Dark Reared Rotifers

The data in table 24 shows that rotifers reared in continuous darkness and exposed to vitamin E (transferred group) showed a significant increase in life-span (6.48 ± 0.12 days) from the regular control (5.47 ± 0.12 days), ethanol control (5.52 ± 0.08 days), or to the 12 hr light/dark control (5.55 ± 0.11 days). However, the transferred group did not have a significant difference in the mean life-span (6.48 ± 0.12 days) with respect to the vitamin E control (6.42 ± 0.10 days) or to the continuous dark control (6.50 ± 0.12 days). No correlation between the two independent life prolongation events; vitamin E or continuous darkness, was seen.

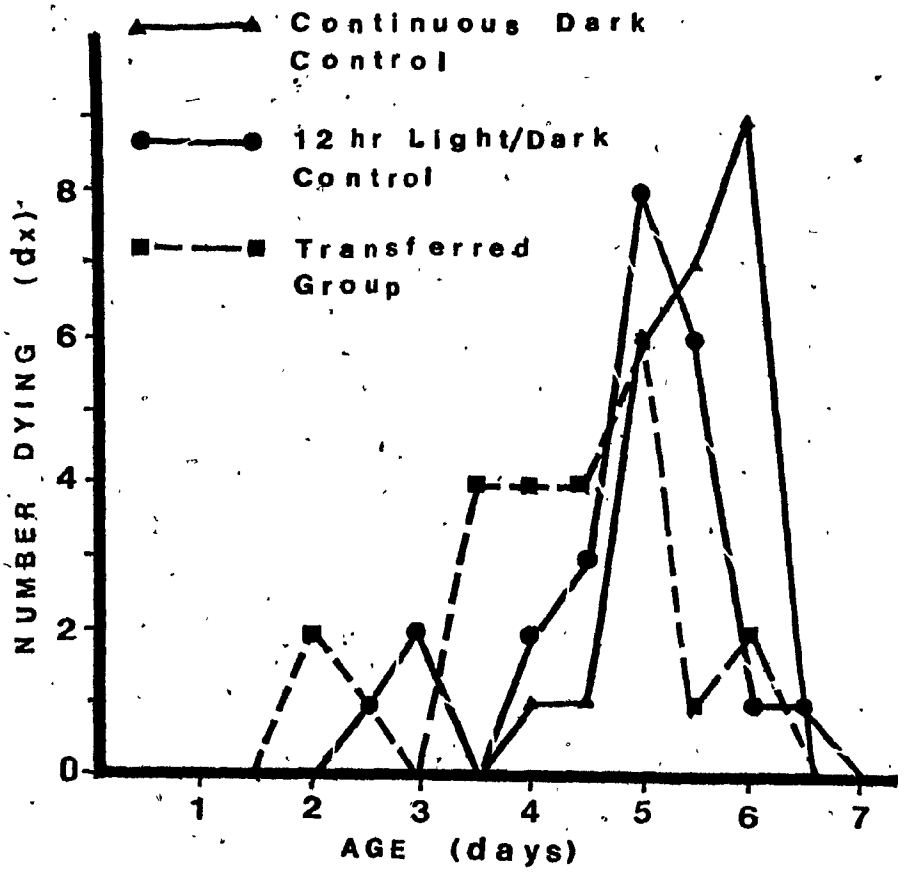


FIGURE 18. The age specific death rates (dx) for A. brightwelli after being reared under continuous darkness and transferred to a 12 hr light/dark cycle with its controls.

TABLE 24

THE EFFECT OF 25 UG/ML OF VITAMIN E ON LIFE-SPAN OF *A. BRIGHTWELLI* REARED IN CONTINUOUS DARK (n = 24)

Experiment	Mean Life-span ± S.E. (days)	Significance With The Post Hoc Tukey Test (alpha)
Control	5.47 ± 0.12	*
Ethanol Control	5.52 ± 0.08	*
12 hr Light/ Dark Control	5.55 ± 0.11	*
Vitamin E Control	6.42 ± 0.10	NSD
Continuous Dark Control	6.50 ± 0.12	NSD
Continuous Dark and Vitamin E	6.48 ± 0.12	NSD

* alpha = 0.01 from transferred group

NSD - No significant difference from vit. E or continuous dark control.

DISCUSSION

This discussion will contain sections to explain how rotifers are affected by the use of neutral red, by the addition of vitamin E, by exposure to ultraviolet radiation and after deprivation of light. The prereproductive developmental stage was the most sensitive to experimental intervention. Since this is the common link which connects all of the experiments, it will be discussed in detail. The results will also be discussed in relation to some of the current theories of aging.

Neutral Red

Biological stains are valuable aids for the visualization and identification of specific organs or structures. One of the earliest studies involving vital dyes was the well known study of Vogt (1929) on the amphibian blastula. Vital dyes were used to develop the "fate map" of the embryonic surface, which is used to follow the developmental fate of the stained cells. The dyes were maintained in contact with the cells throughout the gastrulation period. Vital dyes can penetrate the tissue and not induce an immediate degenerative change. Neutral red (C.I. No. 50040) in particular, has been used to stain living protozoa and for the vital staining of blood cells (Lillie 1977).

Neutral red is commonly used in conjunction with Janus green B (C.I. 11050) for the vital staining of Golgi and mitochondria in a single preparation at 5 mg/ml concentration (Humason 1967). Furtheremore neutral red is frequently used as a counter stain at 10 mg/ml concentration for a multitude of uses (Putt 1972).

In the study presented here, the neutral red stain was maintained throughout the life-span for the rotifer; this is the first known study of its kind. As a whole, vital stains are used only for a limited period of time to follow a given developmental stage. The stains are rarely maintained for any length of time since the purpose of the dye is for differentiation or identification of structures or groups of cell. For example Trypan blue, a valuable vital stain due to its uptake by the reticulo-endothelial system (Lillie 1977), is known to cause the formation of caudal hematomas to chick embryos at Hamburger-Hamilton stages 11-15 with 20 ul treatment of 1% trypan blue solution (Rajala and Daplan 1980).

In the present study using the rotifer A. brightwelli, the results obtained for the mean life-span (table 1) and for the reproductive profile (table 2) at various neutral red concentrations show that neutral red did not have any effect upon the life-span or to the reproductive profile of the rotifers.

No previous experiments involving chronic life time exposure to a vital stain have been done. In the present study, rotifers were exposed to neutral red from postnatal stage to the end of their life-span without any negative effects on life-span or reproduction.

Through not quantitative in nature, neutral red can be used as a rough means to ensure that the ingestion of Paramecia has taken place. As table 3 shows, dyed Paramecia at densities greater than 206 ± 11 Paramecia/ml were required to determine that Paramecia had been ingested. This is quite important to verify. Dietary restriction has been shown to increase the life-span in invertebrates such as Tokophyra lemmarum (MacKeen and Mitchell 1975) and A. brightwelli (Verdone-Smith and Enesco 1982). In both of these dietary restriction experiments, the availability of the food source was reduced by lengthening the intervals between feeding. This procedure reduces the food source available. Since it was possible to verify that rotifers were ingesting the neutral red stained Paramecia, the possibility of dietary restriction as a variable in the present experiments is reduced, if not eliminated.

Alpha-tocopherol

A great deal of research has been undertaken on the tocopherols. Varied effects, ranging from cell division regulation (Liepkalns et al. 1982), to increased fertility

and lengthened life-span (Enesco and Verdone-Smith 1980, Kahn and Enesco 1981) have been shown to take place when vitamin E supplementation was provided either to vertebrates or invertebrates.

Mode of Action

The activity of vitamin E has been separated into its hydroxyl functions and its effects caused by metabolites of vitamin E (Boguth 1969). The chemical properties of the vitamin E molecules can be attributed both to its free phenolic hydroxyl group, which can be acylated, etherified or phosphorylated, and also to the fact that vitamin E is a mono-ether of a hydroquinone which is easily oxidized, giving it antioxidant properties (Sebrell and Harris 1972). Initial oxidation products of the tocopherol degradation can be reduced to its original form by vitamin C (Kutsky 1973, Vasington et al. 1960). However, the quenching of the free radicals normally causes a irreversible change resulting in the destruction of the vitamin E molecules (Vasington et al. 1960). The alpha-tocopherol form of vitamin E can deactivate approximately 40 singlet oxygen molecules through the hydroxy functon of the chromanol ring before being destroyed (Fragata and Bellemare 1980). Vitamin E is also thought to have a regulatory role in enzyme production. Vitamin E may exert direct genetic control by acting as a repressor of various enzyme

syntheses (Olson 1967).

Influence of Vitamin E on life-span

The results obtained in this study show that vitamin E supplementation increases life-span in A. brightwelli. Similar results were obtained for all parameters whether vitamin E was solubilized in Tween 80 or in ethanol, so that the vitamin E effects may be discussed without concern for uncontrolled effects of the solubilizing agents. The survivorship curves (Figure 1) also show that in addition to the increase in mean life-span, the maximum age has been extended from 7 1/2 days to 8 days when vitamin E was added to the rotifers.

The rotifer life-span can be divided into three stages: prereproductive, reproductive and postreproductive. Only the prereproductive period of rotifer development was lengthened by vitamin E supplementation. The increase in the total mean life-span can be accounted for by the increase in the prereproductive stage of development alone. This early developmental stage is the only period that could be modified. Furthermore, it was also determined that the vitamin E had to be present during the prereproductive period in order for the vitamin E to increase the life-span of A. brightwelli. The age specific death rate (dx) period was also shifted to a later age than in the controls for the vitamin E supplemented group

(Figure 9).

The results reported here are in agreement with the findings of Enesco and Verdone-Smith (1980), who showed that Vitamin E supplementation extended the life-span of the rotifer Philodina. Vitamin E increased mean life-span of the Philodina by around 9% (from 18.72 to 20.45 days).

Increased longevity of nematodes has also been reported with vitamin E supplementation. Epstein and Gershon (1972) showed that Caenorhabditis elegans had a 23% increase in maximal life-span (from 56 ± 3 to 69 ± 4 days) when vitamin E was added to their medium. Kahn and Enesco (1981) showed that Turbatrix aceti had a 34% increase in mean life-span (from 45.40 ± 29.6 to 60.68 ± 30.6 days) when supplemented with vitamin E. A 10 day increase in life expectancy (58 to 68 days) was also found when T. aceti was supplemented with vitamin E. Subsequent studies by Kahn-Thomas and Enesco (1980b) revealed that the vitamin E must be present during the prereproductive developmental stage of T. aceti in order to influence life-span.

Ledvina and Hodanove (1980) showed that vitamin E produced a slight 2% increase in the mean life-span (from 690.4 ± 168.1 to 704.2 ± 209.1 days) and 28.6% increase in the maximum life-span (from 933 to 1200 days) in female mice. Though vitamin E did not increase the life-span of

cultured human diploid cells, a synergistic interaction of vitamin E with the cell culture serum has been shown to extend the life-span of cultured human diploid cells (Packer and Smith 1977; a retraction of Packer and Smith 1974).

The increase in the mean life-span found for A. brightwelli (17% increase from 5.46 ± 0.13 to 6.38 ± 0.12 days; vitamin E/Tween) is in agreement with other research on both vertebrates or invertebrates. However, it is important to note that A. brightwelli is the shortest lived species in which vitamin E has ever been tried for life-span or fecundity studies. The fact that the vitamin E supplementation increased the mean life-span of the short-lived A. brightwelli indicates that vitamin E influences longevity whether the life-span of the organism is short (6 days for A. brightwelli) or long (3 years for mice). The life-span comparison between different species is presented in table 25.

Vitamin E at Different Developmental Stage

The results of this study shows that vitamin E had to be present during the prereproductive stage of rotifer development in order for the extension of the mean life-span to occur.

Similar findings were obtained by Kahn and Enesco (1981) for the nematode Turbatrix aceti. Vitamin E

TABLE 25

INFLUENCE OF VITAMIN E ON LIFE-SPAN;
A COMPARISON BETWEEN DIFFERENT SPECIES

Species	Mean Life-span (days)		Maximum Longevity (days)		Reference
	Control	Vit. E -	Control	Vit. E	
mice	690.4 ± 168.1	704.2 ± 209.1	933	1200 *	Ledvina & Hodanove 1980
Turbatrix aceti (nematode)	45.40 ± 29.6	60.68 ± 30.6 *	-	-	Kahn and Enesco 1981
Philodina (rotifer)	18.72	20.45 *	22	22	Enesco and Verdone-Smith 1979
A. brightwelli	5.46 ± 0.13	6.38 ± 0.12 *	6.5	7.5	Present Study

* Determined to be significant

supplementation during the prereproductive stage alone resulted in a significant increase in life-span. Zuckerman and Geist (1983) also showed that the nematode Caenorhabditis elegans required vitamin E during the prereproductive stage in order to extend the life-span. Thus the critical period for vitamin E supplementation is at the prereproductive developmental stage.

Free Radical Theory of Aging

Most authors who have studied the effects of vitamin E on life-span interpret their results in terms of the free radical theory of aging. The increase in the life-span of A. brightwelli is also best interpreted in relation to the free radical theory of aging.

Free radicals are highly reactive compounds due to their unpaired electron in the outer orbital. The free radical theory of aging postulates that aging changes are the results of accumulated free radical damages (Lamb 1977, Harman 1968). Free radicals are formed as transient intermediates in the normal cellular metabolism as well as from spontaneous random reactions. Free radicals can attack membranes and DNA molecules to form cross-linkages of these molecules. Due to its proven free radical scavenging ability, vitamin E (Matsushita et al. 1978, Mc Cay et al. 1978) could act to reduce or prevent such cross-linkages. The membrane stabilizing effect of vitamin

E could also cause the cell membranes to retain their stability with age (Hochschild 1971, Mc Cay et al. 1978). If lysosomal membrane damages are reduced by a free radical scavenger, this would inhibit leakage of hydrolytic enzymes to viable cells.

Possible Synergism Between Vitamin E and Solubilizing Agent

One could argue that though Tween 80, the agent initially used to solubilize the vitamin E, did not increase the life-span since a synergistic effect between vitamin E and Tween 80 may have been present. This is quite plausible since vitamin E is well known to be a synergist with selenium (Chen et al. 1982, Hoekstra 1975), with other vitamins (Kutsky 1973) and even with other antioxidants (Bougle et al. 1982, Lippman 1981). To counter this argument, as well as to facilitate the solubilization and to reduce the chance of oxidation of vitamin E, ethanol was used as an alternative solubilizing agent. The results presented in table 10 compare the Tween solubilized and ethanol solubilized vitamin E at the 25 ug/ml concentration. The data show that the ethanol solubilized vitamin E also increased the mean life-span of the rotifers as compared to ethanol controls. Mean life-span of rotifers was not statistically different when the vitamin E was dissolved in ethanol or dissolved in Tween 80.

Ethanol is known to be an antioxidant (Dr. R. M.

Roy: personal communication) and may have added to the antioxidant action of the vitamin E to a minor degree. Being quite volatile, ethanol can rapidly evaporate from the medium; in contrast Tween 80 has no way of being removed from the medium. From the present study, ethanol and Tween 80 were both deemed to be harmless at the cencentrations used. Synergism between Tween 80 or ethanol and vitamin E was not found to be operative.

Fecundity

Evans and Bishop (1922) first reported that vitamin E plays a role in the fertility of laboratory rats. Vitamin E deficient rats and hamsters have a low number of offspring due to fetal resorption, but this can be corrected through the addition of vitamin E (Rao and Mason 1975). Vitamin E also enhances the fertility of white leghorn chickens (Friedrichson et al. 1980). Due to its importance in fertility, vitamin E has been called the "antisterility vitamin" (Kutsky 1973, Sebrell and Harris 1972).

In addition to vertebrates, many invertebrates are known to require vitamin E for reproduction. The cricket, Cacheta domestica requires vitamin E for spermatogenic activity and egg production (Vieira 1967). The free living nematode, Turbatrix aceti produces a larger number of live offspring due to the addition of vitamin E to its medium

(Kahn-Thomas and Enesco 1982a).

In the data reported here, no increase in the length of the reproductive period or in the number of offspring of A. brightwelli was seen following vitamin E supplementation.

In looking at the fecundity data, the present study is best compared with a similar study where another rotifer species, Philodina, was supplemented with vitamin E (Enesco and Verdone-Smith 1980). Philodina also reproduces asexually, like A. brightwelli. The researchers reported that vitamin E supplementation resulted in an increase in the mean life-span of the Philodina as well as a significant increase in the average number of offspring per rotifer. A significant increase in the length of the reproductive period was also reported. It appears that the vitamin E induced a longer reproductive period which allowed the Philodina to produce a greater number of offspring. Philodina has a 20 day life-span as compared to the 6 day life-span of A. brightwelli. Because of its exceedingly short life-span, it is probable that the length of the reproductive period is under more rigid control in A. brightwelli, and not subject to experimental modulation.

The presence of vitamin E triggers the occurance of sexual reproduction in some rotifer species including A. brightwelli (Gilbert and Thompson 1968, Gilbert 1975).

This is attained through the induction of meiotic rather than mitotic maturation division in the oocytes of the females to give birth to mictic females (first vitamin E generation) which in turn can give birth to male A. brightwelli (second vitamin E generation). Since the present study only involved rotifers treated for a single generation with vitamin E medium, only amictic female reproduction was seen. In any case, there is no known physiological difference between the mictic and amictic females (Pennak 1978). Thus, this specific meiosis inducing effect of vitamin E on rotifers is not a factor in interpreting any of the results reported here.

Although not related to the data presented here, it is of interest to note the induction of meiosis due to vitamin E supplementation can be seen as an important ecological signal. Though predatory in nature, A. brightwelli is known to eat algal or higher plant material which has vitamin E as a neutral lipid (Gilbert and Thompson 1968). In fact the presence of vitamin E is ubiquitous to most algae (Skinner and Sturm 1968). Rotifers may eat plant material when little animal prey is available, indicative of poor environmental conditions. The sexual reproduction induced by vitamin E permits new genetic variations to occur through meiosis and recombination. The rotifer species can continue to

proliferate and adapt to environmental changes when needed. In addition, production of rotifer resting eggs from the sexual reproduction gives environmental protection till the conditions are right for the rotifers to hatch.

Limited Range of Vitamin E

The effect of the vitmin E on life-span extension was only operative at a specific concentration. A very limited range of effective vitamin E concentration has been reported by other investigators for other systems. Yasunaga et al. (1982) reported that vitamin E acted as an immunopotentiation agent at an optimal dosage of 5 to 20 IU/kg/day for mice. Yet at dosages over 80 IU/kg/day, vitamin E acted as a toxicant to the mice. In much the same manner, Rao and Mason (1975) have shown that fetal resorption in vitamin E deficient rats may be prevented through the introduction of 25 mg/kg/day of d-alpha-tocopheryl hydroquinone. At higher daily doses of 125 mg/kg/day, the excess vitamin E interacted with vitamin K, resulting in an antivitamin K effect. This antivitamin K effect on embryos caused by the excess tocopherol resulted in internal hemorrhages, retarded development and death of the rat fetuses. Thus vitamin E is also seen to have optimal results at a given limited range in a number of different systems.

The need for vitamin E has been well established in man (Goldbloom 1963, Horwitt 1976). Susceptibility to hemolysis, edema formation, and development of anemia have been linked to vitamin E deficiency for infants (Wasserman and Taylor 1972). However, the harmful effects due to megavitamin E supplementation in man is still open to speculation (Farrell and Bieri 1975, Kellerher and Losowsky 1970). The lack of correlation between the intake and absorption of vitamin E would well play a role in the lack of harmful effect seen by some researchers in man (Kelleher and Losowsky 1970, Losowsky et al. 1972).

Body Size

In this study, vitamin E supplementaion caused a general increase in both the length and width of A. brightwelli from the new born stage (0.5 days) through the reproductive stage (3.0 days: see table 12A and 12B). However, as the rotifer aged, neither the length nor width of the vitamin E group was significantly different from the control (seen graphically in Figure 10). The maximum size attained by the end of the life cycle for control and vitamin E treated rotifers is the same. Although vitamin E accelerated the growth rate of A. brightwelli, it did not increase maximal size. Such acceleration in the growth rate was also reported for the dipteran insect Agria affinis due to the addition of vitamin E (House 1966).

The present study is in agreement with a similar size increases seen for A. brightwelli clone 4B61; the same clone which was used in this study, by Gilbert (1975) when exposed to vitamin E. Vitamin E has been reported to cause an increase in body size of the rotifer through both cytoplasmic growth and nuclear division (Gilbert 1973). The vitamin E content of a female rotifer is known to be proportional to the degree of her body wall outgrowth response for Asplanchna sieboldi. The gastric glands and vitellarium are the only two organs which undergo cell division in the Asplanchna. A vitamin E dose as low as 0.2 ng/rotifer was sufficient produce the body wall outgrowth for newborn A. sieboldi clone 10C3-1-5 (Gilbert and Birky 1971). Similar outgrowth was also found in other A. sieboldi clones, clone 10C6 (Kabay and Gilbert 1978) and clone 12C1 (Gilbert 1975). Such outgrowth of the body altered the body size in all clones of the same species studied. When another rotifer, Brachionus calyciflorus, was cultured for several generations with 5×10^{-5} M of d-alpha-tocopherol, there was an increase in the mean body size (Gilbert 1974). Gilbert (1973, 1974) has also shown that cannibalism and the uptake of vitamin E caused the increase in body size of the rotifer A. sieboldi.

There are several advantages in attaining a larger body size. Working with A. sieboldi, Gilbert (1975) showed

there was a direct correlation between the size of an individual and the size of its prey. A tocopherol induced increase in size may permit the newborn rotifers to eat a different food size range such that its survival is enhanced in nature. The size variation thus shows that vitamin E may act as a signal for growth in cell size.

Maly (1973) showed that in diaptomid copepods, Diaptomus shoshone and Diaptomus coloradensis, a larger female was able to produce a larger number of offspring. The offspring from larger female D. shoshone was able to hatch at a earlier time than the smaller female. In contrast, the larger vitamin E treated A. brightwelli showed no increase in reproduction or any alteration in the reproductive period.

Body size for the rotifer A. sieboldi is not influenced by temperature, food density, pH or osmolarity differences (Kabay and Gilbert 1978). These variables were not employed in the present study. Also, Maly (1978) showed that for the copepod, Diaptomus shoshone, size variations are present due to intraspecific interactions and temperature variations.

Mode of Vitamin E Uptake

Previous experiments by other investigators on vitamin E in an aquatic environment have not clearly defined the mode of vitamin E uptake by the aquatic

organism. When Paramecia were prepared with vitamin E, such that vitamin E would only be available through the ingestion of Paramecia as mentioned previously, an increase in life-span due to an increased prereproductive stage (table 13) and also a size increase (table 14) were seen. This indicates that the mode of vitamin E uptake by A. brightwelli is dietary in nature.

Ultraviolet Irradiation

Life-Span

As shown in the results, a logarithmic decrease in the mean life-span was seen with increasing doses of U.V. irradiation. The age specific death rate (dx) calculations (Figure 13) shows that U.V. doses equal to and higher than 300 J/m^2 caused the rotifers to die at an earlier age than their respective controls. The decreasing life expectancy values (table 15) further shows that higher U.V. dosages significantly lower the life expectancy of the rotifers.

Sacher and Grahn (1964) showed a similar decline in the survival values with increasing dosage of gamma irradiation on LAF_1 mice. It is well documented that the level of reduction in the life-span is directly related to the level of the radiation dose for a number of experimental animals as well as in man (Sacher 1977).

Genetic Mutation Theory of Aging

U.V. irradiation is well known to produce DNA damage through pyrimidine dimer formation (Hart et al. 1977) and by induction of sister chromatid exchange (Speit et al. 1982). The gradual decline in life-span with increasing U.V. irradiation lends support to the somatic mutation theory of aging. Though there are variations of this theory (Somatic Theory; Lamb 1977, Codon Restriction theory; Strehler 1977, Orgel's Error theory; Orgel 1963, 1970), the general idea is that aging is due to the accumulation of mutations in the genetic makeup of the organism.

An accumulation of DNA damage is known to occur with an increase in age (Dell'Orco and Whittle 1981, Nakanishi et al. 1979, Wheeler and Lett 1974). The radiation induced life shortening effects are well documented by Walburn (1975). Hart and Setlow (1974) further showed a correlation between DNA repair and life-span for a number of different organisms thereby showing the importance of the maintenance and stability of the genetic makeup of the organism.

The decrease in both the mean and expected life-span, from the 300 J/m^2 dose on for A. brightwelli is in agreement with and best explained by the genetic mutation theory of aging.

Possible Stimulatory Effect

The U.V. experiments were initiated to see whether there would be a stimulatory effect of U.V. radiation which would actually increase life-span. However, no stimulatory effect was observed at any of the various whole body radiation dosages applied to the rotifers. This finding differs from results of other investigators who used low levels of ionizing irradiation in experiments of similar design. Low levels of ionizing radiation increased the life-span in female Drosophila melanogaster (Lamb 1965). Lorenz et al. (1954) determined that daily gamma radiation as low as 0.11 rad was able to increase the average life-span of mice. Carlson and Jackson (1959) also determined a similar life-span increases at 1 rad/day of gamma irradiation on the Sprague-Dawley rat. Growth stimulation in Paramecium tetraurelia under low-level chronic gamma irradiation rate of 2 rad/day was also reported (Croute et al. 1982).

Paradoxial life-span increases were also seen when high ionizing irradiation were used. X-ray dosages from 2,000 rads to 200,000 rads resulted in a substantial enhancement of life-span in the marine hydroid, Campanularia flexuosa (Strehler 1964). Dauer et al. (1965) showed that in the male adult house flies, Musca domestica L., high X-ray exposures at 10,000 and 15,000 rads showed

an increase in the mean survival rate. Similar life-span increases in an vertebrate was seen in chronically gamma irradiated guinea pigs (Rust et al. 1966).

Stimulatory life-span increases may have been due to one or a combination of factors. Hart and Setlow (1974) have shown that there is a correlation between DNA repair capability and life-span for a number of mammalian species. A human premature aging syndrome, Hutchinson-Gilford progeria, is characterized by a deficiency in DNA repair (Epstein et al. 1973). When low doses of radiation are administered, the regular DNA repair mechanism (Cleaver 1978, Paterson 1978) may be stimulated to repair DNA beyond the norm. Thus, both radiation induced damage and age-related damage to DNA may be repaired, resulting in an increased life-span.

Aside from an enhanced DNA repair mechanism, the irradiation may have killed pathogens directly by the ionizing radiation or indirectly through the enhancement of antibody formation or via other resistance mechanism. The irradiation may have diminished the random deleterious environmental variables, found in the control group, so as to result in improved survival (Sacher 1963).

Ultraviolet irradiation, used in the present study, is not an ionizing form of radiation. The stimulatory effect may result only from ionizing irradiation. It is

also possible that the exposure points used in this study was not at the stimulatory dose. It may be that a specific dose which would increase the mean life-span exists at a lower exposure dose than that used for this study.

Developmental Sensitivity to U.V. Irradiation

In the previous section, vitamin E induced life-span increases was seen to be the direct result of a prolonged prereproductive developmental stage. The U.V. irradiation during different developmental stages (table 17) further shows the sensitivity of this stage to U.V. irradiation. The U.V. irradiation was only detrimental as to lower the average life-span only during the prereproductive stage of rotifer development.

Alpha-tocopherol on Life-span of U.V. Irradiated Rotifers

Being a nonionizing form of radiation, U.V. light will not mediate free radical formation (Blaylock and Trabalka 1978) but will form pyrimidine dimers in DNA (Harm 1980). On a theoretical basis, one would predict that direct free radical scavenging actions of vitamin E should not influence the degree of radiation damage by ionizing irradiation. However, the results of this study (table 18) shows that the vitamin E was able to counteract the lethality of U.V. irradiation on A. brightwelli. This conclusion confirms the findings of Lennartz and Bovee

(1981), where 1 x 10^{-4} M concentration of alpha-tocopheryl-succinate was able to totally counter the U.V. (253.7 nm) lethality on Blepharisma americanum; a large pink ciliate. Vitamin E was also able to inhibit the U.V. induced (280-365 nm) erythema on the rabbit skin (Roshchupkin et al. 1979). Suppression of U.V. induced tumor formation (1.97 J/cm^2/day for 80 days) in female albino hairless mice was also reported with dietary dl-alpha-tocopherol in conjunction with ascorbic acid and butylated hydroxytouene; all are known antioxidants (Black 1974, Black and Chan 1975). Vitamin E supplementation also prevented the elevation in the malonaldehyde content of rat liver lysosomes exposed to 400 nm U.V. irradiation (Torinuka et al. 1980).

Aside from the direct free radical scavenging actions, vitamin E may control translational and transcriptional events of the genetic material (Olson 1967). Vitamin E may therefore slow the aging process by removing DNA damage or by means of regulatory control.

Light and Dark Experiments

Longevity

An unexplained one day increase in the average life-span was seen for rotifers reared under continuous darkness (table 19). The age specific death rate (dx; Figure 16) was shifted to 1/2 day later for the continuous dark group

than the control and 1 1/2 day later than the continuous light group. However, the continuous light group did not show any significant difference in its life-span even though the age specific death rate occured one day earlier than the control group. Analysis of the developmental stages revealed that the prolongation in the prereproductive stage was the factor which increased the mean life-span of A. brightwelli.

Detection of prey by the rotifers is through touch or by biochemical stimuli (Pennak 1978). Paramecia ingestion by A. brightwelli (table 21) shows ingestion of Paramecia occurs by the continuous dark group thereby eliminating dietary restriction as the factor which increased the life-span.

The continuous dark group was started from a group of 20 rotifers, which were maintained as a separate subpopulation for a minimum of 20 generations. Since the life-span of the continuous dark group was significantly longer than that of the 12 hour light/dark control group, it was of interest to find out what was responsible for this difference. Physiological adaptation to dark conditions or genetic drift were the two possibilites to account for the increase in the life-span. If the continuous dark group was to maintain the same extended life-span when brought back to the 12 hour light/dark

condition, the alteration in life-span could be explained by genetic drift. That is, a small subpopulation had taken on new hereditary characteristics that would not be altered by changing environmental conditions. If, on the other hand the rotifers life-span were altered by returning them to light grown conditions, it would be most likely that some physiological adaptation to dark had taken place.

The total life-span was greatly reduced when the rotifers reared in continuous darkness were returned to light (table 22). The prereproductive stage remained fairly stable in length, but the failure of adaptation of the rotifers to light was shown by the shortened reproductive period. The decrease in life-span due to the shortened reproductive stage shows the sensitivity of this stage to light for rotifers reared in continuous darkness. The results presented here support the explanation of physiological adaptation.

Research involving circadian light/dark rhythm has been done for quite some time. However, studies involving altered longevity due to continuous darkness seems to be quite new. Allemand et al. (1973) first reported an increase in the life-span of male and female adult Drosophila melanogaster reared in continuous darkness. The maximum life-span was also seen to increase for both

male and female Drosophila. The care and handling were carried out under a dark red light with a wave-length greater than 675 nm. Pittendrigh and Minis (1972) showed that the life-span in D. melanogaster decreases when their circadian light cycles were altered.

Adaptation is well known to occur in plants due to its environmental habitat (Grime 1966). Sexual reproduction was caused by an increase in the photoperiod of the rotifer Notommata copeus (Clement and Pourriot 1972, 1974). Varied adaptative process can occur which would eventually fix into the genetic characteristic of the organism (Strickberger 1976, Levitan and Montagu 1971).

Light induced changes of retinal polypeptides in vivo has been seen in the Drosophila, possibly due to light-induced post-translational modification (Matsumoto et al. 1982). Variations in hormones are known to occur for mammals due to photoperiod alterations (Ottenweller and Hedge 1982, Vaughan et al. 1982).

Since only the prereproductive stage increased, the biochemical adaptative changes acted specifically on this initial postnatal stage, resulting in a prolonged life-span. The increase in life-span is therefore seen to be correlated with the length of the prereproductive stage.

There may be a limited minimum length of darkness which causes a prolongation of life, thereby making it a

circadian function. Circadian light rhythm experiments were not carried out. However, the life-span increased after being reared in continuous darkness, but this group showed a decrease in life-span when brought to a 12 hour light/dark cycle. Thus the length of darkness may be more important than the length of light if the increase in the life-span was circadian in nature.

Fecundity

The reproductive data (table 23) shows that the average reproductive and offspring number/rotifer were not affected by the different light conditions. The rotifer responds differently than other species in this respect. Mocquard et al. (1978) report that a decrease in the photoperiod prevented reproduction in the crustacea, Porcellio dilatatus. Photoperiod was also seen to influence ovarian development in the crayfish, Orconectes nais (Rice and Armitage 1974). The oocytes from the long-day crayfish was smaller than normal or short-day crayfish. The regulation of the testis in the male golden hamsters was also seen to be mediated by a photoperiodic response (Elliott et al. 1972). Thus, the cyclic photoperiod variations cited above have altered reproduction in a number of species. However, the experiment presented here using A. brightwelli did not involve circadian light variations. Instead, the effects after continuous light

or continuous dark conditions were studied. Fecundity values for the rotifer may differ if circadian light/dark variations were introduced, but this was not examined in the present study.

The reproductive profile of A. brightwelli may not be purely a photoperiod mediated factor. This would explain why rotifers reared with or without light were unaffected in reproductive potential by light or dark conditions.

The ovarian maturation and egg laying in the crayfish Orconectes virilis, requires proper proportions of both temperature and photoperiod. However, reproductive maturity for the juvenile hamster was attained regardless of the photoperiod (Gaston and Menaker 1967). Thus fecundity need not be dependent on the photoperiod. In the rotifer, Notommata copeus, a loss in photoperiod sensitivity has been seen (Clement and Pourriot 1980). This loss is transmissible to successive generations and is caused not by chromosomal variations but probably through external environmental conditions. A. brightwelli, in the present study could have had a loss in photoperiod sensitivity but this was not studied directly. However, the increased sensitivity to light for the continuous dark group during the reproductive stage shows that a gain in sensitiviy was attained.

Alpha-tocopherol in Continuous Dark Grown Rotifers

Rotifers reared in continuous darkness, whether supplemented with or without vitamin E, showed a similar prolongation of life-span (see table 24). The results show that synergism was not present between continuous darkness and alpha-tocopherol.

Watanabe et al. (1980a) reported a greater decrease in the weight of genital organs for non-vitamin E supplemented rats in the dark environment. This would seem to indicate that vitamin E and the lack of light were somehow related. However, the circadian light and dark length was seen to be of greater significance. Total tocopherol content in rat plasma increased under a light/dark rhythm than those under constant darkness (Watanabe et al. 1980b). Thus light/dark length is again seen to be of greater importance.

Importance of Prereproductive Stage

In the present study, increase in total life-span of A. brightwelli was caused by an increase in the length of the prereproductive stage. The life-span prolongation was only present when vitamin E supplementation occured during the prereproductive stage. This stage was also the most sensitive stage to U.V. irradiation.

Kahn and Enesco (1981) showed that vitamin E supplementation was required from the prereproductive period

to increase the life-span of the nematode Turbatrix aceti. Zuckerman and Geist (1983) also showed that vitamin E should be introduced early in the prereproductive stage to elicite the life-span increase in the nematode Caenorhabditis elegans.

Due to the short life-span of the rotifers, the specific time of the prereproductive stage which is involved in the prolongation have not been isolated. The importance of the prereproductive period is quite evident. However, Snell and King (1977) has shown that long lived A. brightwelli spend the largest part of their life in the reproductive stage at 20°C and 25°C. The rate of reproduction and life-span are suggested to be inversely related for A. brightwelli. Yet in going from 25 down to 20°C, the prereproductive period increased at a greater rate than the reproductive time period.

The data of Verdone-Smith and Enesco (1982) on rotifers examines the correlation between total life-span and the length of the reproductive and prereproductive periods. In these studies, the effect of temperature on life-span was examined. At 17.5°C; the temperature optimum, a long life-span was correlated with a long reproductive period. When temperature was decreased to 15°C, the mean life-span was not significantly different from rotifers which were reared at 17.5°C. However, at 15°C a long life-

span was correlated with a long prereproductive period. Thus the correlation between reproductive period and life-span is only true up to 17.5°C. This again suggests that the prereproductive period is most susceptible to environmental manipulation. The experiment performed in the present study was always maintained at 19°C so that temperature was not a variable.

From the results, it can be concluded that both vitamin E or growth in continuous darkness influences life-span specifically by extending the prereproductive stage of development of A. brightwelli.

The prereproductive period is the time at which cells are enlarging. Cell division is being completed and yolk is being synthesized to support new embryo formation. The "rate of living" theory (Lamb 1977) could be invoked to suggest that if metabolism is slowed down or speeded up by any environmental changes, its influence is most directly expressed during the prereproductive period.

SUMMARY

Supplementation with vitamin E and growth under continuous darkness both resulted in an increase in the mean life-span of A. brightwelli. Analysis of the developmental stages indicated that the lengthening of the prereproductive stage was the major factor contributing to the prolongation of the life-span when the rotifers were grown under continuous darkness or with vitamin E supplementation. Aside from the increase in the life-span, vitamin E increased the size of the newborn rotifers.

Ultraviolet irradiation had the greatest detrimental life-shortening effect during the prereproductive stage. However, vitamin E supplementation was able to counter U.V. irradiation damages by acting as a U.V. protective agent. The presence of vitamin E was also found to be necessary during the prereproductive period in order to have a life prolonging effect.

The increase in life-span for rotifers after being reared in continuous darkness appeared to have been due to adaptation. However, such rotifers became light sensitive as to result in a decreased life-span in light.

The prereproductive period was the most susceptible developmental stage for environmental manipulations and was

seen to be a major factor in the prolongation of the life-span in A. brightwelli.

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APPENDIX I

Stock Buffer Solution

For culturing Paramecia/rotifers

COMPONENT	MOLECULAR WEIGHT	AMOUNT USED
distilled H_2O	-	250.0 ml
NaH_2PO_4	137.99	34.5 g
NaOH	40.0	4.0 g and appropriate amount of 0.5 M solution

Distilled water, NaH_2PO_4 and NaOH mixed together in a 500 ml Erlenmeyer flask. Appropriate volume of 0.5 M NaOH solution added to obtain pH of 6.8. Use 7.0 ml for every liter of Medium.

APPENDIX II

Chromic Acid Solution

Used to clean all glassware.

COMPONENT	AMOUNT USED
H_2O	15.0 ml
$Na_2Cr_2O_7$	15.0 g
concentrated H_2SO_4	320.0 ml

Water and sodium dichromate were mixed together in a 1000-ml Erlenmeyer flask. Potassium dichromate may also be used instead of sodium dichromate. Concentrated sulfuric acid was slowly added, while swirling, over a one hour period. The prepared chromic acid was cooled to room temperature and transferred to a storage bottle.

Preparation of chromic acid was as indicated in Skoog and West (1976). The solution was reused until the green color of chromium (III) ion was detected.

APPENDIX III

The following section contains all the lifetables generated by the computer program in Appendix IV. The meaning of the symbols are listed below (from Krebs 1978).

x = age interval of the organism.

nx = number of survivors at start of age interval x.

lx = proportion of individuals surviving to start of age interval x.

dx = number dying during the age interval x to x + 1.

qx = rate of mortality during the age interval x to x + 1.

ex = mean expectation of life for organisms alive at start of age x.

Lx = number of individuals alive on the average during the age interval x to x + 1.

Tx = the remaining life-span of organism at the given age interval.

TABLE 26

NEUTRAL RED CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	48	1.000	0	0.000	5.13	48.00	492.00
0.5 - 1.0	48	1.000	0	0.000	4.63	48.00	444.00
1.0 - 1.5	48	1.000	0	0.000	4.13	48.00	396.00
1.5 - 2.0	48	1.000	0	0.000	3.63	48.00	348.00
2.0 - 2.5	48	1.000	0	0.000	3.13	48.00	300.00
2.5 - 3.0	48	1.000	0	0.000	2.63	48.00	252.00
3.0 - 3.5	48	1.000	1	0.021	2.13	47.50	204.00
3.5 - 4.0	47	0.979	4	0.085	1.66	45.00	156.50
4.0 - 4.5	43	0.896	5	0.116	1.30	40.50	111.50
4.5 - 5.0	38	0.792	13	0.342	0.93	31.50	71.00
5.0 - 5.5	25	0.521	9	0.360	0.79	20.50	39.50
5.5 - 6.0	16	0.333	7	0.438	0.59	12.50	19.00
6.0 - 6.5	9	0.188	7	0.778	0.36	5.50	6.50
6.5 - 7.0	2	0.042	2	1.000	0.25	1.00	1.00
7.0 - 7.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 27

NEUTRAL RED AT 0.1 MICROGRAM/ML

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	48	1.000	0	0.000	4.90	48.00	470.00
0.5 - 1.0	48	1.000	0	0.000	4.40	48.00	422.00
1.0 - 1.5	48	1.000	1	0.021	3.90	47.50	374.0
1.5 - 2.0	47	0.979	0	0.000	3.47	47.00	326.50
2.0 - 2.5	47	0.979	0	0.000	2.97	47.00	279.50
2.5 - 3.0	47	0.979	0	0.000	2.47	47.00	232.50
3.0 - 3.5	47	0.979	2	0.043	1.97	46.00	185.50
3.5 - 4.0	45	0.938	2	0.044	1.55	44.00	139.50
4.0 - 4.5	43	0.896	5	0.116	1.11	40.50	95.50
4.5 - 5.0	38	0.792	18	0.474	0.72	29.00	55.00
5.0 - 5.5	20	0.417	8	0.400	0.65	16.00	26.00
5.5 - 6.0	12	0.250	9	0.750	0.42	7.50	10.00
6.0 - 6.5	3	0.063	2	0.667	0.42	2.00	2.50
6.5 - 7.0	1	0.021	1	1.000	0.25	0.50	0.50
7.0 - 7.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 28

NEUTRAL RED AT 0.5 MICROGRAM/ML

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	48	1.000	0	0.000	5.05	48.00	485.00
0.5 - 1.0	48	1.000	0	0.000	4.55	48.00	437.0
1.0 - 1.5	48	1.000	0	0.000	4.05	48.00	389.00
1.5 - 2.0	48	1.000	0	0.000	3.55	48.00	341.00
2.0 - 2.5	48	1.000	1	0.021	3.05	47.50	293.00
2.5 - 3.0	47	0.979	1	0.021	2.61	46.50	245.50
3.0 - 3.5	46	0.958	1	0.022	2.16	45.50	199.00
3.5 - 4.0	45	0.938	4	0.089	1.71	43.00	153.50
4.0 - 4.5	41	0.854	5	0.122	1.35	38.50	110.50
4.5 - 5.0	36	0.750	8	0.222	1.00	32.00	72.00
5.0 - 5.5	28	0.583	14	0.500	0.71	21.00	40.00
5.5 - 6.0	14	0.292	7	0.500	0.68	10.50	19.00
6.0 - 6.5	7	0.146	4	0.571	0.61	5.00	8.50
6.5 - 7.0	3	0.063	1	0.333	0.58	2.50	3.50
7.0 - 7.5	2	0.042	2	1.000	0.25	1.00	1.00
7.5 - 8.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 29

NEUTRAL RED AT 0.75 MICROGRAM/ML

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	48	1.000	[illegible]	0.000	5.02	48.00	482.00
0.5 - 1.0	48	1.000	0	0.000	4.52	48.00	434.00
1.0 - 1.5	48	1.000	0	0.000	4.02	48.00	386.00
1.5 - 2.0	48	1.000	0	0.000	3.52	48.00	338.00
2.0 - 2.5	48	1.000	0	0.000	3.02	48.00	290.00
2.5 - 3.0	48	1.000	0	0.000	2.52	48.00	242.00
3.0 - 3.5	48	1.000	0	0.000	2.02	48.00	194.00
3.5 - 4.0	48	1.000	3	0.063	1.52	46.50	146.00
4.0 - 4.5	45	0.938	9	0.200	1.11	40.50	99.50
4.5 - 5.0	36	0.750	12	0.333	0.82	30.00	59.00
5.0 - 5.5	24	0.500	9	0.375	0.60	19.50	29.00
5.5 - 6.0	15	0.313	13	0.867	0.32	8.50	9.50
6.0 - 6.5	2	0.042	2	1.000	0.25	1.00	1.00
6.5 - 7.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 30

NEUTRAL RED AT 1.6 MICROGRAM/ML

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	48	1.000	0	0.000	5.21	48.00	500.00
0.5 - 1.0	48	1.000	2	0.042	4.71	47.00	452.00
1.0 - 1.5	46	0.958	0	0.000	4.40	46.00	405.00
1.5 - 2.0	46	0.958	0	0.000	3.90	46.00	359.00
2.0 - 2.5	46	0.958	0	0.000	3.40	46.00	313.00
2.5 - 3.0	46	0.958	0	0.000	2.90	46.00	267.00
3.0 - 3.5	46	0.958	0	0.000	2.40	46.00	221.00
3.5 - 4.0	46	0.958	0	0.000	1.90	46.00	175.00
4.0 - 4.5	46	0.958	2	0.043	1.40	45.00	129.00
4.5 - 5.0	44	0.917	16	0.364	0.95	36.00	84.00
5.0 - 5.5	28	0.583	8	0.286	0.86	24.00	48.00
5.5 - 6.0	20	0.417	10	0.500	0.60	15.00	24.00
6.0 - 6.5	10	0.208	6	0.600	0.45	7.00	9.00
6.5 - 7.0	4	0.083	4	1.000	0.25	2.00	2.00
7.0 - 7.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 31

VITAMIN E/TWEEN 80 REGULAR CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.21	24.00	250.00
0.5 - 1.0	24	1.000	0	0.000	4.71	24.00	226.00
1.0 - 1.5	24	1.000	0	0.000	4.21	24.00	202.00
1.5 - 2.0	24	1.000	0	0.000	3.71	24.00	178.00
2.0 - 2.5	24	1.000	0	0.000	3.21	24.00	154.00
2.5 - 3.0	24	1.000	0	0.000	2.71	24.00	130.00
3.0 - 3.5	24	1.000	0	0.000	2.21	24.00	106.00
3.5 - 4.0	24	1.000	2	0.083	1.71	23.00	82.00
4.0 - 4.5	22	0.917	1	0.045	1.34	21.50	59.00
4.5 - 5.0	21	0.875	4	0.190	0.89	19.00	37.50
5.0 - 5.5	17	0.708	8	0.471	0.54	13.00	18.50
5.5 - 6.0	9	0.375	8	0.889	0.31	5.00	5.50
6.0 - 6.5	1	0.042	1	1.000	0.25	0.50	0.50
6.5 - 7.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 32

5 MICROGRAM/ML OF TWEEN 80 CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.17	24.00	248.00
0.5 - 1.0	24	1.000	0	0.000	4.67	24.00	224.00
1.0 - 1.5	24	1.000	0	0.000	4.17	24.00	200.00
1.5 - 2.0	24	1.000	0	0.000	3.67	24.00	176.00
2.0 - 2.5	24	1.000	0	0.000	3.17	24.00	152.00
2.5 - 3.0	24	1.000	0	0.000	2.67	24.00	128.00
3.0 - 3.5	24	1.000	0	0.000	2.17	24.00	104.00
3.5 - 4.0	24	1.000	0	0.000	1.67	24.00	80.00
4.0 - 4.5	24	1.000	1	0.042	1.17	23.50	56.00
4.5 - 5.0	23	0.958	9	0.391	0.71	18.50	32.50
5.0 - 5.5	14	0.583	7	0.500	0.50	10.50	14.00
5.5 - 6.0	7	0.292	7	1.000	0.25	3.50	3.50
6.0 - 6.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 33

5 MICROGRAM/ML OF VITAMIN E

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.42	24.00	260.00
0.5 - 1.0	24	1.000	0	0.000	4.92	24.00	236.00
1.0 - 1.5	24	1.000	0	0.000	4.42	24.00	212.00
1.5 - 2.0	24	1.000	0	0.000	3.92	24.00	188.00
2.0 - 2.5	24	1.000	0	0.000	3.42	24.00	164.00
2.5 - 3.0	24	1.000	0	0.000	2.92	24.00	140.00
3.0 - 3.5	24	1.000	1	0.042	2.42	23.50	116.00
3.5 - 4.0	23	0.958	0	0.000	2.01	23.00	92.50
4.0 - 4.5	23	0.958	1	0.043	1.51	22.50	69.50
4.5 - 5.0	22	0.917	4	0.182	1.07	20.00	47.00
5.0 - 5.5	18	0.750	5	0.278	0.75	15.50	27.00
5.5 - 6.0	13	0.542	9	0.692	0.44	8.50	11.50
6.0 - 6.5	4	0.167	3	0.750	0.38	2.50	3.00
6.5 - 7.0	1	0.042	1	1.000	0.25	0.50	0.50
7.0 - 7.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 34

10 MICROGRAM/ML TWEEN 80 CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.15	24.00	247.00
0.5 - 1.0	24	1.000	0	0.000	4.65	24.00	223.00
1.0 - 1.5	24	1.000	0	0.000	4.15	24.00	199.00
1.5 - 2.0	24	1.000	0	0.000	3.65	24.00	175.00
2.0 - 2.5	24	1.000	0	0.000	3.15	24.00	151.00
2.5 - 3.0	24	1.000	0	0.000	2.65	24.00	127.00
3.0 - 3.5	24	1.000	0	0.000	2.15	24.00	103.00
3.5 - 4.0	24	1.000	0	0.000	1.65	24.00	79.00
4.0 - 4.5	24	1.000	4	0.167	1.15	22.00	55.00
4.5 - 5.0	20	0.833	7	0.350	0.83	16.50	33.00
5.0 - 5.5	13	0.542	6	0.462	0.63	10.00	16.50
5.5 - 6.0	7	0.292	4	0.571	0.46	5.00	6.50
6.0 - 6.5	3	0.125	3	1.000	0.25	1.50	1.50
6.5 - 7.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 35

10 MICROGRAM/ML OF VITAMIN E

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.52	24.00	265.00
0.5 - 1.0	24	1.000	0	0.000	5.02	24.00	241.00
1.0 - 1.5	24	1.000	0	0.000	4.52	24.00	217.00
1.5 - 2.0	24	1.000	0	0.000	4.02	24.00	193.00
2.0 - 2.5	24	1.000	0	0.000	3.52	24.00	169.00
2.5 - 3.0	24	1.000	0	0.000	3.02	24.00	145.00
3.0 - 3.5	24	1.000	0	0.000	2.52	24.00	121.00
3.5 - 4.0	24	1.000	0	0.000	2.02	24.00	97.00
4.0 - 4.5	24	1.000	1	0.042	1.52	23.50	73.00
4.5 - 5.0	23	0.958	4	0.174	1.08	21.00	49.50
5.0 - 5.5	19	0.792	6	0.316	0.75	16.00	28.50
5.5 - 6.0	13	0.542	9	0.692	0.48	8.50	12.50
6.0 - 6.5	4	0.167	2	0.500	0.50	3.00	4.00
6.5 - 7.0	2	0.083	2	1.000	0.25	1.00	1.00
7.0 - 7.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 36

25 MICROGRAM/ML OF TWEEN 80 CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.04	24.00	242.00
0.5 - 1.0	24	1.000	0	0.000	4.54	24.00	218.00
1.0 - 1.5	24	1.000	0	0.000	4.04	24.00	194.00
1.5 - 2.0	24	1.000	0	0.000	3.54	24.00	170.00
2.0 - 2.5	24	1.000	0	0.000	3.04	24.00	146.00
2.5 - 3.0	24	1.000	0	0.000	2.54	24.00	122.00
3.0 - 3.5	24	1.000	0	0.000	2.04	24.00	98.00
3.5 - 4.0	24	1.000	0	0.000	1.54	24.00	74.00
4.0 - 4.5	24	1.000	3	0.125	1.04	22.50	50.00
4.5 - 5.0	21	0.875	6	0.286	0.65	18.00	27.50
5.0 - 5.5	15	0.625	13	0.867	0.32	8.50	9.50
5.5 - 6.0	2	0.083	2	1.000	0.25	1.00	1.00
6.0 - 6.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 37

25 MICROGRAM/ML OF VITAMIN E

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	6.10	24.00	293.00
0.5 - 1.0	24	1.000	0	0.000	5.60	24.00	269.00
1.0 - 1.5	24	1.000	0	0.000	5.10	24.00	245.00
1.5 - 2.0	24	1.000	0	0.000	4.60	24.00	221.00
2.0 - 2.5	24	1.000	0	0.000	4.10	24.00	197.00
2.5 - 3.0	24	1.000	0	0.000	3.60	24.00	173.00
3.0 - 3.5	24	1.000	0	0.000	3.10	24.00	149.00
3.5 - 4.0	24	1.000	0	0.000	2.60	24.00	125.00
4.0 - 4.5	24	1.000	0	0.000	2.10	24.00	101.00
4.5 - 5.0	24	1.000	1	0.042	1.60	23.50	77.00
5.0 - 5.5	23	0.958	2	0.087	1.16	22.00	53.50
5.5 - 6.0	21	0.875	7	0.333	0.75	17.50	31.50
6.0 - 6.5	14	0.583	8	0.571	0.50	10.00	14.00
6.5 - 7.0	6	0.250	5	0.833	0.33	3.50	4.00
7.0 - 7.5	1	0.042	1	1.000	0.25	0.50	0.50
7.5 - 8.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 38

50 MICROGRAM/ML OF TWEEN 80 CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.21	24.00	250.00
0.5 - 1.0	24	1.000	0	0.000	4.71	24.00	226.00
1.0 - 1.5	24	1.000	0	0.000	4.21	24.00	202.00
1.5 - 2.0	24	1.000	0	0.000	3.71	24.00	178.00
2.0 - 2.5	24	1.000	0	0.000	3.21	24.00	154.00
2.5 - 3.0	24	1.000	0	0.000	2.71	24.00	130.00
3.0 - 3.5	24	1.000	0	0.000	2.21	24.00	106.00
3.5 - 4.0	24	1.000	0	0.000	1.71	24.00	82.00
4.0 - 4.5	24	1.000	3	0.125	1.21	22.50	58.00
4.5 - 5.0	21	0.875	7	0.333	0.85	17.50	35.50
5.0 - 5.5	14	0.583	5	0.357	0.64	11.50	18.00
5.5 - 6.0	9	0.375	7	0.778	0.36	5.50	6.50
6.0 - 6.5	2	0.083	2	1.000	0.25	1.00	1.00
6.5 - 7.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 39

50 MICROGRAM/ML OF VITAMIN E

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.04	24.00	242.00
0.5 - 1.0	24	1.000	0	0.000	4.54	24.00	218.00
1.0 - 1.5	24	1.000	0	0.000	4.04	24.00	194.00
1.5 - 2.0	24	1.000	0	0.000	3.54	24.00	170.00
2.0 - 2.5	24	1.000	0	0.000	3.04	24.00	146.00
2.5 - 3.0	24	1.000	0	0.000	2.54	24.00	122.00
3.0 - 3.5	24	1.000	0	0.000	2.04	24.00	98.00
3.5 - 4.0	24	1.000	2	0.083	1.54	23.00	74.00
4.0 - 4.5	22	0.917	4	0.182	1.16	20.00	51.00
4.5 - 5.0	18	0.750	6	0.333	0.86	15.00	31.00
5.0 - 5.5	12	0.500	5	0.417	0.67	9.50	16.00
5.5 - 6.0	7	0.292	5	0.714	0.46	4.50	6.50
6.0 - 6.5	2	0.083	1	0.500	0.50	1.50	2.00
6.5 - 7.0	1	0.042	1	1.000	0.25	0.50	0.50
7.0 - 7.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 40

100 MICROGRAM/ML OF TWEEN 80 CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.27	24.00	253.00
0.5 - 1.0	24	1.000	0	0.000	4.77	24.00	229.00
1.0 - 1.5	24	1.000	0	0.000	4.27	24.00	205.00
1.5 - 2.0	24	1.000	0	0.000	3.77	24.00	181.00
2.0 - 2.5	24	1.000	1	0.042	3.27	23.50	157.00
2.5 - 3.0	23	0.958	0	0.000	2.90	23.00	133.50
3.0 - 3.5	23	0.958	0	0.000	2.40	23.00	110.50
3.5 - 4.0	23	0.958	0	0.000	1.90	23.00	87.50
4.0 - 4.5	23	0.958	0	0.000	1.40	23.00	64.50
4.5 - 5.0	23	0.958	6	0.261	0.90	20.00	41.50
5.0 - 5.5	17	0.708	8	0.471	0.63	13.00	21.50
5.5 - 6.0	9	0.375	5	0.556	0.47	6.50	8.50
6.0 - 6.5	4	0.167	4	1.000	0.25	2.00	2.00
6.5 - 7.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 41

100 MICROGRAM/ML OF VITAMIN E

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.00	24.00	240.00
0.5 - 1.0	24	1.000	0	0.000	4.50	24.00	216.00
1.0 - 1.5	24	1.000	0	0.000	4.00	24.00	192.00
1.5 - 2.0	24	1.000	0	0.000	3.50	24.00	168.00
2.0 - 2.5	24	1.000	0	0.000	3.00	24.00	144.00
2.5 - 3.0	24	1.000	0	0.000	2.50	24.00	120.00
3.0 - 3.5	24	1.000	0	0.000	2.00	24.00	96.00
3.5 - 4.0	24	1.000	1	0.042	1.50	23.50	72.00
4.0 - 4.5	23	0.958	5	0.217	1.05	20.50	48.50
4.5 - 5.0	18	0.750	4	0.222	0.78	16.00	28.00
5.0 - 5.5	14	0.583	10	0.714	0.43	9.00	12.00
5.5 - 6.0	4	0.167	3	0.750	0.38	2.50	3.00
6.0 - 6.5	1	0.042	1	1.000	0.25	0.50	0.50
6.5 - 7.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 42

VITAMIN E/ETHANOL REGULAR CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.21	24.00	250.00
0.5 - 1.0	24	1.000	0	0.000	4.71	24.00	226.00
1.0 - 1.5	24	1.000	0	0.000	4.21	24.00	202.00
1.5 - 2.0	24	1.000	0	0.000	3.71	24.00	178.00
2.0 - 2.5	24	1.000	0	0.000	3.21	24.00	154.00
2.5 - 3.0	24	1.000	0	0.000	2.71	24.00	130.00
3.0 - 3.5	24	1.000	0	0.000	2.21	24.00	106.00
3.5 - 4.0	24	1.000	2	0.083	1.71	23.00	82.00
4.0 - 4.5	22	0.917	1	0.045	1.34	21.50	59.00
4.5 - 5.0	21	0.875	4	0.190	0.89	19.00	37.50
5.0 - 5.5	17	0.708	8	0.471	0.54	13.00	18.50
5.5 - 6.0	9	0.375	8	0.889	0.31	5.00	5.50
6.0 - 6.5	1	0.042	1	1.000	0.25	0.50	0.50
6.5 - 7.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 43

ETHANOL CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.35	24.00	257.00
0.5 - 1.0	24	1.000	0	0.000	4.85	24.00	233.00
1.0 - 1.5	24	1.000	0	0.000	4.35	24.00	209.00
1.5 - 2.0	24	1.000	0	0.000	3.85	24.00	185.00
2.0 - 2.5	24	1.000	1	0.042	3.35	23.50	161.00
2.5 - 3.0	23	0.958	0	0.000	2.99	23.00	137.50
3.0 - 3.5	23	0.958	0	0.000	2.49	23.00	114.50
3.5 - 4.0	23	0.958	0	0.000	1.99	23.00	91.50
4.0 - 4.5	23	0.958	0	0.000	1.49	23.00	68.50
4.5 - 5.0	23	0.958	6	0.261	0.99	20.00	45.50
5.0 - 5.5	17	0.708	7	0.412	0.75	13.50	25.50
5.5 - 6.0	10	0.417	4	0.400	0.60	8.00	12.00
6.0 - 6.5	6	0.250	5	0.833	0.33	3.50	4.00
6.5 - 7.0	1	0.042	1	1.000	0.25	0.50	0.50
7.0 - 7.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 44

VITAMIN E/ETHANOL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	6.56	24.00	315.00
0.5 - 1.0	24	1.000	0	0.000	6.06	24.00	291.00
1.0 - 1.5	24	1.000	0	0.000	5.56	24.00	267.00
1.5 - 2.0	24	1.000	0	0.000	5.06	24.00	243.00
2.0 - 2.5	24	1.000	0	0.000	4.56	24.00	219.00
2.5 - 3.0	24	1.000	0	0.000	4.06	24.00	195.00
3.0 - 3.5	24	1.000	0	0.000	3.56	24.00	171.00
3.5 - 4.0	24	1.000	0	0.000	3.06	24.00	147.00
4.0 - 4.5	24	1.000	0	0.000	2.56	24.00	123.00
4.5 - 5.0	24	1.000	0	0.000	2.06	24.00	99.00
5.0 - 5.5	24	1.000	1	0.042	1.56	23.50	75.00
5.5 - 6.0	23	0.958	3	0.130	1.12	21.50	51.50
6.0 - 6.5	20	0.833	4	0.200	0.75	18.00	30.00
6.5 - 7.0	16	0.667	12	0.750	0.38	10.00	12.00
7.0 - 7.5	4	0.167	4	1.000	0.25	2.00	2.00
7.5 - 8.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 45

REGULAR CONTROL FOR U.V. EXPERIMENT

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.48	24.00	263.00
0.5 - 1.0	24	1.000	0	0.000	4.98	24.00	239.00
1.0 - 1.5	24	1.000	0	0.000	4.48	24.00	215.00
1.5 - 2.0	24	1.000	0	0.000	3.98	24.00	191.00
2.0 - 2.5	24	1.000	0	0.000	3.48	24.00	167.00
2.5 - 3.0	24	1.000	0	0.000	2.98	24.00	143.00
3.0 - 3.5	24	1.000	0	0.000	2.48	24.00	119.00
3.5 - 4.0	24	1.000	1	0.042	1.98	23.50	95.00
4.0 - 4.5	23	0.958	2	0.087	1.55	22.00	71.50
4.5 - 5.0	21	0.875	5	0.238	1.18	18.50	49.50
5.0 - 5.5	16	0.667	4	0.250	0.97	14.00	31.00
5.5 - 6.0	12	0.500	5	0.417	0.71	9.50	17.00
6.0 - 6.5	7	0.292	5	0.714	0.54	4.50	7.50
6.5 - 7.0	2	0.083	0	0.000	0.75	2.00	3.00
7.0 - 7.5	2	0.083	2	1.000	0.25	1.00	1.00
7.5 - 8.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 46

50 J/m^2 U.V. DOSE

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.44	24.00	261.00
0.5 - 1.0	24	1.000	0	0.000	4.94	24.00	237.00
1.0 - 1.5	24	1.000	0	0.000	4.44	24.00	213.00
1.5 - 2.0	24	1.000	0	0.000	3.94	24.00	189.00
2.0 - 2.5	24	1.000	0	0.000	3.44	24.00	165.00
2.5 - 3.0	24	1.000	0	0.000	2.94	24.00	141.00
3.0 - 3.5	24	1.000	0	0.000	2.44	24.00	117.00
3.5 - 4.0	24	1.000	0	0.000	1.94	24.00	93.00
4.0 - 4.5	24	1.000	2	0.083	1.44	23.00	69.00
4.5 - 5.0	22	0.917	3	0.136	1.05	20.50	46.00
5.0 - 5.5	19	0.792	6	0.316	0.67	16.00	25.50
5.5 - 6.0	13	0.542	10	0.769	0.37	8.00	9.50
6.0 - 6.5	3	0.125	3	1.000	0.25	1.50	1.50
6.5 - 7.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 47

50 J/m^2 CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.65	24.00	271.00
0.5 - 1.0	24	1.000	0	0.000	5.15	24.00	247.00
1.0 - 1.5	24	1.000	0	0.000	4.65	24.00	223.00
1.5 - 2.0	24	1.000	0	0.000	4.15	24.00	199.00
2.0 - 2.5	24	1.000	0	0.000	3.65	24.00	175.00
2.5 - 3.0	24	1.000	0	0.000	3.15	24.00	151.00
3.0 - 3.5	24	1.000	0	0.000	2.65	24.00	127.00
3.5 - 4.0	24	1.000	0	0.000	2.15	24.00	103.00
4.0 - 4.5	24	1.000	1	0.042	1.65	23.50	79.00
4.5 - 5.0	23	0.958	3	0.130	1.21	21.50	55.50
5.0 - 5.5	20	0.833	5	0.250	0.85	17.50	34.00
5.5 - 6.0	15	0.625	9	0.600	0.55	10.50	16.50
6.0 - 6.5	6	0.250	4	0.667	0.50	4.00	6.00
6.5 - 7.0	2	0.083	1	0.500	0.50	1.50	2.00
7.0 - 7.5	1	0.042	1	1.000	0.25	0.50	0.50
7.5 - 8.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 48

150 J/m^2 U.V. DOSE

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.06	24.00	243.00
0.5 - 1.0	24	1.000	0	0.000	4.56	24.00	219.00
1.0 - 1.5	24	1.000	0	0.000	4.06	24.00	195.00
1.5 - 2.0	24	1.000	0	0.000	3.56	24.00	171.00
2.0 - 2.5	24	1.000	0	0.000	3.06	24.00	147.00
2.5 - 3.0	24	1.000	1	0.042	2.56	23.50	123.00
3.0 - 3.5	23	0.958	1	0.043	2.16	22.50	99.50
3.5 - 4.0	22	0.917	1	0.045	1.75	21.50	77.00
4.0 - 4.5	21	0.875	3	0.143	1.32	19.50	55.50
4.5 - 5.0	18	0.750	5	0.278	1.00	15.50	36.00
5.0 - 5.5	13	0.542	6	0.462	0.79	10.00	20.50
5.5 - 6.0	7	0.292	4	0.571	0.75	5.00	10.50
6.0 - 6.5	3	0.125	0	0.000	0.92	3.00	5.50
6.5 - 7.0	3	0.125	2	0.667	0.42	2.00	2.50
7.0 - 7.5	1	0.042	1	1.000	0.25	0.50	0.50
7.5 - 8.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 49

150 J/m^2 CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.35	24.00	257.00
0.5 - 1.0	24	1.000	0	0.000	4.85	24.00	233.00
1.0 - 1.5	24	1.000	0	0.000	4.35	24.00	209.00
1.5 - 2.0	24	1.000	0	0.000	3.85	24.00	185.00
2.0 - 2.5	24	1.000	0	0.000	3.35	24.00	161.00
2.5 - 3.0	24	1.000	0	0.000	2.85	24.00	137.00
3.0 - 3.5	24	1.000	0	0.000	2.35	24.00	113.00
3.5 - 4.0	24	1.000	0	0.000	1.85	24.00	89.00
4.0 - 4.5	24	1.000	4	0.167	1.35	22.00	65.00
4.5 - 5.0	20	0.833	4	0.200	1.08	18.00	43.00
5.0 - 5.5	16	0.667	5	0.313	0.78	13.50	25.00
5.5 - 6.0	11	0.458	8	0.727	0.52	7.00	11.50
6.0 - 6.5	3	0.125	1	0.333	0.75	2.50	4.50
6.5 - 7.0	2	0.083	1	0.500	0.50	1.50	2.00
7.0 - 7.5	1	0.042	1	1.000	0.25	0.50	0.50
7.5 - 8.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 50

200 J/m^2 U.V. DOSE

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.06	24.00	243.00
0.5 - 1.0	24	1.000	0	0.000	4.56	24.00	219.00
1.0 - 1.5	24	1.000	0	0.000	4.06	24.00	195.00
1.5 - 2.0	24	1.000	0	0.000	3.56	24.00	171.00
2.0 - 2.5	24	1.000	0	0.000	3.06	24.00	147.00
2.5 - 3.0	24	1.000	0	0.000	2.56	24.00	123.00
3.0 - 3.5	24	1.000	0	0.000	2.06	24.00	99.00
3.5 - 4.0	24	1.000	2	0.083	1.56	23.00	75.00
4.0 - 4.5	22	0.917	4	0.182	1.18	20.00	52.00
4.5 - 5.0	18	0.750	4	0.222	0.89	16.00	32.00
5.0 - 5.5	14	0.583	5	0.357	0.57	11.50	16.00
5.5 - 6.0	9	0.375	9	1.000	0.25	4.50	4.50
6.0 - 6.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 51

200 J/m^2 CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.71	24.00	274.00
0.5 - 1.0	24	1.000	0	0.000	5.21	24.00	250.00
1.0 - 1.5	24	1.000	0	0.000	4.71	24.00	226.00
1.5 - 2.0	24	1.000	0	0.000	4.21	24.00	202.00
2.0 - 2.5	24	1.000	0	0.000	3.71	24.00	178.00
2.5 - 3.0	24	1.000	0	0.000	3.21	24.00	154.00
3.0 - 3.5	24	1.000	1	0.042	2.71	23.50	130.00
3.5 - 4.0	23	0.958	0	0.000	2.32	23.00	106.50
4.0 - 4.5	23	0.958	1	0.043	1.82	22.50	83.50
4.5 - 5.0	22	0.917	1	0.045	1.39	21.50	61.00
5.0 - 5.5	21	0.875	4	0.190	0.94	19.00	39.50
5.5 - 6.0	17	0.708	8	0.471	0.60	13.00	20.50
6.0 - 6.5	9	0.375	6	0.667	0.42	6.00	7.50
6.5 - 7.0	3	0.125	3	1.000	0.25	1.50	1.50
7.0 - 7.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 52

300 J/m^2 U.V. DOSE

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	4.48	24.00	215.00
0.5 - 1.0	24	1.000	0	0.000	3.98	24.00	191.00
1.0 - 1.5	24	1.000	0	0.000	3.48	24.00	167.00
1.5 - 2.0	24	1.000	0	0.000	2.98	24.00	143.00
2.0 - 2.5	24	1.000	0	0.000	2.48	24.00	119.00
2.5 - 3.0	24	1.000	3	0.125	1.98	22.50	95.00
3.0 - 3.5	21	0.875	2	0.095	1.73	20.00	72.50
3.5 - 4.0	19	0.792	4	0.211	1.38	17.00	52.50
4.0 - 4.5	15	0.625	4	0.267	1.18	13.00	35.50
4.5 - 5.0	11	0.458	3	0.273	1.02	9.50	22.50
5.0 - 5.5	8	0.333	3	0.375	0.81	6.50	13.00
5.5 - 6.0	5	0.208	1	0.200	0.65	4.50	6.50
6.0 - 6.5	4	0.167	4	1.000	0.25	2.00	2.00
6.5 - 7.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 53

300 J/m^2 CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.13	24.00	246.00
0.5 - 1.0	24	1.000	0	0.000	4.63	24.00	222.00
1.0 - 1.5	24	1.000	0	0.000	4.13	24.00	198.00
1.5 - 2.0	24	1.000	0	0.000	3.63	24.00	174.00
2.0 - 2.5	24	1.000	0	0.000	3.13	24.00	150.00
2.5 - 3.0	24	1.000	0	0.000	2.63	24.00	126.00
3.0 - 3.5	24	1.000	0	0.000	2.13	24.00	102.00
3.5 - 4.0	24	1.000	1	0.042	1.63	23.50	78.00
4.0 - 4.5	23	0.958	4	0.174	1.18	21.00	54.50
4.5 - 5.0	19	0.792	4	0.211	0.88	17.00	33.50
5.0 - 5.5	15	0.625	7	0.467	0.55	11.50	16.50
5.5 - 6.0	8	0.333	7	0.875	0.31	4.50	5.00
6.0 - 6.5	1	0.042	1	1.000	0.25	0.50	0.50
6.5 - 7.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 54

600 J/m^2 U.V. DOSE

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	3.73	24.00	179.00
0.5 - 1.0	24	1.000	0	0.000	3.23	24.00	155.00
1.0 - 1.5	24	1.000	0	0.000	2.73	24.00	131.00
1.5 - 2.0	24	1.000	0	0.000	2.23	24.00	107.00
2.0 - 2.5	24	1.000	0	0.000	1.73	24.00	83.00
2.5 - 3.0	24	1.000	0	0.000	1.23	24.00	59.00
3.0 - 3.5	24	1.000	7	0.292	0.73	20.50	35.00
3.5 - 4.0	17	0.708	13	0.765	0.43	10.50	14.50
4.0 - 4.5	4	0.167	3	0.750	0.50	2.50	4.00
4.5 - 5.0	1	0.042	0	0.000	0.75	1.00	1.50
5.0 - 5.5	1	0.042	1	1.000	0.25	0.50	0.50
5.5 - 6.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 55

600 J/m^2 CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	4.98	24.00	239.00
0.5 - 1.0	24	1.000	0	0.000	4.48	24.00	215.00
1.0 - 1.5	24	1.000	0	0.000	3.98	24.00	191.00
1.5 - 2.0	24	1.000	0	0.000	3.48	24.00	167.00
2.0 - 2.5	24	1.000	0	0.000	2.98	24.00	143.00
2.5 - 3.0	24	1.000	0	0.000	2.48	24.00	119.00
3.0 - 3.5	24	1.000	0	0.000	1.98	24.00	95.00
3.5 - 4.0	24	1.000	2	0.083	1.48	23.00	71.00
4.0 - 4.5	22	0.917	4	0.182	1.09	20.00	48.00
4.5 - 5.0	18	0.750	6	0.333	0.78	15.00	28.00
5.0 - 5.5	12	0.500	5	0.417	0.54	9.50	13.00
5.5 - 6.0	7	0.292	7	1.000	0.25	3.50	3.50
6.0 - 6.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 56

1200 J/m^2 U.V. DOSE

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	30	1.000	0	0.000	3.05	30.00	183.00
0.5 - 1.0	30	1.000	0	0.000	2.55	30.00	153.00
1.0 - 1.5	30	1.000	0	0.000	2.05	30.00	123.00
1.5 - 2.0	30	1.000	1	0.033	1.55	29.50	93.00
2.0 - 2.5	29	0.967	7	0.241	1.09	25.50	63.50
2.5 - 3.0	22	0.733	6	0.273	0.86	19.00	38.00
3.0 - 3.5	16	0.533	7	0.438	0.59	12.50	19.00
3.5 - 4.0	9	0.300	7	0.778	0.36	5.50	6.50
4.0 - 4.5	2	0.067	2	1.000	0.25	1.00	1.00
4.5 - 5.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 57

1200 J/m^2 CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	30	1.000	0	0.000	5.12	30.00	307.00
0.5 - 1.0	30	1.000	0	0.000	4.62	30.00	277.00
1.0 - 1.5	30	1.000	0	0.000	4.12	30.00	247.00
1.5 - 2.0	30	1.000	0	0.000	3.62	30.00	217.00
2.0 - 2.5	30	1.000	0	0.000	3.12	30.00	187.00
2.5 - 3.0	30	1.000	0	0.000	2.62	30.00	157.00
3.0 - 3.5	30	1.000	0	0.000	2.12	30.00	127.00
3.5 - 4.0	30	1.000	1	0.033	1.62	29.50	97.00
4.0 - 4.5	29	0.967	2	0.069	1.16	28.00	67.50
4.5 - 5.0	27	0.900	12	0.444	0.73	21.00	39.50
5.0 - 5.5	15	0.500	7	0.467	0.62	11.50	18.50
5.5 - 6.0	8	0.267	6	0.750	0.44	5.00	7.00
6.0 - 6.5	2	0.067	1	0.500	0.50	1.50	2.00
6.5 - 7.0	1	0.033	1	1.000	0.25	0.50	0.50
7.0 - 7.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 58

4800 J/m^2 U.V. DOSE

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	30	1.000	0	0.000	1.57	30.00	94.00
0.5 - 1.0	30	1.000	0	0.000	1.07	30.00	64.00
1.0 - 1.5	30	1.000	16	0.533	0.57	22.00	34.00
1.5 - 2.0	14	0.467	10	0.714	0.43	9.00	12.00
2.0 - 2.5	4	0.133	3	0.750	0.38	2.50	3.00
2.5 - 3.0	1	0.033	1	1.000	0.25	0.50	0.50
3.0 - 3.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 59
4800 J/m^2 CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	30	1.000	0	0.000	5.20	30.00	312.00
0.5 - 1.0	30	1.000	0	0.000	4.70	30.00	282.00
1.0 - 1.5	30	1.000	0	0.000	4.20	30.00	252.00
1.5 - 2.0	30	1.000	0	0.000	3.70	30.00	222.00
2.0 - 2.5	30	1.000	0	0.000	3.20	30.00	192.00
2.5 - 3.0	30	1.000	1	0.033	2.70	29.50	162.00
3.0 - 3.5	29	0.967	1	0.034	2.28	28.50	132.50
3.5 - 4.0	28	0.933	2	0.071	1.86	27.00	104.00
4.0 - 4.5	26	0.867	3	0.115	1.48	24.50	77.00
4.5 - 5.0	23	0.767	5	0.217	1.14	20.50	52.50
5.0 - 5.5	18	0.600	4	0.222	0.89	16.00	32.00
5.5 - 6.0	14	0.467	7	0.500	0.57	10.50	16.00
6.0 - 6.5	7	0.233	5	0.714	0.39	4.50	5.50
6.5 - 7.0	2	0.067	2	1.000	0.25	1.00	1.00
7.0 - 7.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 60

LIGHT/DARK CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.31	24.00	255.00
0.5 - 1.0	24	1.000	0	0.000	4.81	24.00	231.00
1.0 - 1.5	24	1.000	0	0.000	4.31	24.00	207.00
1.5 - 2.0	24	1.000	0	0.000	3.81	24.00	183.00
2.0 - 2.5	24	1.000	0	0.000	3.31	24.00	159.00
2.5 - 3.0	24	1.000	1	0.042	2.81	23.50	135.00
3.0 - 3.5	23	0.958	0	0.000	2.42	23.00	111.50
3.5 - 4.0	23	0.958	0	0.000	1.92	23.00	88.50
4.0 - 4.5	23	0.958	3	0.130	1.42	21.50	65.50
4.5 - 5.0	20	0.833	4	0.200	1.10	18.00	44.00
5.0 - 5.5	16	0.667	2	0.125	0.81	15.00	26.00
5.5 - 6.0	14	0.583	11	0.786	0.39	8.50	11.00
6.0 - 6.5	3	0.125	2	0.667	0.42	2.00	2.50
6.5 - 7.0	1	0.042	1	1.000	0.25	0.50	0.50
7.0 - 7.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 61

REARED IN CONTINUOUS DARKNESS

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	6.31	24.00	303.00
0.5 - 1.0	24	1.000	0	0.000	5.81	24.00	279.00
1.0 - 1.5	24	1.000	0	0.000	5.31	24.00	255.00
1.5 - 2.0	24	1.000	0	0.000	4.81	24.00	231.00
2.0 - 2.5	24	1.000	0	0.000	4.31	24.00	207.00
2.5 - 3.0	24	1.000	0	0.000	3.81	24.00	183.00
3.0 - 3.5	24	1.000	0	0.000	3.31	24.00	159.00
3.5 - 4.0	24	1.000	0	0.000	2.81	24.00	135.00
4.0 - 4.5	24	1.000	0	0.000	2.31	24.00	111.00
4.5 - 5.0	24	1.000	1	0.042	1.81	23.50	87.00
5.0 - 5.5	23	0.958	3	0.130	1.38	21.50	63.50
5.5 - 6.0	20	0.833	2	0.100	1.05	19.00	42.00
6.0 - 6.5	18	0.750	10	0.556	0.64	13.00	23.00
6.5 - 7.0	8	0.333	2	0.250	0.63	7.00	10.00
7.0 - 7.5	6	0.250	6	1.000	0.25	3.00	3.00
7.5 - 8.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 62

REARED IN CONTINUOUS LIGHT

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.00	24.00	240.00
0.5 - 1.0	24	1.000	0	0.000	4.50	24.00	216.00
1.0 - 1.5	24	1.000	0	0.000	4.00	24.00	192.00
1.5 - 2.0	24	1.000	0	0.000	3.50	24.00	168.00
2.0 - 2.5	24	1.000	0	0.000	3.00	24.00	144.00
2.5 - 3.0	24	1.000	0	0.000	2.50	24.00	120.00
3.0 - 3.5	24	1.000	2	0.083	2.00	23.00	96.00
3.5 - 4.0	22	0.917	0	0.000	1.66	22.00	73.00
4.0 - 4.5	22	0.917	2	0.091	1.16	21.00	51.00
4.5 - 5.0	20	0.833	8	0.400	0.75	16.00	30.00
5.0 - 5.5	12	0.500	6	0.500	0.58	9.00	14.00
5.5 - 6.0	6	0.250	5	0.833	0.42	3.50	5.00
6.0 - 6.5	1	0.042	0	0.000	0.75	1.00	1.50
6.5 - 7.0	1	0.042	1	1.000	0.25	0.50	0.50
7.0 - 7.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 63

TRANSFERRED GROUP

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	4.00	24.00	192.00
0.5 - 1.0	24	1.000	0	0.000	3.50	24.00	168.00
1.0 - 1.5	24	1.000	0	0.000	3.00	24.00	144.00
1.5 - 2.0	24	1.000	2	0.083	2.50	23.00	120.00
2.0 - 2.5	22	0.917	1	0.045	2.20	21.50	97.00
2.5 - 3.0	21	0.875	0	0.000	1.80	21.00	75.50
3.0 - 3.5	21	0.875	4	0.190	1.30	19.00	54.50
3.5 - 4.0	17	0.708	4	0.235	1.04	15.00	35.50
4.0 - 4.5	13	0.542	4	0.308	0.79	11.00	20.50
4.5 - 5.0	9	0.375	6	0.667	0.53	6.00	9.50
5.0 - 5.5	3	0.125	1	0.333	0.58	2.50	3.50
5.5 - 6.0	2	0.083	2	1.000	0.25	1.00	1.00
6.0 - 6.5	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 64

12 HOUR LIGHT/DARK CONTROL

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	4.56	24.00	219.00
0.5 - 1.0	24	1.000	0	0.000	4.06	24.00	195.00
1.0 - 1.5	24	1.000	0	0.000	3.56	24.00	171.00
1.5 - 2.0	24	1.000	0	0.000	3.06	24.00	147.00
2.0 - 2.5	24	1.000	1	0.042	2.56	23.50	123.00
2.5 - 3.0	23	0.958	2	0.087	2.16	22.00	99.50
3.0 - 3.5	21	0.875	0	0.000	1.85	21.00	77.50
3.5 - 4.0	21	0.875	2	0.095	1.35	20.00	56.50
4.0 - 4.5	19	0.792	3	0.158	0.96	17.50	36.50
4.5 - 5.0	16	0.667	8	0.500	0.59	12.00	19.00
5.0 - 5.5	8	0.333	6	0.750	0.44	5.00	7.00
5.5 - 6.0	2	0.083	1	0.500	0.50	1.50	2.00
6.0 - 6.5	1	0.042	1	1.000	0.25	0.50	0.50
6.5 - 7.0	0	0.000	0	0.000	0.00	0.00	0.00

TABLE 65

CONTROL REARED IN CONTINUOUS LIGHT

x	nx	lx	dx	qx	ex	Lx	Tx
0.0 - 0.5	24	1.000	0	0.000	5.21	24.00	250.00
0.5 - 1.0	24	1.000	0	0.000	4.71	24.00	226.00
1.0 - 1.5	24	1.000	0	0.000	4.21	24.00	202.00
1.5 - 2.0	24	1.000	0	0.000	3.71	24.00	178.00
2.0 - 2.5	24	1.000	0	0.000	3.21	24.00	154.00
2.5 - 3.0	24	1.000	0	0.000	2.71	24.00	130.00
3.0 - 3.5	24	1.000	0	0.000	2.21	24.00	106.00
3.5 - 4.0	24	1.000	1	0.042	1.71	23.50	82.00
4.0 - 4.5	23	0.958	1	0.043	1.27	22.50	58.50
4.5 - 5.0	22	0.917	6	0.273	0.82	19.00	36.00
5.0 - 5.5	16	0.667	7	0.438	0.53	12.50	17.00
5.5 - 6.0	9	0.375	9	1.000	0.25	4.50	4.50
6.0 - 6.5	0	0.000	0	0.000	0.00	0.00	0.00

APPENDIX IV

Computer Program To Determine Statistical Significance

There are many features build into this program, written in the BASIC language, to determine the statistical significance. The user is initially asked, via the program, for upper and lower limits of the data so that the computer would check and give an audio and visual warning if too high or too low a value is accidentally inputted.

Equal or unequal sample size may be used for any of the groups. The number of groups and the sample size in each group are only limited by the amount of memory which are usable by the computer. However, a maximum of 10 groups with a maximum of 48 sample was used for the present study as the default value. This maximum default value may be altered by changing line 420 (max. size of group) and line 430 (max. sample size).

The following calculations are performed via the program and the user has the option to print the results onto a printer and placing a heading on top of the results.

1) Standard Deviation
2) Standard Error of Mean
3) Mean for Each Group
4) One Way ANOVA
5) Tukey Test

During the input of the data, typing the following characters will manipulate the data accordingly. Features I and II are normally set automatically at the very beginning but are used when unequal sizes are used.

I) "N" or "n" - (Next) Used to indicate end of present group. The program goes to the next group and awaits for data.

II) "E" or "e" - (End) No more data will be placed in, therefore start the calculation.

III) "H" or "h" - (Help) A wrong data was placed and it needs to be changed or to see every data that was placed in.

This program will also detect and print an appropriate error message on the screen if anything goes wrong with the machine.

```
1 '                    STATISTICAL PROGRAM
5 '
10 '    Masaaki Sawada - November 1982 (Assistance of
20 '                     Mr. Hiroyuki Sawada)
30 '
40 '          This section of the program asks the user for
50 '    the limits of the data.  The data, in turn, is
60 '    checked as to conform with the limits.  Once the
70 '    limits are set, and the user places the data, the
80 '    computer automatically performs the analysis.  The
90 '    user may over-ride the automatic feature by typing
100 '   "n" or "N" to go to the next group and/or "e" or
110 '   "E" to indicate the end of data input and the start
120 '   of the analysis.
130 '         If an error exists with the machine, the
140 '   program is directed to the "HELP" feature; starts
150 '   from line 10000 on.  The program also makes sure
160 '   that the data are only numerical values.  A warning
170 '   is sounded, and an appropriate message is indicated,
180 '   whenever anything goes wrong or to call attention to
190 '   the user.
200 '
210 '
220 CLS
230 input "print all values on screen?      (y/n);A$
240 IF A$="y" OR A$="Y" THEN FLAG=1:GOTO 270
250 IF A$="n" OR A$="N" THEN 270
260 BEEP:PRINT "type Y for YES or N for NO":GOTO 230
270 ON ERROR GOTO 10330
280 CLS
290 DEFINT A,L,N
300 DEFSNG B,C,D,G,M,Q,S
310 'MIN.INPUT=10
320 'MAX.INPUT=300
330 INPUT "What caption do you want";C$
340 INPUT "what value is too low as input";MIN.INPUT
350 INPUT "what value is too high as input";MAX.INPUT
360 IF MIN.INPUT<MAX.INPUT THEN 400
370 PRINT "the low value must be less than the high value"
380 PRINT "try again":BEEP
390 GOTO 340
400 INPUT "how many groups max.(ENTER only = 10)";MAX.G
```

```
410 INPUT "how many values/group (ENTER only = 48)";MAX.N
420 IF MAX.G=0 THEN MAX.G=10
430 IF MAX.N=0 THEN MAX.N=48
440 'MAX.G=10
450 'MAX.N=48     'changes with experim.
460 DIM GRP(MAX.G,MAX.N)
470 DIM N.PER.GRP(MAX.G)
480 DIM M.OF.GRP(MAX.G)
490 DIM GRPS.SUM(MAX.G)
500 G=G+1:N=0
510 N=N+1
520 IF N>MAX.N THEN G=G+1:N=1:BEEP
530 IF G>MAX.G THEN N.OF.GRPS=MAX.G:GOTO 830
540 PRINT "input group ";G;" # ";N
550 INPUT A$:IF A$="" THEN PRINT "no value",:GOTO 720
560 A=ASC(A$)
570 IF A=72 OR A=104 THEN N.OF.GRPS=G:GOTO 10000   'help
580 IF A=78 OR A=110 THEN BEEP:PRINT:GOTO 500 'next group
590 IF A=69 OR A=101 THEN 830                    'end
600 IF A<48 OR A>57 THEN PRINT "not number",:GOTO 720
610 N.PER.GRP(G)=N
620 IF G<>MAX.G-1 OR N<>1 THEN GOTO 640
630 BEEP:BEEP:PRINT "only 1 more group allowed"
640 IF N<>MAX.N-1 THEN GOTO 660
650 BEEP:BEEP:PRINT "only 1 more input for this group"
660 IF N<>MAX.N+1 THEN GOTO 690
670 G=G+1:N=1:BEEP:PRINT "grp: ";G;"no. ";N;
680 PRINT N.OF.GRPS,G
690 GRP(G,N)=VAL(A$)
700 IF GRP(G,N)>MIN.INPUT AND GRP(G,N)<MAX.INPUT THEN 510
710 PRINT "outside limits",
720 FOR A=1000 TO 3000 STEP 1000:SOUND A,1:NEXT
730 PRINT "try again"
740 GOTO 530
750 '
760 '
770 '
780 '        This section calculates the "Standard
790 '  Deviation" as well as the "Standard Error Of Mean".
800 '
810 '
820 '
830 PRINT SPC(40-(LEN(C$)/2)) C$
840 IF FLAG=1 THEN LPRINT SPC(40-(LEN(C$)/2)) C$
850 IF N=1 THEN G=G-1
860 N.OF.GRPS=G
870 FOR G=1 TO N.OF.GRPS
880   STEP2=0:STEP3=0
```

```
890    FOR N=1 TO N.PER.GRP(G)
900      STEP2=STEP2+GRP(G,N)
910      STEP3=STEP3+GRP(G,N)↑2
920    NEXT
930    STEP4=(STEP2↑2)/N.PER.GRP(G)
940    STEP5=STEP3-STEP4
950    STEP6=STEP5/(N.PER.GRP(G)-1)
960    S.D=SQR(STEP6)
970    PRINT:PRINT "S.D.is ";S.D;" ";
980    IF FLAG=1 THEN LPRINT:LPRINT "S.D. ";S.D;" ";
990    S.E.M=S.D/(SQR(N.PER.GRP(G)))
1000 ' PRINT "S.E.M. grp ";G;"is";S.E.M
1010   IF FLAG=1 THEN LPRINT "S.E.M. grp";G;"is";S.E.M
1020 NEXT
1030 '
1040 '
1050 '
1060 '        This section calculates the values for the
1070 '  ANOVA and, in turn, uses the values determined in
1080 '  the ANOVA calculation to perform the Post Hoc Tukey
1090 '  Test.  The mean for each of the group is also
1100 '  calculated in this section.
1110 '
1120 '
1130 INPUT "type <ENTER> to continue";A$
1140 'IF N=1 THEN N.OF.GRPS=N.OF.GRPS-1
1150 FOR G=1 TO N.OF.GRPS
1160   FOR N=1 TO N.PER.GRP(G)
1170     GRPS.SUM(G)=GRPS.SUM(G)+GRP(G,N)
1180     SQ.OF.GRPS.SUM=SQ.OF.GRPS.SUM+GRP(G,N)↑2
1190   NEXT
1200 NEXT
1210 '
1220 FOR G=1 TO N.OF.GRPS
1230   GRAND.TOTAL=GRAND.TOTAL+GRPS.SUM(G)
1240   SSB=SSB+GRPS.SUM(G)↑2/N.PER.GRP(G)
1250   MEASUREMENTS=MEASUREMENTS+N.PER.GRP(G)
1260 NEXT
1270 '
1280 CORRECTION.TERM=GRAND.TOTAL↑2/MEASUREMENTS
1290 SST=SQ.OF.GRPS.SUM-CORRECTION.TERM
1300 SSB=SSB-CORRECTION.TERM
1310 SSW=SST-SSB
1320 DF.FOR.SST=MEASUREMENTS-1
1330 DF.FOR.SSB=N.OF.GRPS-1
1340 DF.FOR.SSW=DF.FOR.SST-DF.FOR.SSB
1350 MST=SST/DF.FOR.SSW
1360 MSB=SSB/DF.FOR.SSB
```

```
1370 MSW=SSW/DF.FOR.SSW
1380 F=MSB/MSW
1390 PRINT "F",F
1400 IF FLAG=1 THEN LPRINT "F",F
1410 PRINT "MSw",MSW
1420 IF FLAG=1 THEN LPRINT "MSw",MSW
1430 PRINT "df.for.ssb",DF.FOR.SSB
1440 IF FLAG=1 THEN LPRINT "df.for.ssb",DF.FOR.SSB
1450 PRINT "df.for.ssw",DF.FOR.SSW
1460 IF FLAG=1 THEN LPRINT "df.for.ssw",DF.FOR.SSW
1470 '
1480 FOR G=1 TO N.OF.GRPS
1490   M.OF.GRP(G)=GRPS.SUM(G)/N.PER.GRP(G)
1500   PRINT "mean of group ";G;" is ";M.OF.GRP(G)
1510   IF FLAG<>1 THEN GOTO 1530
1520 LPRINT "mean of group ";G;" is ";M.OF.GRP(G)
1530 NEXT
1540 FOR G=1 TO N.OF.GRPS
1550   BAR.N=BAR.N+1/N.PER.GRP(G)
1560 NEXT
1570 BAR.N=N.OF.GRPS/BAR.N
1580 STAND.ERROR=SQR(MSW/BAR.N)
1590 PRINT "standard error of mean is",STAND.ERROR
1600 IF F<>1 THEN GOTO 1620
1610 LPRINT "standard error of mean is ",STAND.ERROR
1620 INPUT "input q ",Q
1630 IF FLAG=1 THEN LPRINT "the Q used is ";Q
1640 C.DIFF=Q*STAND.ERROR
1650 PRINT "c.diff is ";C.DIFF
1660 IF FLAG=1 THEN LPRINT "c.diff is ";C.DIFF
1670 FOR G=1 TO N.OF.GRPS:PRINT M.OF.GRP(G):NEXT
1680 PRINT "input any key to continue"
1690 IF F<>1 THEN GOTO 1710
1700 FOR G=1 TO N.OF.GRPS:LPRINT M.OF.GRP(G):NEXT
1710 A$=INKEY$:IF A$="" THEN 1710
1720 A=0
1730 FOR G=1 TO N.OF.GRPS-1
1740   FOR GR=G+1 TO N.OF.GRPS
1750     BUN=ABS(M.OF.GRP(G)-M.OF.GRP(GR))
1760     IF BUN<=C.DIFF THEN 1810
1770     PRINT "group ";G;" vs group ";GR
1780     PRINT "    ";BUN;"    "
1790     IF FLAG=1 THEN LPRINT "group ";G;" vs group ";GR
1800     IF FLAG=1 THEN LPRINT "    ";BUN;"    "
1810     A=A+1
1820     IF A<>8 THEN 1850
1830       A=0
1840       INPUT "type <ENTER> to continue",A$
```

```
1850   NEXT
1860 NEXT
1870 INPUT "try new q value? (y/n)";A$
1880 IF A$="y" OR A$="Y" THEN 1620
1890 INPUT "Do you want to do more (Y/N)";A$
1900 IF (A$="Y") OR (A$="y") THEN CLEAR: GOTO 220
1910 STOP
1920 '
1930 '
1940 '
1950 '        This is the."HELP" feature of the program.
1960 '  It permits the user to see all the inputted data,
1970 '  as well as to change any of the data.  The
1980 '  correction of data are invoked by typing "h" at any
1990 '  point during input or the correction of the data.
2000 '        The function and the status of the computer
2010 '  is checked.  If any error is detected,  an
2020 '  appropriate error message will be shown and the
2030 '  computer will stop operation.
2040 '
2050 '
10000 CLS
10010 PRINT SPC(10) "MENU"
10020 PRINT:PRINT "to go back, type      B"
10030 PRINT "to see a group        S"
10040 PRINT "to change one         C"
10050 A$=INKEY$:IF A$="" THEN 10050
10060 A=ASC(A$):A=A AND &HDF
10070 IF A=66 THEN 540
10080 IF A<>83 THEN 10220          'see
10090   PRINT:PRINT "see"
10100   INPUT "group # ";GR
10110   IF GR<1 OR GR>N.OF.GRPS THEN 10100
10120     A=0
10130     FOR NU=1 TO N.PER.GRP(GR)
10140       PRINT "group ";GR;" # ";NU;" : ";GRP(GR,NU)
10150       A=A+1
10160       IF A<>20 THEN 10190
10170         A=0
10180         INPUT "type <ENTER> to continue",A$
10190     NEXT
10200 INPUT "type <ENTER> to go back to menu",A$
10210 GOTO 10000
10220 IF A<>67 THEN 10340
10230 PRINT:PRINT "change value"
10240 INPUT "type group and #",GR,NU 'change grp
10250 IF GR<1 OR NU<1 THEN 10240
10260 IF GR<=N.OF.GRPS THEN GOTO 10280
```

```
10270 BEEP:PRINT:PRINT "does not exist":GOTO 10240
10280 IF NU>N.PER.GRP(GR) THEN GOTO 10270
10290 PRINT "enter value or <ENTER> if change not needed"
10300 PRINT "group";GR;" # ";NU;"was";GRP(GR,NU)
10310 INPUT ;A$
10320 IF A$<>"" THEN GRP(GR,NU)=VAL(A$)
10330 GOTO 10000
10340 GOTO 10000          'future expansion
10350 IF ERR=6 THEN PRINT "overflow"
10360 IF ERR=7 THEN PRINT "out of mem"
10370 IF ERR=11 THEN PRINT "division by zero"
10380 IF ERR<>17 THEN GOTO 10400
10390 PRINT "program was modified, cannot continue"
10400 IF ERR=72 THEN PRINT "diskette is useless,get help"
10410 IF ERR=71 THEN PRINT "no diskette"
10420 IF ERR=70 THEN PRINT "write is not allowed"
10430 IF ERR=67 THEN PRINT "directory is full"
10440 IF ERR=61 THEN PRINT "disk is full"
10450 IF ERR=57 THEN PRINT "input/output error"
10460 IF ERR<>51 THEN GOTO 10480
10470 BEEP:PRINT "machine is malfunctioning, call dealer"
10480 IF ERR<>27 THEN 10570
10490 PRINT "  Out of paper"
10500 PRINT "add more and type ENTER"
10510 PRINT "to continue printing"
10520 PRINT "  To stop printing, type the
10530 PRINT "number "1", "2" to start it"
10540 PRINT "typed, printing stops"
10550 INPUT A$:IF A$="1" THEN FLAG=0:IF A$="2" THEN FLAG=1
10560 RESUME
10570 IF ERR=24 THEN PRINT "printer off":GOTO 10510
10580 PRINT ERR,ERL
10590 SOUND 2000,20:IF ERR>19 THEN RESUME ELSE STOP
```

Sample Data Input And Program Output For Statistical Program

Since the data for any experiment in the present study was long, the following sample data were used to show the program output. The "q" value requested by the program comes from the standard "Significant Studentized Range" table. Input is required after a "?".

Sample Data

Group 1	Group 2	Group 3
3.21	3.77	4.72
3.73	3.58	5.20
3.91	3.87	5.10
3.83	-	5.33

Output

```
What caption do you want?  This Is A Sample Run
what value is too low as input?  2
what value is too high as input?  6
how many groups max.(ENTER only = 10)? 3
how many values/group (ENTER only = 48)? 4
input group  1  #  1
? 3.21
input group  1  #  2
? 3.73
input group  1  #  3
? 3.91
only 1 more input for this group
input group  1  #  4
? 3.83
input group  2  #  1
? 3.77
only 1 more group allowed
input group  2  #  2
? 3.58
input group  2  #  3
? 3.87
only 1 more input for this group
input group  2  #  4
? n
input group  3  #  1
? 4.72
input group  3  #  2
? 5.20
```

```
input group 3  #  3
? 5.10
only 1 more input for this group
input group  3  #  4
? 5.33
                        This Is A Sample Run
S.D.is  .3153818  S.E.M. grp 1 is .1576909

S.D.is  .1473154  S.E.M. grp 2 is 8.505255E-02

S.D.is  .2624708  S.E.M. grp 3 is .1312354
type  ENTER  to continue?
F               35.79947
MSw             6.855965E-02
df.for.ssb      2
df.for.ssw      8
mean of group  1  is  3.67
mean of group  2  is  3.74
mean of group  3  is  5.0875
standard error of mean is     .1380013
input q? 3.26
the Q used is 3.26
c.diff is  .4498841
input any key to continue
group  1  vs group  3
    1.4175
group  2  vs group  3
    1.3475
try new q value? (y/n)?
```

The "q" value used was for alpha = 0.05. Therefore group 1 vs group 3 and group 2 vs group 3 were found to be significant at alpha = 0.05.

Computer Program to Determine Life Table Values

This program is written in the BASIC language. All the life table data, as seen in appendix III, were obtained with this program. The only data required are the number of individuals alive per given age interval. The age intervals are set for every half day but may be altered for any age interval.

```
10 '                    LIFE TABLE PROGRAM
20 '
30 '    Masaaki Sawada - January 1983 (Assistance of
40 '                     Mr. Hiroyuki Sawada)
50 '
60 '        This program is used to determine all the life
70 '   table values.  Since the data are not large, there
80 '   are no error correction capabilities.
90 '
100 '
110 DEF SEG=&HB800
120 DEFSNG A-T
140 X$="day "
150 INPUT "what caption do you want";B$
160 MIN=1     :U=0 'print/noprint
170 MAX=150
180 ON ERROR GOTO 0
190 GOTO 240
200 'on a goto
210 ON A GOTO 10
220 'sections
230 ON A GOTO 10
240 WIDTH 80
250 SCREEN 2
260 INPUT "print on paper (Y/N)";A$
270 IF (A$="Y") OR (A$="y") THEN U=1
280 FOR Z=0 TO 20:ZZ=Z/2
290 PRINT "number alive on ";X$;ZZ;"-";ZZ+.5;
300 INPUT N(Z)
310 IF N(Z)<=MAX THEN 340
320  BEEP:PRINT "too big, try a smaller no."
330 GOTO 290
340 IF N(Z)=INT(N(Z)) THEN 370
350  BEEP:PRINT "you can't have a fraction of a creature"
360 GOTO 290
370 IF N(Z)<MIN THEN X=Z:GOTO 390
380 NEXT
390 '
```

```
400 IF X>2 THEN 450
410  PRINT "you can't do anything with less than 2"
420  BEEP:BEEP:BEEP
430  PRINT "start all over again"
440  GOTO 280
450 '
460 '
470 '
480 '        This section is the computational part of the
490 '  program.
500 '
510 '
520 FOR Z=0 TO X-1
530  D(Z)=N(Z)-N(Z+1)
540  Q(Z)=D(Z)/N(Z)
550 LL(Z)=(N(Z)+N(Z+1))/2
560 NEXT
570 '
580 FOR Z=0 TO X
590  L(Z)=N(Z)/N(0)
600 NEXT
610 '
620 FOR Z=0 TO X
630  FOR Y=Z TO X
640   T(Z)=LL(Y)+T(Z)
650  NEXT
660 NEXT
670 FOR Z=0 TO X-1
680  E(Z)=T(Z)*.5/N(Z) '0.5 because age interval is 1/2
690 NEXT
710 '
720 '
730 '         This section controls the format of the life
740 '  table values.
750 '
760 '
770 CLS
780 PRINT SPC(40-(LEN(B$)/2)) B$
790 IF U=1 THEN LPRINT SPC(40-(LEN(B$)/2)) B$
800 PRINT STRING$(79,45)
810 IF U=1 THEN LPRINT STRING$(79,45)
820 PRINT"       x         nx       lx       dx       qx";
830 PRINT"     ex       Lx         Tx"
840 IF U=1 THEN LPRINT"       x         nx       lx       ";
850 IF U=1 THEN LPRINT" dx       qx         ex       ";
860 IF U=1 THEN LPRINT"Lx          Tx"
870 PRINT STRING$(79,45)
880 IF U=1 THEN LPRINT STRING$(79,45).
```

```
890 FOR Z=0 TO X
900  PRINT USING "####.#";Z/2;
910 PRINT " -";
920  PRINT USING "##.#";Z/2+.5;
930 IF U=1 THEN LPRINT USING"####.#";Z/2;
940 IF U=1 THEN LPRINT " -";
950 IF U=1 THEN LPRINT USING"##.#";Z/2+.5;
960  PRINT USING "#######";N(Z);
970  IF U=1 THEN LPRINT USING "#######";N(Z);
980  PRINT USING "######.###";L(Z);
990  IF U=1 THEN LPRINT USING "######.###";L(Z);
1000  PRINT USING "#######";D(Z);
1010  IF U=1 THEN LPRINT USING "#######";D(Z);
1020  PRINT USING "######.###";Q(Z);
1030  IF U=1 THEN LPRINT USING "######.###";Q(Z);
1040  PRINT USING "######.##";E(Z);
1050  IF U=1 THEN LPRINT USING "######.##";E(Z);
1060  PRINT USING "#######.##";LL(Z);
1070  IF U=1 THEN LPRINT USING "#######.##";LL(Z);
1080  PRINT USING "########.##";T(Z)
1090  IF U=1 THEN LPRINT USING "########.##";T(Z)
1100 NEXT
1110 INPUT "do you want to do more life tables (Y/N)";C$
1120 IF (C$="Y") OR (C$="y") THEN CLEAR:GOTO 130
```

Sample Data Input and Program Output For Life Tables

The data for this example comes from the 4800 J/m^2 U.V. dose group. The output is similar to table 58 therefore the program output is not shown in this section.

Example

```
what caption do you want? 4800 J/m^2
print on paper (Y/N)? n
number alive on day  0 - .5 ? 30
number alive on day  .5 - 1 ? 30
number alive on day  1 - 1.5 ? 30
number alive on day  1.5 - 2 ? 14
number alive on day  2 - 2.5 ? 4
number alive on day  2.5 - 3 ? 1
number alive on day  3 - 3.5 ? 0
```

APPENDIX V

Photomicrographs

Zeiss-Ultraphot II Phase Contrast Microscope with the Zeiss Ikon automatic photomicrographic camera attachment was used to take the photomicrographs. Kodak Panatomic-X black and white; ASA 32, and Kodacolor II color; ASA 100, film were used.

PLATE 1. A 2 1/2 day old A. brightwelli (1 cm = 70 um). Ingestion of Paramecia stained in 0.75 ug/ml of neutral red induces the red coloration in the gut.

PLATE 2. Two 2 1/2 day old A. brightwelli (1 cm = 70 um). The rotifer on the left ingested regular Paramecia while the one on the right ingested Paramecia stained in 0.75 ug/ml of neutral red.

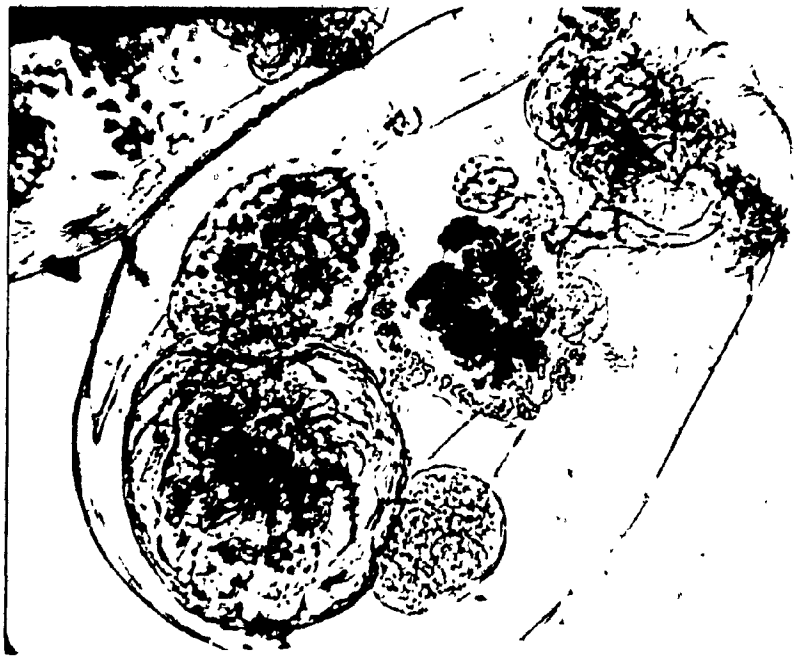

PLATE 3. A 6 day old A. brightwelli (1 cm = 70 um).

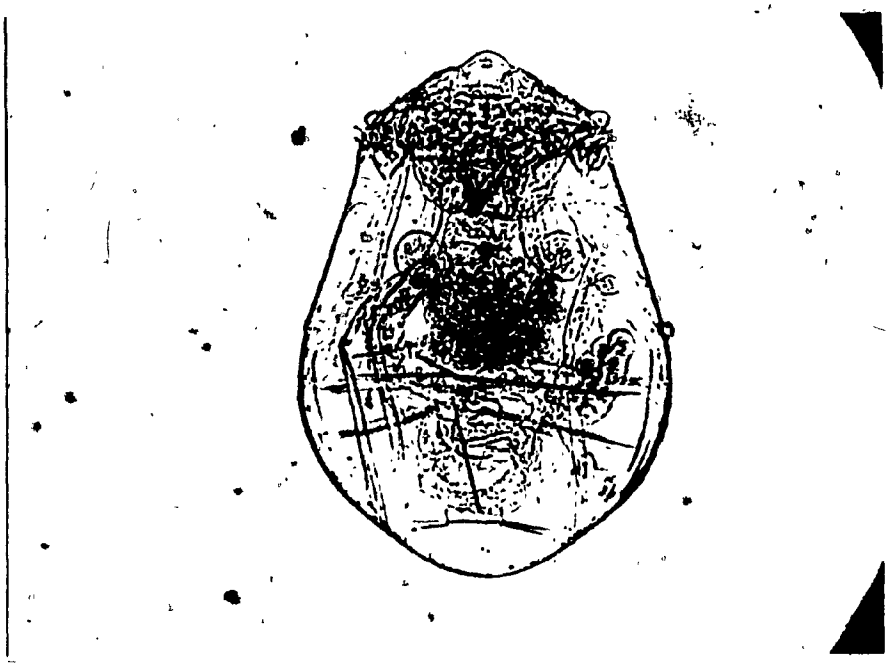

PLATE 4. A 1/2 day old A. brightwelli (1 cm = 70 um).

PLATE 5. A 4 day old A. brightwelli showing its ovoviviparous birth of an offspring (1 cm = 80 um).