AN *IN VIVO* STUDY OF THE EFFECTS OF α-TOCOPHEROL ON THE FREE-LIVING NEMATODE *TURBATRIX ACETI*

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ABSTRACT

AN *IN VIVO* STUDY OF THE EFFECTS OF α-TOCOPHEROL ON THE FREE-LIVING NEMATODE *TURBATRIX ACETI*

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The effects of dietary dl-α-tocopherol were studied in an *in vivo* system, using *Turbatrix aceti* because of its relative simplicity. The experiments tested the effects of α-tocopherol on maturation, growth, reproduction and senescence. Three generations were examined in order to determine the developmental stages at which α-tocopherol affects maturation, growth and reproduction.

α-Tocopherol's effects on senescence were studied firstly by comparing reproductive capacity and survivorship of senescing nematodes cultured in control and α-tocopherol media, and secondly, by observing the survivorship of control and α-tocopherol nematodes whose life spans had been affected by various dosages of gamma irradiation. The interaction of α-tocopherol with some of the nutrients essential for optimum development of *Turbatrix aceti* was also studied.

It was found that the addition of α-tocopherol increases the reproductive capacity of this nematode by ensuring optimum development and maturation. α-Tocopherol

also significantly increased the survivorship of both irradiated and non-irradiated nematodes. The results could be explained either as a direct effect of the presence of α-tocopherol or as the result of increased availability of nutrients caused by the presence of α-tocopherol.

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INTRODUCTION

The research that will be reported on examined the processes of maturation and aging in the nematode Turbatrix aceti. In particular, the influence of α-tocopherol (vitamin E) on these processes was examined. To introduce this topic the background of research on maturation and aging in the nematode will be examined, and then the role of vitamin E in biological systems will be reviewed.

Free-living nematodes have in recent years become very popular as models for the examination of complex biological processes. Many investigators believe that the basic mechanisms involved in most biological processes may be similar in most animals (Zuckerman 1976). Free-living nematodes are presently being used as models in many research areas, such as behaviour, genetics, development, nutrition, biochemistry and aging. Croll (1976), and Duggal (1978), among other investigators, have analyzed the behaviour of several species of nematodes. Genetic studies include those of Beguet (1972) and Beguet and Brun (1972). A great deal of elaborate work in genetics has also been done by Brenner and his co-workers (1974).

A number of free-living nematodes are especially advantageous for studies in development and embryology, due to their great simplicity and favourable genetic characteristics. Classical studies established the strictly determinate cleavage of nematode eggs and led to the concept of cell linage (Boveri, 1899). Other classical observations of nematode embryology have been thoroughly reviewed by Chitwood and Chitwood (1974). These investigators revealed that in these organisms development follows a rigidly fixed program.

In 1928, Pai used the species Turbatrix aceti to study general processes of development. Honda (1925) did cytological studies on bisexual and hermaphroditic free-living nematodes. Laufer (1980) recently reported on a detailed embryological study on Caenorhabditis elegans.

The free-living nematode C. elegans is one of the most commonly used species. It is particlularly suitable for genetic studies because of its ease of culture, well-studied life cycle, short life span and normally hermaphroditic mode of reproduction. A large number of mutant strains have been isolated (Brenner, 1974; Epstein et al., 1974; Vanderslice, 1976) exhibiting a wide range of morphological and behavioural modifications. A well characterized gonadogenesis has also been

described for a number of species (Honda, 1925; Hirsh et al., 1976), but little has been reported on the species Turbatrix aceti. Pasternak and Samoiloff (1971) and Anya (1976), have studied various factprs which influence spermatogenesis in a number of nematodes.

A great deal of work has been done in the area of nutrition, where investigators have used nematode maturation and fertility to evaluate the importance of certain nutrients (Dougherty et al., 1959; Sayre et al., 1963; Bolla,1979). The popularity or free-living nematodes in biology led to a great number of investigations toward the development of axenic culture methods. Turbatrix aceti was one of the first free-living nematodes grown axenically, in the absence of any other free-living forms (Zimmerman, 1921). As studies involving nematodes became more complex, the need for a chemically-defined medium arose. In the past decade, a number of investigators have worked on the production of a totally chemically-defined medium. To date, investigators have only been successful in developing media in which all the components are commercially available, with the exception of a proteinaceous growth factor, which is necesary for reproduction (Buecher et al., 1966; Vanflateren,

1974). In spite of the advantages of axenic cultivation, a number of investigators have felt that growth is more normal under xenic conditions and that nematodes in axenic culture suffer from nutritional deficiency (Sulston et al., 1974). This is probably true since a perfect axenic media has not yet been developed.

A number of studies have been done to test various axenic media for the cultivation of free-living nematodes. Satisfactory media for the species C. briggsae were reported by Dougherty et al. (1959), Buecher et al. (1966), Sayre et al. (1963). Rothstein and Cook (1966) investigated the conditions for routine axenic cultures of three species of free-living nematodes, namely, R. anomala, T. aceti and P. revividus. Several species of nematodes have been cultured serially in axenic media (Hansen & Cryan, 1966). However, the chemically-defined medium in current use (CbMM) (Buecher, Hansen & Yarwood, 1966), does not support continuous growth in a number of nematode species, unless supplemented with a growth factor. In spite of the numerous studies, some controversy still remains as to the optimum axenic medium for the cultivation of Turbatrix aceti.

Heated liver extract among other proteinaceous

factors has been shown to support growth and maturation for several generations in commonly used (Sayre et al., 1963). Buecher and his co-workers (1970) showed an association of biological activity and precipitate formation, and attempted to substitute the proteinacious portion of the growth factor with several commercially available proteins. A number of investigators believe that the essential growth factor consists principally of organic materials which might provide nutrients in the proper particulate form (Vanfleteren, 1974; Buecher et al., 1970). Vanfleteren (1976) showed that several purified heme proteins were highly active when properly precipitated and added to media containing sterols. Iron-building proteins, such as ferritin haemin and transferrin haemin, have also shown growth promoting activities when allowed to interfere with heamin chloride and form a precipitate. Less specific iron-building materials, like bovine serum albumin and egg albumin, only permit limited development, with longer generation time. The make-up of the growth factor has been much studied. This essential factor is thought to consist of three distinct components: sterols (Rothstein, 1968), a heme moeity (Hieb et al., 1970) and a proteinaciuos fraction (Sayre et al., 1961).

Attempts to biochemically identify and purify the essential factor have proven unsuccessful so far.

Free-living nematodes have proven to be very useful in aging research. Because nematode aging will also be looked at in this study, we will review some of the work done in this field. As early as 1927, Peters, using the light microscope, noticed that young nematodes of the species Turbatrix aceti had intestines of narrow, even width whereas those of older nematodes were often distended. In 1928, Pai reported differences between the reproductive and digestive organs of young and old nematodes. He also noticed that movement slowed with age. In neither of these studies were the ages of the nematodes reported.

Zuckerman in a review (1976) has defined a number of parameters which characterize senescence in free-living nematodes, based on his own studies, and those of other investigators. C. briggsae was found to undergo changes in osmotic fragility, specific gravity and membrane surface charge similar to those found in red blood cells during senescence (Zuckerman et al.; 1971, 1972). According to Kisiel et al. (1975) virgin nematodes of the species Turbatrix aceti do not undergo age-related changes in osmotic fragility and

specific gravity, although these investigators did find some age-related changes in non-virgin females. Two hypotheses were proposed on the basis of the similarities between these nematodes and aging red blood cells. One attributes the changes to intracellular concentration gradiants, and the other attributes them to changes in membrane premeability.

The first behavioural age-related changes in Turbatrix aceti were noticed by Pai (1928). Zuckerman et al. (1971) showed a progressive decline in movement with age. A number of investigators have found a decrease in fecundity with age in C. briggsae (Zuckerman et al., 1974); Turbatrix aceti (Kisiel, 1974) and C. elegans (Beguet & Brun , 1972; Croll, 1977). Beguet and Brun (1972) and Beguet (1972) investigated the effects of parental senescence on succeeding generations in C. elegans. These investigators observed that the fecundity of offspring of old parents was lower than that of offspring of young parents, and that this effect lasted for four generations. They also observed that the progeny of older parents had earlier sexual maturation, fewer spermatozoa produced in the F_1 generation, and increased mortality of F_2 eggs than did progeny of young parents. The mechanisms responsible for these

effects were not clear, but it was proposed by these and other investigators that the factor responsible for the reduced fecundity was in the cytoplasm of the maternal oocyte. Two mechanisms were proposed to explain this cytoplasmic alteration in old parents: increased DNA in cellular organelles, and changes in enzyme activities with age. Similar studies have been carried out with other organisms. The difficulty in clarifying these mechanisms probably lies in the difficulty in differentiating between genetic and environmental influences.

A great deal of work has been done on the morphological changes that occur with age. Subcelluar changes have been seen with the electron microscope in C. briggsae, C. elegans and T. aceti (Zuckerman et al., 1971; Epstein et al., 1972; Himmelhoch, 1973; Kisiel et al., 1975). Morphological changes observed include alterations in the structural integrity of cellular organelles with age (Zuckerman et al., 1971). Kisiel et al. (1975) found mitochondria and lipid droplets encroached between the terminal webs and the bacillary layer in T. aceti. Zuckerman (1976) suggested that such disruptions could interfere with food absorption, thus producing death.

Accumulation of lipofuscin (aging pigment) is an aging parameter commonly used to evaluate senescence in nematodes. This pigment is believed to be derived from the peroxidation of lipids and proteins (Fletcher et al., 1973). Lipofuscin accumulation has been described in C. briggsae (Kisiel, 1974) and to a lesser extent in T. aceti (Kisiel, 1975) on the basis of electron microscope evidence. Research on age pigment accumulation in nematodes was initiated by Buecher and Hansen (1974). These investigators used evidence obtained from spectrofluorometric studies. Quantitative accumulation of lipofuscin with age in a synchronized population has not yet been shown in nematodes.

The importance of understanding biological processes at the molecular level has led a great number of investigators toward biochemical studies. However, a problem which has been encountered by researchers in this field, and which has not yet been solved, is obtaining an age-synchronous population of nematodes large enough for proper biochemical investigation. Aging studies similar to those mentioned so far can be made on individual nematodes

or on small numbers, but valid biochemical studies require large masses of nematodes. Although a number of investigators have attempted to synchronize large masses of nematodes, a totally successful method, without the use of chemicals or abnormal growing conditions, has not yet been reported. Studies such as those designed to determine enzyme activity and protein synthesis in nematodes have been faced with this problem. A number of studies have been reported in which synchronization was achieved with the aid of chemical compounds (DNA synthesis inhibitors).

Rothstein et al. (1974), observed a linear drop with age in the specific activities of a number of enzymes in the species Turbatrix aceti. Bolla and Brot (1975) observed a decline in specific activities of both DNA polymerase and aldolase and an increase in RNA polymerase in aged Turbatrix aceti. Age-related changes were also found in tRNA for arginine and tyrosine (Reitz & Sanadi, 1972). These and other observations of changes in protein accumulation in aging nematodes (Reznik & Gershon, 1979; Prasanna, 1979) have encouraged a number of investigators to persue further aging studies using free-living nematodes. Gershon and his co-workers have recently reviewed a number

of aging theories in light of the results obtained from aging studies performed on nematodes.

A great number of investigations on the accumulation of altered molecules in senescent organisms were inspired by Orgel's theory of "error catastrophe" (1963). Understood in this theory is that miscoding is the main mechanism that leads to the accumulation of altered proteins with age. Based on studies done on eutelic nematodes, Reznick and Gershon (1979) and Gershon (1979) have postulated that the mechanism responsible for the accumulation of aberrant proteins is post-translational, and can be attributed to a defect in the degradation system which develops with age. It should be noted that biochemical studies on nematodes involve populations synchronized with the aid of chemical compounds such as DNA synthesis inhibitors. The influence of such compounds on the results obtained in aging studies is not clear (Zuckerman, 1976).

Suitability of Turbatrix Aceti for this Study

It would be useful at this point to briefly describe the species used in this study and mention the characteristics which recommend it for this purpose. Pai (1927, 1928) observed that maturation

started on day seventeen after birth and lasted for twenty-nine days at 21°C. Females had an average of seventy-two offspring. A life span of forty-five days in females and forty-eight in males was observed. Most studies were carried out under xenic conditions, until Rothstein and Cook's study in 1966 showed growth and reproduction in axenic media at 30°C.

Several recent reports have been published on the fecundity, duration of the reproductive period, longevity and senescence of Turbatrix aceti (Zuckerman, 1974; Kisiel et al., 1975). Aside from the general advantages which Turbatrix aceti shares with other species of free-living nematodes, such as ease of maintenance, availability of information and brevity of life span, fixed cell number, simple and well-differentiated cell systems, it also has a transparent cuticle which allows a view of internal organs under the light microscope until fairly late in life. In addition to these advantages the low pH at which this species is maintained proved useful in preventing bacterial contamination.

Biological Role of Vitamin E

Vitamin E was discovered when male and female rats failed to reproduce normally on diets which turned out to be deficient in Vitamin E. In spite of the large

number of investigations that have used reproductive processes to elucidate the mode of action of vitamin E, its first recognized function as an anti-sterility factor for the laboratory rat has been overshadowed by its demonstrated function in maintaining the structural and functional integrity of muscle, membranes, and, in some animals, peripheral vascular systems. The activitiy of α-tocopherol has been classified by Boguth (1969) into two main areas: effects attributable to the hydroxy functions of the molecule, and effects brought about by metabolites of α-tocopherol.

Many workers have demonstrated that disease symptoms caused by vitamin E-deficiency can be cured or prevented by a number of antioxidants. As a result, it has been suggested by these investigators that the sole function of α-tocopherol in biological systems is that of a lipid antioxidant, preventing the formation of peroxides and products of their subsequent degradation from highly unsaturated lipids (Dam, 1957). This theory has caused much controversy. The weight of evidence at present supports the theory that antioxidants other than α-tocopherol can substitute for vitamin E, but that the effectiveness of such substitution is dependent on the structure of the particular compound (McCay, 1980). Tappel (1962)

regards all manifestations of vitamin E-deficiency as secondary to in vivo damage of sensitive cell structures produced by lipid peroxidation. Green and his co-workers (1962) have opposed this theory, because some of the unusual functions of vitamin E cannot be related to its antioxidant properties. There have been a number of theories advanced about the antioxidant effects of α-tocopherol. In particular, the possible role of α-tocopherol in cellular metabolism has led to a great deal of research in this area. One hypothesis on the mode of action of α-tocopherol is that it is involved in a specific, although thus far undefined, regulatory role (Wasserman, 1974; Olson, 1974; Green, 1970).

A number of theories have been proposed about the regulatory role of vitamin E in the metabolism of nucleic acids and proteins. The work by Dinning and his co-workers (1957, 1962) has been among those studies most cited. These investigators found an increase in DNA content in vitamin E-deficient rabbit muscle and bone marrow in monkeys, and attributed these findings to an acceleration of DNA synthesis. It has recently been suggested by Bierri and Farrell (1976) that the marked accelaration of DNA synthesis is the result of infiltration by leukocytes and macrophages.

Further studies on DNA synthesis in bone marrow revealed that the increased rate of DNA synthesis could be attributed to an increase in erythropoiesis (Porter et al., 1962). A number of studies on the effects of vitamin E-deficiency on accumulation of RNA have been reported. Olson (1974) has demonstrated an increase in ribosomal RNA in vitamin E-deficient rabbit muscle.

The observed changes in activity in a number of enzyme systems in vitamin E-deficient animals have led a number of investigators to persue studies in this field. A large number of enzymes undergo changes in activity in vitamin E-deficient tissue. The most frequently studied ones have been creatine kinase, and liver xanthine oxidase (Olson, 1974; Catignani, 1974).

Olson (1967) postulated that vitamin E plays a role in the synthesis of proteins. Catignani (1980) has recently reviewed this theory. Studies on the increase in the rate of protein synthesis in vitamin E-deficient animals have shown conflicting evidence about the rates of amino acid incorporation and changes in polysome profiles (Devillers et al., 1973; Olson, 1974). In addition, Catignani (1980) has recently shown evidence for a protein obtained from rat liver supernatant which is capable of binding with tocopherol.

The development of nutritional anemia in vitamin E-deficient animals has led a number of investigators to postulate that vitamin E may have a role in heme synthesis, or in the biosynthesis of heme proteins. Nair and his co-workers (1972) believe that the effect of vitamin E is directly on the biosynthetic pathway controlling the mechanism of the induction and repression of two key enzymes: δALA synthetase and δ-ALA-dehydratase. More recently, however, some evidence against this theory has appeared (Barttlet et al., 1974).

As mentioned above, some of the earliest evidence on the effects of vitamin E in animals has been that of its role in reproduction. There is little convincing evidence of a specific role that controls reproduction. The large amount of evidence on the possible effect of α-tocopherol on protein metabolism has led a number of investigators to suggest that this is due to a failure to understand the mechanism of hormone action at the molecular level. Recent biochemical advances have permitted closer analysis of the inter-relation between a wide variety of hormones and vitamins.

Kitabchi (1980), has recently reviewed the hormonal status of vitamin E-deficiency. The

description by Mason (1954) of testicular degeneration in rats associated the inhibition of spermatogenic activity with nuclear chromolysis of spermatids and secondary spermatocytes, followed by nuclear and cytoplasmic degeneration in the primary spermatocytes and spermatogonia. These investigators found no signs of degeneration of the accessory sex glands. In female rats, estrous, ovulation and implantation processes were normal, and morphological changes occurred in the ovary or uterus. The well-documented resorption of the fetus *in utero* appears to occur because of suppression of hematopoietic development in the yolk sac, allontois, fetal placenta and liver blood vascular system of the fetus and fetal membrane. Mason (1954) attributed this abnormality to asphyxia, starvation leading to coagulation, and necrosis of the fetus.

Kitabchi (1980), suggested that estrous, ovulation, and implantation in the female rats, as well as hormonal control of the accessory sex gland in the male, are unaffected by vitamin E-deficiency. This suggestion may or may not be in accordance with Blandau *et al.* (1949), who suggested that prolonged depletion of vitamin E beyond the first month of reproductive life leads to decreased fecundity due to a uterine,

rather than an ovarian disorder.

Some of the controversy on this subject arose from discrepancies between the results obtained on different species. Studies on closely related species, like the mouse and hamster , have shown inconsistent results with respect to the effects of vitamin E-deficiency on reproduction (Bryan & Mason, 1940). Kitabchi concluded from his and other investigators' findings, that in adrenal tissues, tocopherol appears to have two functions. One appears to be correlated to lipid peroxidation, stimulating steroid hydrolases systems of cellular organelles, and the second one (not related to peroxidation), appears to be involved in modulation of the membrane bound ACTH-stimulated enzyme, adenylate cyclase.

Even the well-documented theory of the effect of α-tocopherol in controlling membrane stability (Lucy & Diplok, 1972) can probably be explained in terms of a relationship between α-tocopherol and proteins. Lucy et al. (1972) have suggested that cell membranes have increased permeability in vitamin E-deficient tissues, and reduced permeability in α-tocopherol supplemented cells. Proteins, as well as lipids, are important components of membranes. Molenaar (1972, 1980) proposed a theory of the function of vitamin E

in relation to the integrity of cellular membranes. He proposed that in vitamin E-deficiency two factors contribute to the pathological symptoms. One is the molecular interactions in which the missing tocopherol would normally take part. The other is the membrane itself, which has an altered function due to the missing tocopherol molecule. According to Molenaar (1980), most of the proposed theories on how vitamin E contributes to the function of the cell can probably be explained in these terms. Furthermore, he suggested that changes in membrane structure and function are dependent on the structure of the specific membrane, thus accounting for the variation in pathological lesions among tissues and species.

Tocopherols have been found to affect several mitochondrial and microsomal functions (Corwin, 1965). Many of them are related to the ability to oxidize. Corwin (1980) has recently reviewed some of the evidence, and has concluded that vitamin E seems to affect the early part of the electron transport chain. He postulated an interaction of α-tocopherol with sulphydryl groups of the dehydrogenases, once again indicating a possible interaction of vitamin E with protein molecules.

Having reviewed some of the theories and research carried out to elucidate how vitamin E contributes to the function of the cell, it remains to examin some of the reasons that have led to the controversy in this field. Inconsistency among experimental results on the mode of action of α-tocopherol can probably be attributed to discrepancies in four important factors: absorption, transport, deposition and storage. These factors are governed by variables in the experimental procedure, making it difficult to correlate and extrapolate findings. The method used for the administration of this vitamin may play an important role in determining the results obtained. In in vivo systems, particularly in higher animals, the method of administration is of prime importance. When α-tocopherol is injected, the solvent, or carrier, plays a major role in the transport, deposition and absorption of this molecule. For example, the recovery of labelled α-tocopherol in lymph, bile and feces varied when different carriers were used as injection vehicles (MacMahon & Thompson, 1970).

The dietary status of the organism of cell in question has a great deal of influence on the observation of the effects of α-tocopherol. The interpretation of the effects of α-tocopherol has

been shown to be complicated by a number of substances present in the diet of most animals. The effects of polyunsaturated fatty acids (PUFA) in increasing vitamin E requirements are well documented for most animals (Dam, 1962). The complicated diets of humans made observation in this respect difficult (Horwitt, 1972). The mechanism whereby PUFA increase α-tocopherol requirements is not clear, although it has been postulated that it involves absorption and storage (Weber & Wiss, 1963). Contrary to these findings, a number of investigators have shown that PUFA do not interfere with the absorption of these lipid molecules(Peake et al., 1972; Bieri & Evarts, 1975). These investigators concluded that although more work is required in this field, the relationship between PUFA and α-tocopherol is not linear. Another complicating factor is that vitamin E-deficient rats have been shown to have better absorption than normal rats (Losowsky, 1972).

The determination of the tocopherol content in a cell or organism has been shown to be complicated by various aspects other that dietary intake. For example, in mammals, the plasma concentration of α-tocopherol correlates highly with plasma concentration of total lipids and cholesterol (Rubinstein, 1968). A complicating factor often

ignored in evaluating the body status of vitamin E was studied by Bieri and Evarts (1975). They showed that the degree of adiposity in the tissue affects the amount of α-tocopherol in the tissue. These investigators found that when normal and genetically obese rats were fed α-tocopherol the obese groups had lower concentrations of α-tocopherol in their heart and lung tissue. These differences occurred in spite of the fact that the obese rats had three times as much α-tocopherol in their plasma than did the normal rats. This indicates that organ storage of α-tocopherol is affected by body adiposity. This is an important factor to consider in trying to correlate the effects of α-tocopherol in different tissues. In addition to differences in tissue storage, different cell types have different abilities to metabolize and absorb α-tocopherol. The differences in the metabolism of the cell types involved have led to some controversy in this area, which remains under investigation.

The above-mentioned complications make comparisons between species risky. The duration and degrees of depletion necessary to bring about certain tissue dysfunctions in some species are difficult to obtain without producing other effects that would mask the vitamin E-deficiency symptoms. For example, in some

species the most pronounced effect of vitamin E depletion is the presence of muscular lesions. However, the absence of lesions in other organs does not necessarily mean that vitamin E is not required in those tissues (Mason & Horwitt, 1972). Although there are many discrepancies between tissues, and between species, most tissues and species are affected by α-tocopherol's antioxidant effects (Mason & Horwitt, 1972).

Discrepancies between *in vivo* and *in vitro* studies have also lead to some controversy over the role of α-tocopherol in biological systems. There is good evidence that tocopherols have an antioxidant activity *in vivo* in adipose tissue (Weber & Wiss, 1963), but there is no clear evidence of peroxidative loss of unsaturated fatty acids and accumulation of products of lipid peroxidation in other tissues. Most support for an antioxidant action of α-tocopherol comes from studies *in vitro*. It has been emphasized by many investigators that supplementation of α-tocopherol to animals *in vivo* results in different findings from those observed in an *in vitro* system. The difference in oxygen tension between *in vitro* and *in vivo* systems and the possibility of cellular disruption in *in vitro*

systems both suggest that higher levels of α-tocopherol are required in in vitro systems.

The classic in vitro hemolysis of vitamin E-deficient red blood cells by hydrogen peroxide (Rose & Gyorgy, 1952), which has been used as an assay for many years, has been criticized by a number of investigators on the ground that it is influenced by factors other than the blood tocopherol content (Horwitt et al., 1968).

The above-mentioned complications leave us with many unanswered questions about the role of α-tocopherol in biological systems. It is clear that the more complex the animal, the more complicating factors there will be. Thus, the search for a correlation between tocopherol deficiency and clinical findings in man has led a number of investigators to study the manifestations of such deficiencies in lower animals.

Objectives of this Study

Because of its simple but well known morphology, its simple axenic diet, its reasonably short life span, its advanced but simple reproductive system and its eutelic characteristics, it was felt that the nematode Turbatrix aceti would lend itself to an

in vivo study in which the effects of α-tocopherol on some of the basic physiological functions, such as development, growth, reproduction, and aging could be clarified. Few studies have been reported in which all of these aspects of α-tocopherol have been studied in one animal. It was thought that this nematode would be useful in the evaluation of both the chronic and short term effects of dietary α-tocopherol. It was also hoped that this small organism would provide an experimental system simple enough to minimize the complicating factors normally encountered in an *in vivo* systen, without encountering the problems of an *in vitro* system.

MATERIALS AND METHODS

Animals

The free-living nematode Turbatrix aceti (Peters, 1927) was the organism chosen for the studies reported here. The axenic cultures of Turbatrix aceti were obtained from Dr. Morton Rothstein (State University of New York at Buffalo).

Turbatrix aceti is a bisexual ovoviviparous nematode which has a fixed cell number throughout its post hatching period, with the exception of the reproductive system and the intestinal epithelium (Pai, 1928). This nematode grows from a size of 0.2-0.3 mm in length and 15 to 20 µm in width in newly hatched animals, to 1.5 - 2.5 mm and 50 - 60 µm in adults.

Preparation of Culture Medium

Basal medium. Basal media for axenic cultuvation was adapted from Sayre et al. (1963) as described by Kisiel, et al. (1974) with some minor alterations; 4% (w/v) Soy Peptone (Sheffield Chemical Co. Union N.Y.), and 3% (w/v) Yeast extract (Difco), were disolved in 90 ml of destilled water and heated for 10 minutes. Glacial acetic acid was added to this clear medium to a final concentration of 2%. All test media were made up to 100 ml in 400 ml

Erlenmyer flasks.

After medium components were added, sponge-stoppered flasks were autoclaved for 7 minutes (fast exhaust).

Heated liver extract. One of the drawbacks in using heated liver extract (HLE) for the axenic cultivation of free-living nematodes is the possible introduction of variables which could be avoided by using a chemically defined medium.

A number of investigators have attempted to modify the original axenic nutrient medium described by Sayre et al. (1963), with the objective of producing a totally chemically defined culture media (Rothstein & Cook, 1966; Buecher et al., 1970). Preliminary experiments in this study, have shown that under the conditions used in this laboratory, "HLE" is essential for the maintenence of optimum growth and development required in the type of experiments performed in this study. HLE was prepared according to Sayre et al. (1963) with the following modifications: freshly obtained beef liver was diced and stored at -4°C over night. Homogenization and extraction of the extract was followed according to the author's recipe. 10 ml aliquots of the filtered supernatant were freeze-activated and stored at -17°C (Sayre et al., 1963). The necessary amount required for the volume of medium being prepared was allowed to thaw at room

temperature just prior to use.

Filter sterilization was performed after freezing by passing the extract through a membrane filters (Millipore Corporation, Bedford, Mass. 01730, USA) of decreasing pore size ranging from 10 µm to .22 µm (LCWP 02500, AAWP 02500, DAWP 02500, HAWP 02500, GSWP 02500). A Millipore prefilter (AP 25025-00) was used to aid in the filtration process. The final addition of the sterile HLE to the basal medium was done under sterile conditions in a laminar flow hood.

Removal of precipitate. The formation of large amounts of precipitate upon addition of HLE due to the low pH of the basal media is inevitable. The importance of the nutrient content present in this precipitate has been reviewed by Vanfleteren (1974). Because media with total precipitate was found to interfere with the accurate visualization required in the experiments of this study, the development of an equillibrium which allowed optimum growth and development as well as clear visualization was essential.

Preliminary experiments showed the most efficient and convenient method to be as follows. Media containing HLE was shaken thoroughly for one minute, and the mixture transfered under sterile conditions into 50 ml sterile test tubes. Stoppered tubes were left standing

for 24 hours, thus allowing the larger particles to settle. The supernatant containing nutrients to promote adequate growth, development and reproduction was then carefully decanted and stored in sterile tissue culture flasks (Corning 25100, Corning Co., Corning, N.Y. 14380). Any special addition to the medium was done at this point. Variations in the medium required under certain experimental conditions will be noted in the respective sections.

The medium was stored and used for a maximum of two weeks. Fresh medium was prepared at the beginning of each experiment. Stored medium was usually used to replenish culture dishes of experiments in progress.

Procedure for evaluating media. Each batch of medium was compared with the previous batch as follows: duplicate flasks of each media were inoculated with an equal number of worms from the same culture. The increase in population size was compared after a determined length of time. One examination was done after 3 days, and all media that showed inconsistancy at this time were discarded. Cultures were kept and a second examination was done after 14 days, for a better estimate of the ability of the media to maintain a continuous population. Experiments utilizing media that showed inconsistant results at this time were discarded.

All experiments involving media checks were done in this manner. Duplicates were tested to ensure consistancy.

Preparation of Vitamin E. dl-α-tocopherol was obtained from ICN Pharmaceuticals (Cleveland, O. 44128) in liquid form. The advantage of this liquid over a powdered form also available is the absence of carriers, and therefore variables, which complicate the controls. Pilot experiments showed that the most convenient method for α-tocopherol supplementation was to dissolve it in the acetic acid present in the growth media used in culture Turbatrix aceti. A range of concentrations of α-tocopherol was suggested by Dr. R.M. Roy (personal communication), based on the Vitamin E concentration effective in radioprotection of invertebrates such as Drosophila. Pilot testing of several dosages for their effect on growth and fertility showed that a dilution of 10^{-4} was effective (see Appendix I). 0.1 ml of α-tocopherol was dissolved in 10 ml of glacial acetic acid. 1 ml of this solution and 1 ml of acetic acid were then added to the basal media, to make up the 2 ml of acetic acid required. Solubility of this lipid in acetic acid was checked under the microscope.

Sterility Checks

Turbatrix aceti's medium acts as its own check on

sterility, rapidly becoming cloudly if contaminated by bacteria. Nontheless, sterility checks using nutrient agar (Difco) were performed on every sub-culture. Inoculated agar plates were incubated for at least 96 hours at 37°C.

In general, bacterial contamination was very rare, but if a culture was suspected of being contaminated, a more rigid procedure was used as a check: 0.1 ml of the sample was pipetted into 1 ml of basal media (adjusted to pH 7.0) and incubated for up to 1 week at 37°C. If the results were not clear possibly due to any interfering precipitate, a loopful of this medium was transferred to fresh media and treated as above (Rothstein, 1974).

All transfers and inoculations were carried out in a laminar flow hood (CCI, Kupsville, Penn.), kept at 20°C. Instruments used in any of the manipulations were sterilized prior to use.

Maintenence of Cultures

For optimum growth, cultures were maintained at a constant temperature of 29°C (Rothstein, 1974).

pH was maintained between 3.9 - 4.1 for all experiments, measurements were recorded for each new batch of medium using a pH meter (Corning 10).

Experiments which ran over a certain length of time were checked for pH changes. Changes were found to be rare.

Culture Dishes. Individual experiments required different types of tissue culture dishes. Three main types of culture dishes were utilized in this study: large scale cultures of Turbatrix aceti were maintained in 25 cm^2 sterile tissue culture flasks (Corning 25100). Smaller scale cultures (mass cultures) were maintained in 35 x 10 mm Petri dishes (Costar 3035; 205 Broadway, Cambridge, Mass. 02139). Individual cultures (individually mated pairs; single females) such as those used in reproduction experiments and survivorship studies, were maintained in 16 mm tissue culture cluster wells (Costar 3524).

All dishes with the exception of the screw cap bottles were sealed in large Tupperware containers to prevent variation in the culture media due to evaporation.

In lengthy experiments media was removed and substituted with fresh media to ensure a constant supply of nutrients and prevent toxic effects due to old media. All culture dishes were supplemented in equal and accurate amounts. Tissue culture flasks were generally filled with 2 - 5 ml of media, Petri dishes were kept filled with 2 ml and individual clusters were kept

filled with 0.5 ml.

Inoculation of Cultures

Stock cultures were maintained to ensure availability of nematodes, and to test new batches of media. Duplicate tissue culture flasks containing 2 - 5 ml of media were inoculated with 10 µl of worms from two-week-old cultures. Subcultures were maintained in the same manner every 14 to 18 days. Pilot experiments showed that under the experimental conditions maintained in this laboratory, cultures left longer than 18 to 21 days did not produce optimum growth when used to inoculate new medium.

If a particular subculture was not producing populations of any consequence, the duplicate flask was checked. If it also showed negligible growth, that media was discarded and the inoculation was repeated in new media (see media test). Control subcultures were kept separate from α-tocopherol. Special inoculated cultures will be described below.

Supplementation and Depletion of α-Tocopherol

Nematodes described in the text as being of a particular origin are nematodes grown in that culture media for several generations; control or α-tocopherol. Supplementation was accomplished by transferring the

nematodes to α-tocopherol supplemented media at the indicated times. Depletion, likewise, was accomplished by transferring nematodes to control media. To minimize the possibility of transferring any α-tocopherol with the animal, nematodes were washed through four consecutive changes of control media. Supplementation or depletion before birth was achieved by transferring the mother of the nematode in question to the new media just prior to birth.

Synchronization

Because of the importance of the accuracy of the age of the organism in some of the experiments of this study, we found it most convenient to synchronize and harvest worms manually. Large numbers were harvested by filtering large populations through 10 µm pore size Millipore membranes (LCWG 02500). This method results in separation of large numbers of 0 - 3 day old worms. Nematodes obtained in this manner were used to start new populations for mass cultures, and to produce offspring of more precisely determined age. This was carried out by allowing a set number of young larvae to grow in a petrie dish. As soon as their first offspring appeared, all parents were transferred to similar dishes, leaving only newborn larvae in the dish. This procedure was repeated every 12 or 24 hours,

depending on the accuracy desired. This method results in newly-born larvae daily, and aged worms at the end of their reproductive period.

To harvest larger numbers of aged worms, parents were removed from their offspring every 3 days until the end of their reproductive period (parents are easily distinguished from their offspring for 3 days).

The apparatus used to transfer young and adult nematodes consisted of a rubber tubing 12 cm long and 0.3 cm in diameter attached to a mouth piece at one end, adapted to a small glass tubing which was stoppered with a small rubber plug with an opening to accomodate a 20 µl micropipette (Corning 70995-20). The glass tubing proved useful as a trap.

Micropipettes were heat-pulled to very narrow points and sterilized (autoclaved) for 15 minutes prior to use. The diameter of the point was adjusted according to our needs by carefully breaking off more or less of the tip. A larger diameter was required to prevent injury to adult worms, whereas a very narrow tip was required to transfer newly-born larvae. This method allowed quick and safe transfer of nematodes as well as very accurate synchronization.

Measurements

Prior to measuring their length, nematodes were

straightened by heating at 57°C for 5 seconds (Hieb & Rothstein, 1974). By this procedure, organisms are rapidly killed and straightened without shrinkage in length. Measurements were taken through a binocular light microscope (Cook Thourghton and Simms). The length was estimated using an ocular micrometer (calibrated with a 1 mm micrometer scale with increments of .01 mm) at a magnification of 6.3 x 10.

Detection of Dead Worms

Dead worms were easily detected by adding a drop of neutral red (1%) to the culture. Dead worms take up stain readily, thus distinguishing them from live worms. Dead worms can also be recognized by their lack of movement.

Counting

Nematodes were counted manually. In most experiments, it was possible to count one worm at a time. Progeny were counted as they were removed from their culture dish with a micropipette. Large populations (mass cultures) were estimated using the method reported by Lower et al. (1966). The actual counting was performed under a disecting microscope (American Optical

Spensor Zoom).

Fertility Experiments

Three experimental procedures were used to evaluate the effects of α-tocopherol on reproduction: single cultures, individually paired cultures and mass cultures.

Mass cultures. Synchronized nematodes were transferred to Petri dishes in numbers sufficient to ensure an adequate male to female ratio for optimum fertilization. This method proved satisfactory for measurement of mating times, and time of onset of the reproductive period, but less adequate for measurement of the length of the reproductive period and the number of progeny.

Single cultures. Nematodes were left in mass cultures during their mating time. After mating, females were isolated, so that their reproductive capacities could be measured. This culture method ensures optimum fertilization, due to the abundance of males in the mass cultures which eliminates the limiting factors in individual males.

Individually mated pairs. Pairs of nematodes were isolated into single culture chambers before the onset of their reproductive periods. This method was used to measure most of the above-mentioned parameters of reproduction, but proved to be relatively inaccurate

in measuring such things as time of onset of reproduction. The probability of both male and female being mature enough to reproduce is diminished in a two nematode culture.

It was felt that the combination of methods would give a more accurate measurement of the effect of α-tocopherol on reproduction than would any one of these methods used by itself.

Survival and Longevity Experiments

48 synchronized virgin nematodes per group were isolated into individual cluster chambers. These individuals were observed every 4 - 5 days for any sign of movement. Dead nematodes are easily recognized by their lack of movement and by their straight bodies.

Separation of Litters

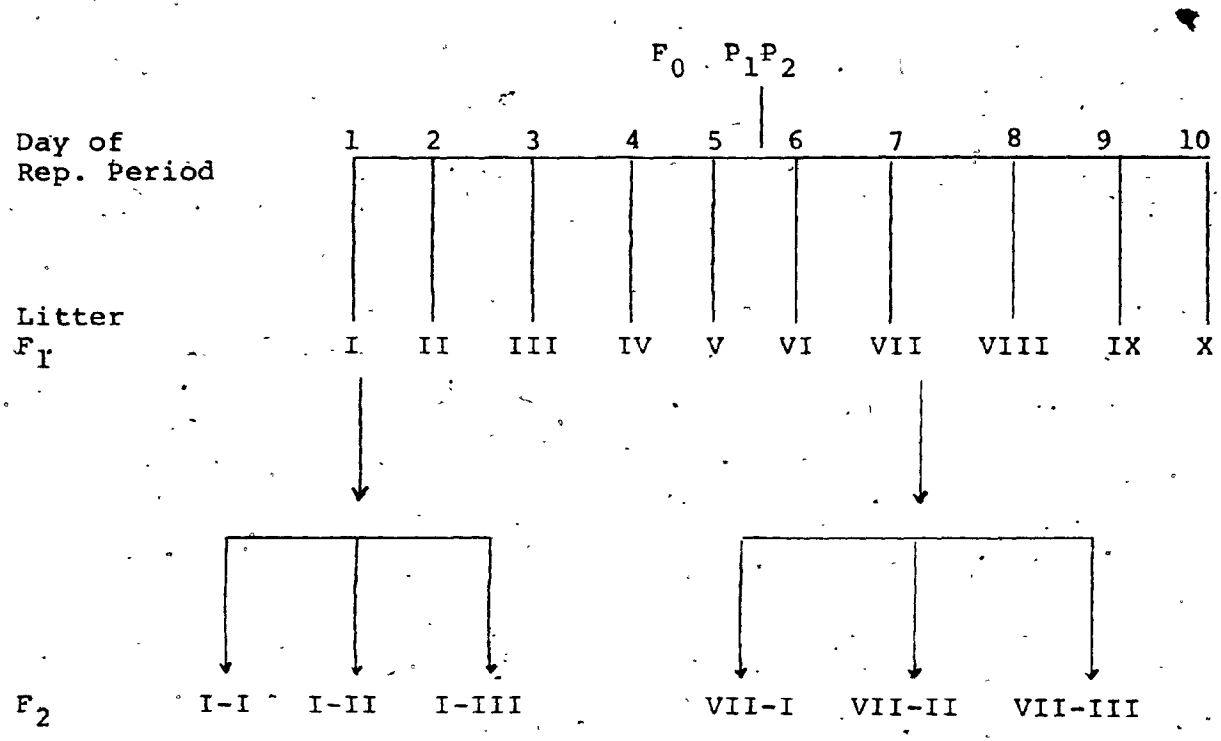
For our experimental purposes a litter is defined as the larvae born in a 24 hour period. Litters were obtained in the usual manner by removing the parents from the new born every 24 hours. Litter numbers were given according to the day of the parents' reproduction period (I = 1st day of reproduction) (see Figure I).

Radiation Culture Conditions

Approximately 200 synchronized nematodes per group (radiation dosage and treatment) were washed through three consecutive changes of very diluted

Figure I

Diagramatic Representation of Experimental Set up for Litter Experiments



basal media (no HLE). Media, rather than water, was used for this purpose to prevent additional stress just prior to irradiation. Radiation was administered in a gamma cell (^{60}Co) at a rate of 30 roentgens/sec.

Pre-irradiation treatments was administered for various generations (control or α-tocopherol origin).

Post-irradiation treatment was administered immediately after radiation (within 5 minutes), by transferring irradiated nematodes to individual chambers containing the respective culture media (control or α-tocopherol). Survivorship was monitored in the usual manner. Non-irradiated nematodes were given similar treatment, to control for the stress caused by the handling involved in these experiments.

Cytology

Unsuccessful attempts at fixing, embedding and sectioning Turbatrix aceti for the light microscope led us to do most of the microscopic observations and photography on live intact worms. This was possible due to their thick, resistant and fairly impermeable cuticle. Using a small drop of glycerine jelly (Galigher & Kozloff, 1971) or albumin, it was possible to maintain an intact young worm under the microscope for approximately 3 minutes. Stillness without death was achieved by replacing these substances with ice

water. This method was more effective for very young nematodes than for old ones. Some observations were done with squashed rather than with whole nematodes. This was easily achieved with adults, specially females, by carefully placing a coverslip on an adult worm which had been placed on a very small drop of water or glycerine. If the mere impact of the coverslip did not cause bursting, slight pressure on the coverslip was applied.

The amount of liquid on the worm, and the age of the worm, are crucial to the success of this procedure. Organs of carefully prepared worms are easily identified. Cells can easily be stained once there is no cuticle interfering with the absorbtion of the stain.

Nematode tissue was stained in two ways:

a) Vital stains were added to cultures approximately 48 hours prior to use. This method was found to aid in the resolution of a number of organs for colour microphotography purposes.

b) Nematodes burst open under the coverslip were stained by adding a drop of a particular stain and drawing it to the other side using absorbant tissue. This method was used for nonvital stains.

The vital stains used were Neutral Red 1% and

Methylene Blue (Galigher & Kozloff, 1964). Nonvital stains were used in the attempt to stain lipids. Oil Red (Schmid GmbH & Co.) and Sudan IV (Schmid GmbH & Co.) were used for this purpose (Galigher & Kozloff, 1964). The type of stain used is indicated under each individual photograph.

Photomicrography

Specimens for microphotography were obtained as explained in the cytology section.

Microscope. A Zeiss Standard Universal Microscope was used for most microscopic examination. Photographs were taken with a special Phase Contrast Condenser and PhZ Neofluor 16X and 40X objective lenses. Dark field was obtained by using a 6.3X objective lens. 6X or 12X ocular lenses were used between the camera and the objective lens. A neutral-density filter was used over the diaphragm of the microscope, to enhance contrast and decrease the intensity of the illumination (12 V illuminator with 60 W, 12 V bulb), for colour film purposes. A green filter was used for black and white film.

Camera. A Zeiss Ikon 35 mm camera, set up with a photocell for exposure control (B-setting at 7 or 8 for automatic exposure) and Kodak Ektachrome slide film (ASA 160, for tungsten light)

were used for all coloured photographs. Black and white photographs were taken with the same camera, using Kodak Plus-X-Pan 160 ASA.

Statistics

Most standard statistical analyses were carried out using formulae described by Rolph and Sokal (1969).

Paired t-tests, analysis of variance and regression analysis were performed with the aid of a T_I Programmable 58/59 Texas Instrument Calculator.

Probit analyses were obtained through our central computer (Statpak). Comparisons between slopes and LD_{50} values were done according to Stanley (1963). A summary of this procedure is described in Appendix II.

RESULTS

The Effect of α-Tocopherol on Growth and Maturation

The first set of experiments was devised to detect the differences in growth patterns between control and α-tocopherol-cultured nematodes. Figure 1, which shows the complete life cycle of Turbatrix aceti, under optimum experimental conditions, is given as a reference for the experiments to follow.

The growth of Turbatrix aceti was measured in length, as has been described in the Materials and Methods section. Stages of development were defined based upon both size and developmental criteria such as molts and degree of differentiation of sex organs. These stages are presented separately for males and females in Table 1. Molting times were estimated from observations of shed cuticles and large change in size per time interval. The most obvious changes in size were noted right after cuticles were shed. Growth patterns were obtained by measuring sets of thirty synchronized nematodes differing in age by twelve hours. This is represented graphically in Figures 2a and 2b for females and males respectively. These graphs emphasize that the worms in α-tocopherol reach a

FIGURE 1. Life cycle of the heterosexual nematode Turbatrix aceti grown in axenic cultures. Stages of development and post development are shown from the time the parental germ cells are formed until death. The adult stage is characterized by the appearence of the first egg in utero.

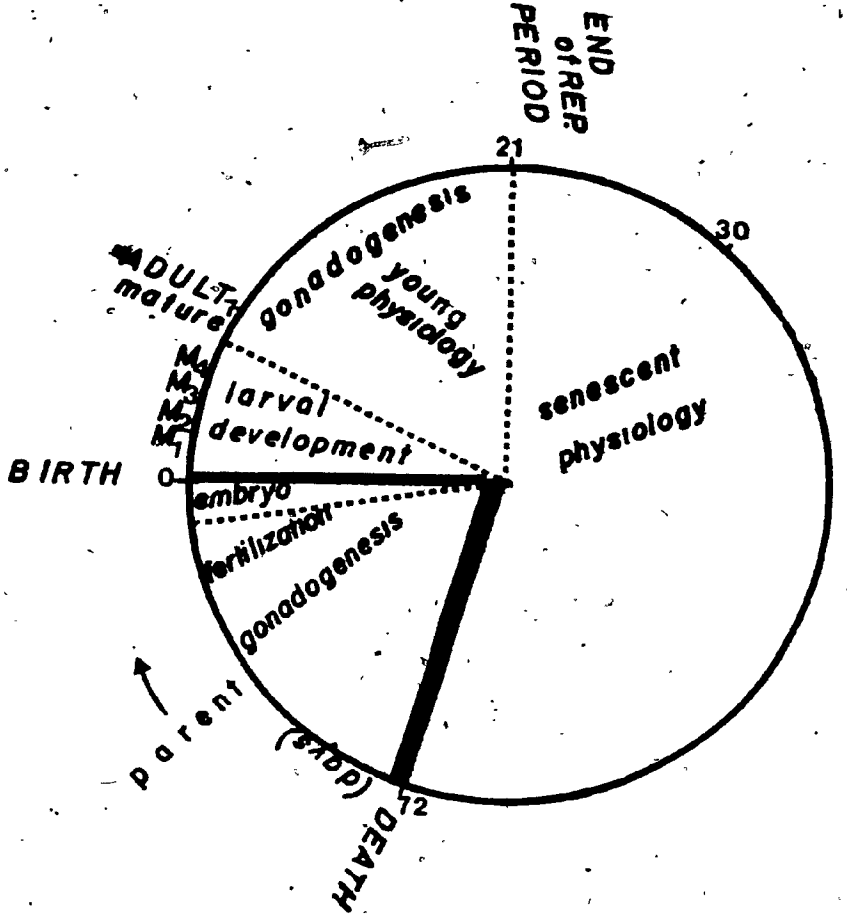

END of REP. PERIOD
21
30
ADULT
mature
gonadogenesis
young physiology
senescent physiology
M4
M3
M2
M1
larval development
BIRTH
0
embryo
fertilization
gonadogenesis
parent
(days)
DEATH
72

Table 1

Criteria for Defining Growth Stages

Stage	Approximate Time of Molt	Length (mm) Male	Length (mm) Female	Light Microscope Morphology Male	Light Microscope Morphology Female
S1		0.25 - 0.30		No difference	
	→ Molt I				
S2		0.35 - 0.40		No difference	
	→ Molt II				
S3		0.40 - 0.50		No difference	
	→ Molt III				
S4		0.55	0.75	Distinguishable shape, but no distinguishable reproductive organs	
	→ Molt IV				
S5		0.60 - 0.70	1.10 - 1.13	No distinguishable reproductive organs	Ovaries
S6		0.90 - 1.10	1.20 - 1.50	Testis	Fully developed eggs
S7		1.10 - 1.20	1.40 - 1.60	Fully developed	Fertilized eggs
S8		1.30	1.65	Mature	Full of fertilized eggs - larvae
S9		1.35	1.75+	Mature	Offspring production

given stages of development more quickly than do the control worms.

The difference in size between control and α-tocopherol treated nematodes at all stages of development defined is further emphasized in Figures 3a and 3b. In these figures, paired histograms represent the mean length of control and α-tocopherol-cultured nematodes on each day. In this experiment, a new set of 30 synchronized nematodes was measured daily. The most pronounced difference in females is observed between 72 and 168 hours (period of most active cell growth). The difference in length between control and α-tocopherol males is less pronounced.

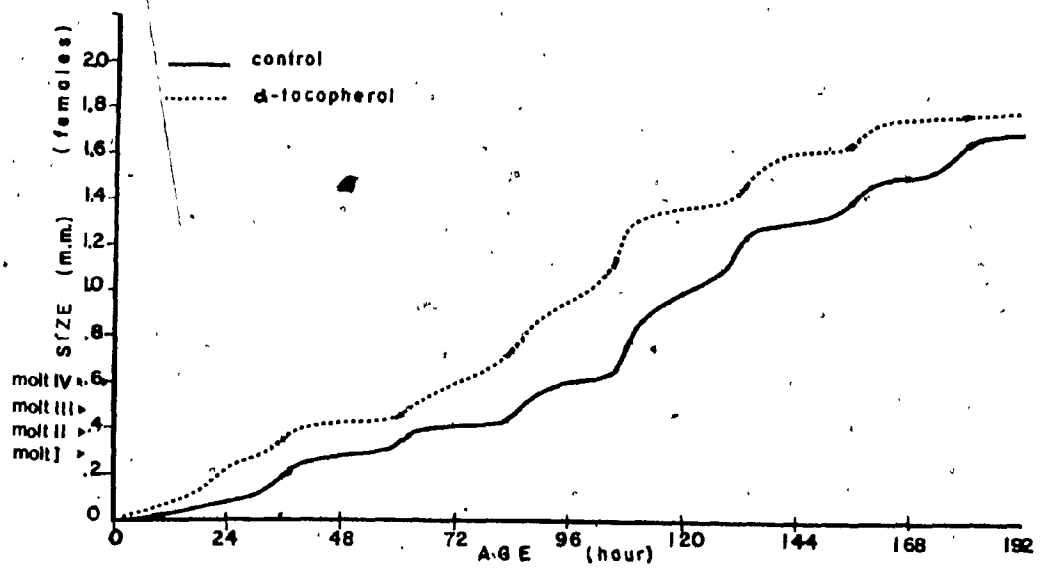
Student t-tests were performed to determine the significance of these differences. Significant differences between control and α-tocopherol treated nematodes for individual days are indicated above the respective bars.

Approximately equal sizes were eventually reached by both groups in both male and female cultures; 1.3 mm and 1.6-1.7 mm respectively. Measurements were only taken for the first 12 days since this is the time of most active growth in this species.

Figure 4 shows the growth rate (change in size per time interval) of control and α-tocopherol treated

FIGURE 2a. Life cycle of female Turbatrix aceti showing the periods of growth and lethargus.

FIGURE 2b. Life cycle of male Turbatrix aceti showing the periods of growth and lethargus.


control
d-tocopherol
SIZE (m.m.) (females)
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0
molt IV
molt III
molt II
molt I
0
24
48
72
96
120
144
168
192
AGE (hour)

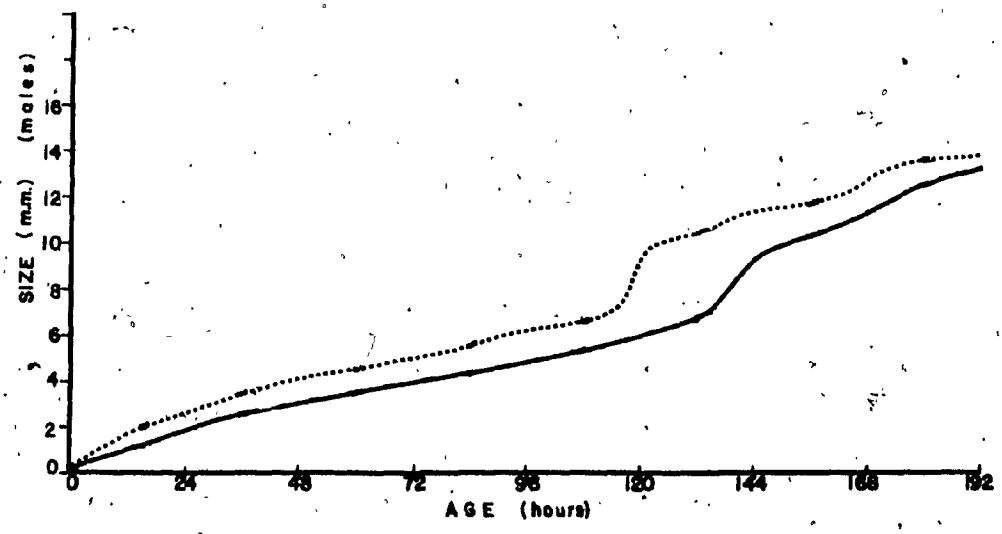
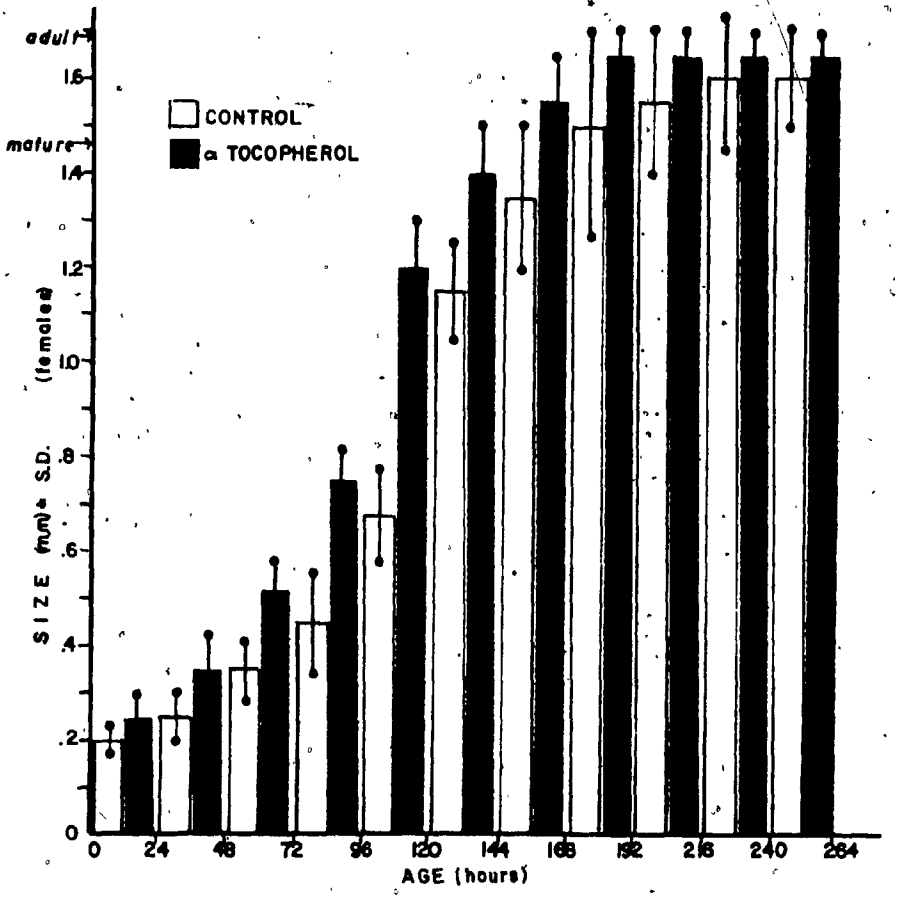

SIZE (m.m.) (males)
16
14
12
10
8
6
4
2
0
0
24
48
72
96
120
144
168
192
AGE (hours)

FIGURE 3a. Histogram illustrating the mean size in mm. at a given time interval of females cultured in control and α-tocopherol media.

FIGURE 3b. Histogram illustrating the mean size in mm. at a given time interval of males cultured in control and α-tocopherol media.


adult
mature
CONTROL
α TOCOPHEROL
SIZE (mm)± S.D. (females)
0
.2
.4
.6
.8
1.0
1.2
1.4
1.6
0
24
48
72
96
120
144
168
192
216
240
264
AGE (hours)

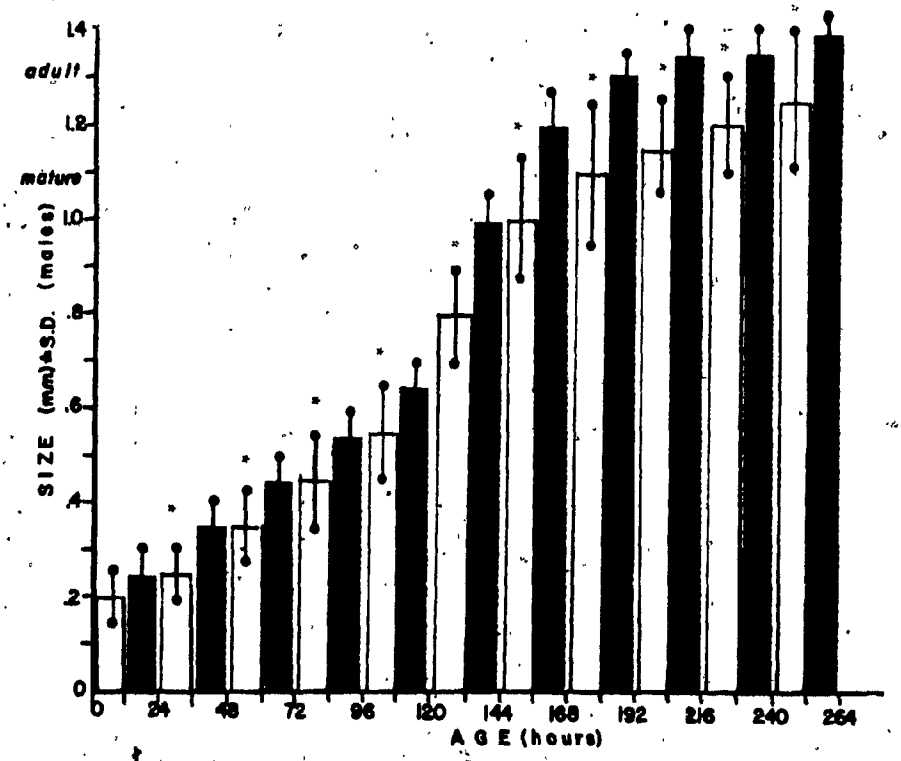
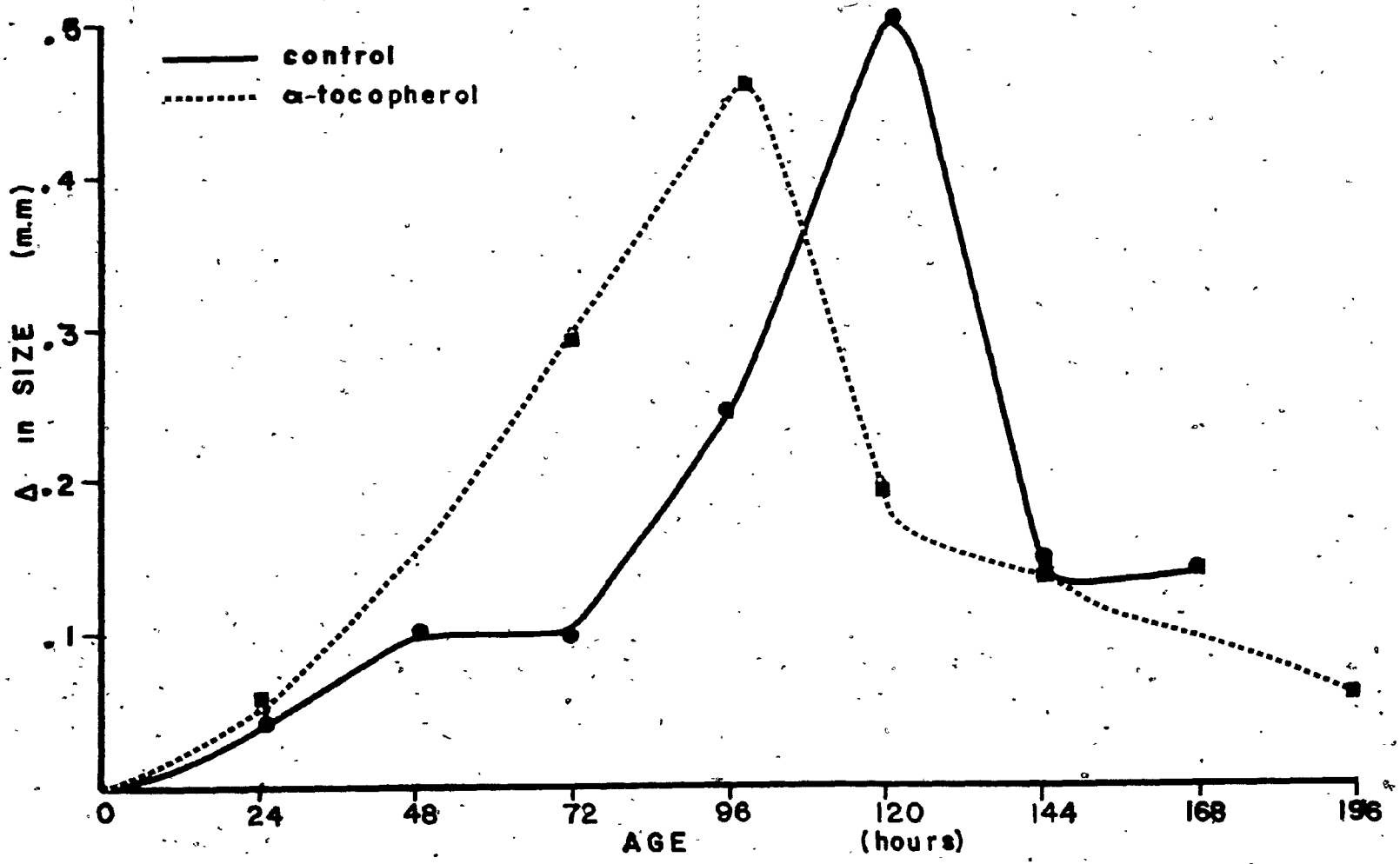

adult
mature
SIZE (mm)±S.D. (males)
0
.2
.4
.6
.8
1.0
1.2
1.4
0
24
48
72
96
120
144
168
192
216
240
264
AGE (hours)

FIGURE 4. Curves representing the mean change in size per time interval, of females cultured in control and α-tocopherol media.

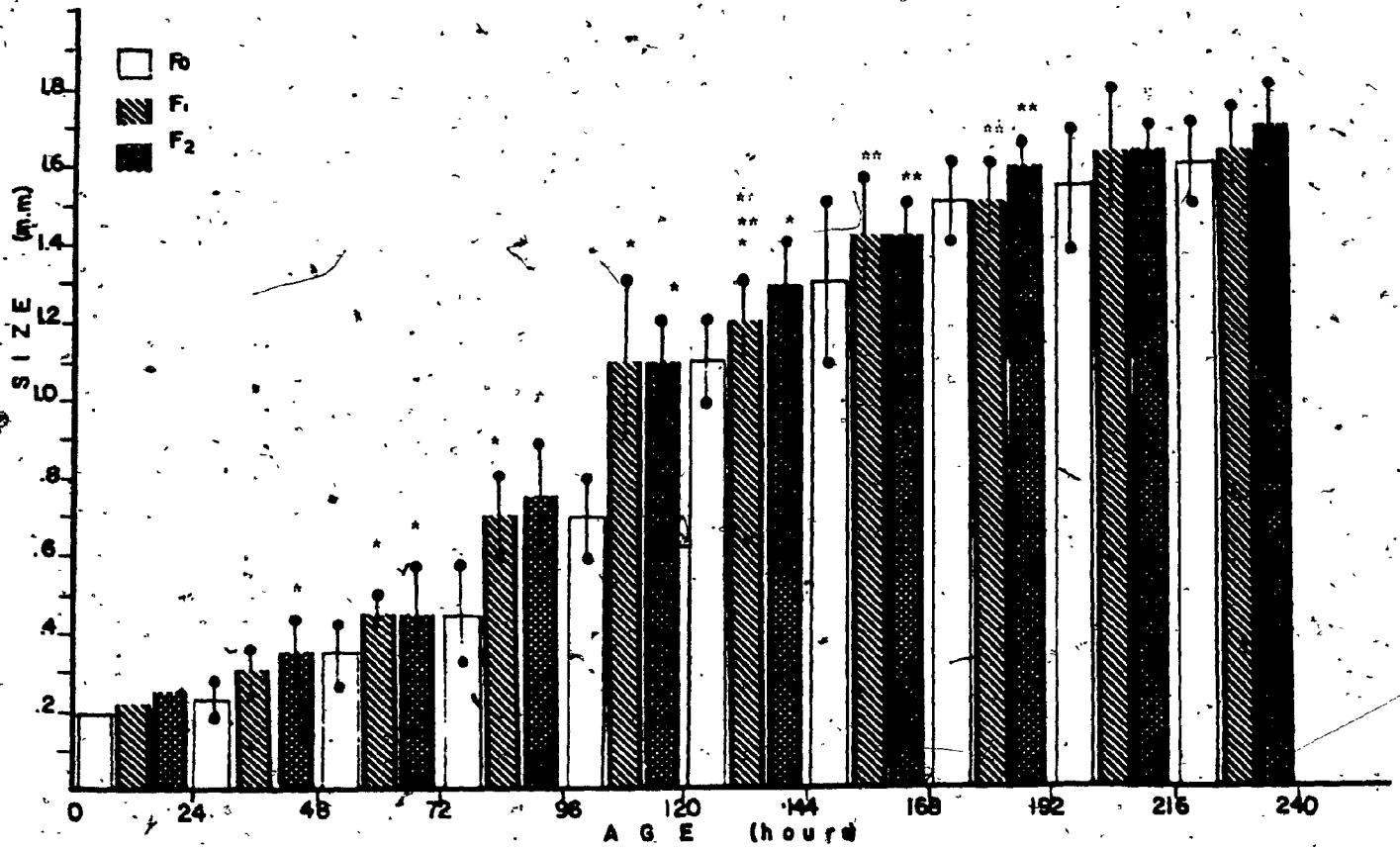

control
α-tocopherol
Δ in SIZE (m.m)
.5
.4
.3
.2
.1
0
24
48
72
96
120
144
168
196
AGE
(hours)

females. Data is presented in this way to show that α-tocopherol treated nematodes go through a maximum growth period approximately 24 hours earlier than control nematodes. Females were used for these experiments because their change in size is more obvious than that of the males. Non-parametric test statistics performed on these values (Wilcoxon Rank Sum Test), indicate that α-tocopherol nematodes grow significantly faster ($\alpha<.05$) than control nematodes.

Similar observations were made on females whose parents (F_0) were supplemented with α-tocopherol just prior to the onset of their reproductive period. The effects of α-tocopherol were evaluated on the growth and development of their offspring (F_1 and F_2). In this experiment, α-tocopherol was present during the development and growth of the reproductive organs of the first generation (F_1), but not during their early embryogenesis. In the second generation (F_2) both somatic and germ cells were formed in α-tocopherol supplemented media.

It should be noted from figure 5 that the most distinct difference in size between the F_0 and the F_1 and F_2 generations is observed between 72 and 120 hours. All three groups eventually reach the same size. Once again student t-tests were performed to

FIGURE 5. Histogram illustrating the mean size in mm. at a given time interval of nematodes supplemented with α-tocopherol after sexual development and the two following generations.


Po
F1
F2
SIZE (m.m)
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0
24
48
72
96
120
144
168
192
216
240
AGE (hours)

test the significance of the difference between groups on each day. Significant differences for these comparisons are indicated by asterisks above the respective bars.

Figure 6 represents the growth rate of these three groups. These curves indicate that nematodes supplemented with α-tocopherol before their reproductive period show a growth rate similar to that observed in control females (Figure 4). The maximum change in size in both these groups takes place on day five. F_1 and F_2 females show a growth rate similar to that observed in α-tocopherol females. Non-parametric test statistics indicate the F_1 and F_2 generations mature significantly faster than the F_0 generation ($\alpha<.05$).

A reverse experiment, where α-tocopherol parents (F_0) transferred from α-tocpherol to control media, is shown in Figure 7. The most striking observation made in these experiments was the significant drop ($\alpha<.05$) in mean growth rate of the progeny of these α-tocopherol-depleted females as compared to the F_0. It should be noted that the F_1 generation shows significantly slower growth between 144 and 216 hours than females cultured in control media. F_2 nematodes appear to recover to a certain extent after 168 hours. Student t-test for daily comparisons were

FIGURE 6. Curves representing the mean change in size per time interval of nematodes supplemented with α-tocopherol after sexual development and the two following generations.

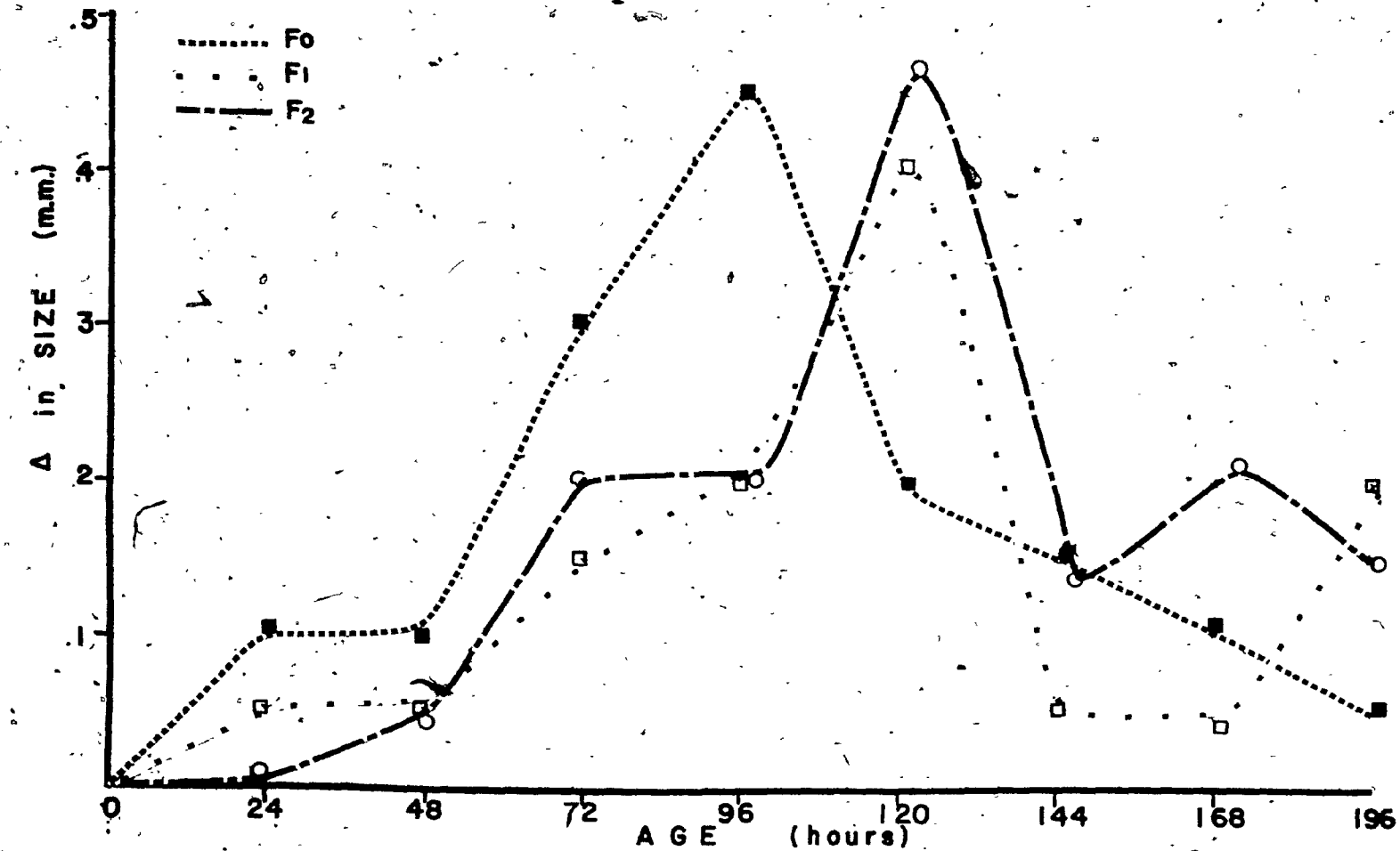

F0
F1
F2
Δ in SIZE (m.m.)
5
4
3
2
1
0
24
48
72
96
120
144
168
196
AGE (hours)

FIGURE 7. Histogram illustrating the mean size in mm. at a given time interval of nematodes of α-tocopherol origin removed from α-tocopherol medium after sexual maturation, and the two following generations.

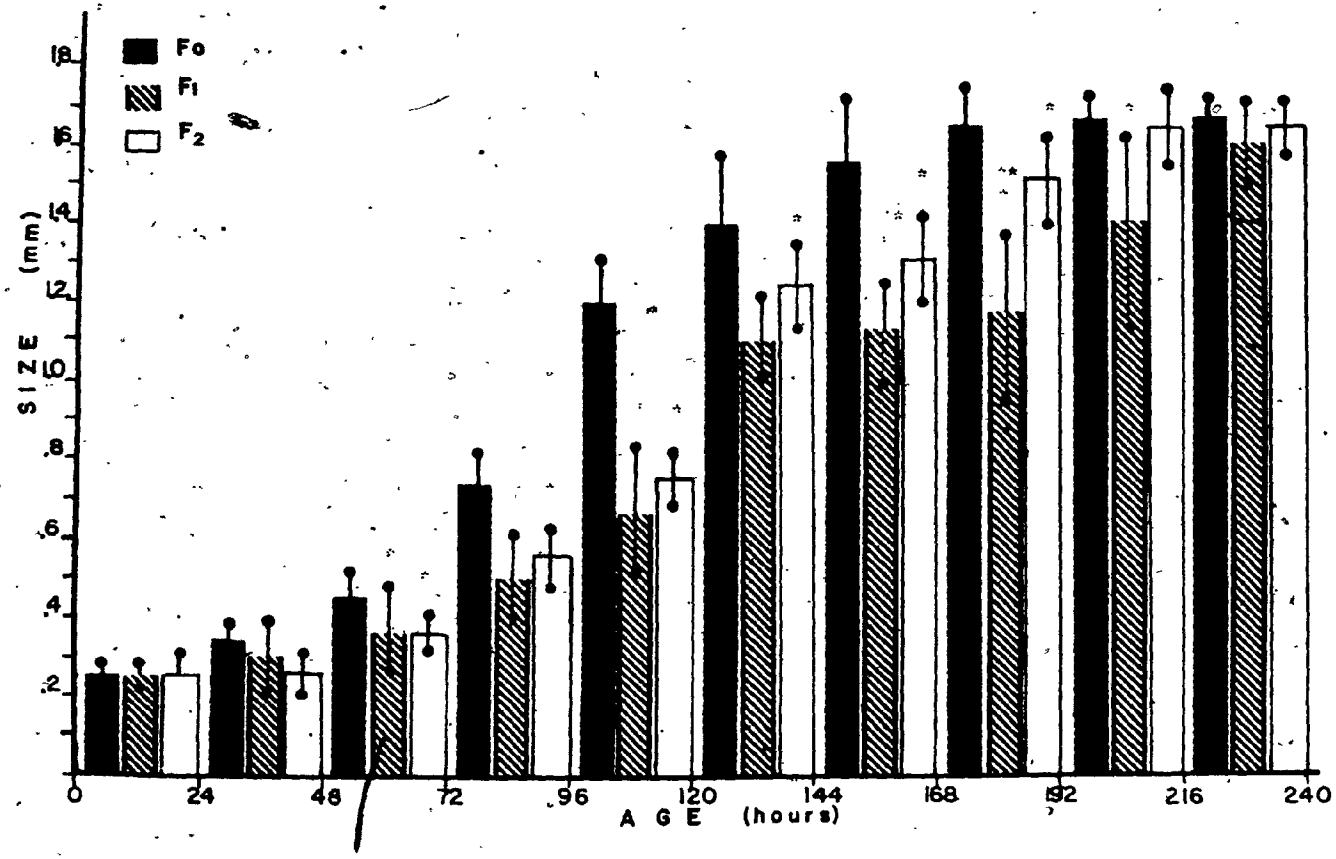
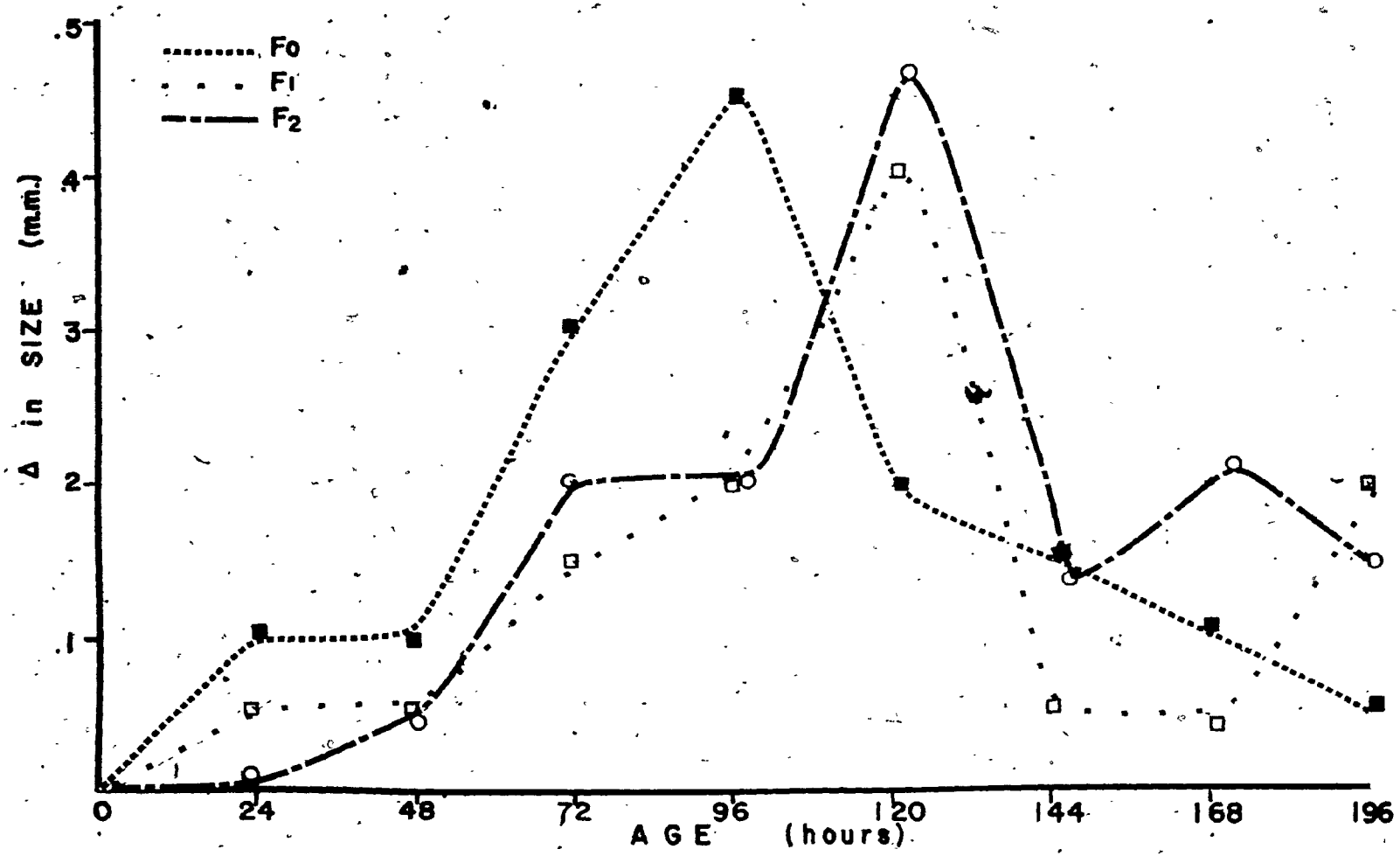

Fo
F1
F2
SIZE (mm)
.2
.4
.6
.8
1.0
1.2
1.4
1.6
1.8
0
24
48
72
96
120
144
168
192
216
240
AGE (hours)

FIGURE 8. Curves illustrating the mean change in size per time interval of nematodes of α-tocopherol origin removed from α-tocopherol medium after sexual maturation and the two following generations.


F0
F1
F2
Δ in SIZE (m.m.)
.5
.4
.3
.2
.1
0
24
48
72
96
120
144
168
196
AGE (hours)

performed in the usual manner. Significant differences ($\alpha<.05$) are indicated by asterisks.

Growth curves obtained on these data however indicate that both the F_1 and F_2 generations have a growth rate similar to that observed in control cultured females. Non parametric test statistics (Sign test) indicate that the F_1 and F_2 generations mature significantly more slowly than the α-tocopherol-depleted F_0 generation.

The low mean growth rate of these α-tocopherol-depleted nematodes is probably due to the increased number of dauerlarvae (larvae that are arrested in their growth, in the presence of adverse environmental conditions), formed as a result of their transfer from α-tocopherol to control media. These results should be considered when evaluating the fertility experiments described below.

In summary, it has been shown that α-tocopherol supplementation in the nematodes' media increases the mean growth rate of F_1 and F_2 generations, and that this change in size is somewhat slower than that observed in nematodes of α-tocopherol origin. It has also been shown that removing α-tocopherol nematodes from α-tocopherol media significantly affects the growth of their progeny when removed before the onset of reproduction.

The purpose of the next set of experiments was to determine the difference in growth between progeny that did not go through embryogenesis in α-tocopherol (early litters, I, II, III), and progeny whose parents were in α-tocopherol media while they were going through embryogenesis (late litters, VI, VII, VIII). This was accomplished by adding α-tocopherol to parents just before the birth of the early litters, thereby allowing later litters to go through embryogenesis in α-tocopherol media. A similar experiment was also done in which α-tocopherol was remover instead of added.

Before this experiment was carried out a preliminary experiment was performed to determine the degree of variation in growth and maturation among litters of control and α-tocopherol-treated nematodes.

Table 2 shows the mean ages during which different litter groups go through two of the more clearly defined developmental stages (S_5, S_8). The results indicate that control litters IV, V and VI, have a significantly faster growth and maturation rate ($\alpha<.05$), than other litter groups. Litter IX of the control group shows a significantly slower maturation ($\alpha<.05$) compared with the other litters in this group. α-Tocopherol-cultured litters show no clear trend.

Table 2

Growth and Maturation of Successive Litters as Influenced by Parental Age and by α-Tocopherol

Day of Rep. Period of F_0	Litter Group	Litter	Mean Age (Hours) ± S.D. S5 Control	S5 α-Toc.	S8 Control	S8 α-Toc.
1		I	132 ± 16	96 ± 16	229 ± 16	168 ± 16
2	a	II	144 ± 16	108 ± 16	240 ± 16	192 ± 16
3		III	144 ± 16	96 ± 16	216 ± 16	168 ± 16
4		IV	108 ± 16	96 ± 16	204 ± 16	156 ± 16
5	b	V	108 ± 16	108 ± 16	192 ± 16	192 ± 16
6		VI	132 ± 16	96 ± 16	192 ± 16	168 ± 16
7		VII	132 ± 16	108 ± 16	240 ± 16	156 ± 16
8	c	VIII	144 ± 16	108 ± 16	240 ± 16	192 ± 16
9		IX	156 ± 24	120 ± 16	252 ± 16	168 ± 16
Wilcoxon Rank Sum Test Control vs α-Toc.			$T_{s_{(9)}} = 1^*$		$T_{s_{(9)}} = 0^*$	

* $\alpha < .05$

Each litter represents 48 nematodes (n = 48)

This suggests that α-tocopherol keeps maturation constant among litters.

A Wilcoxon Rank Sum Test comparing all control litters with α-tocopherol litters further indicates that the overall maturation rate in control nematodes is significantly slower than that observed in α-tocopherol nematodes.

With these results in mind α-tocopherol supplementation and depletion experiments were set up avoiding litter groups IV, V and VI. Table 3a shows these results. Late litters (litter group b) supplemented with α-tocopherol (C/α-Toc.), show a significantly earlier maturation rate by the S_8 stage ($\alpha<.05$), than early litters (a) in this group. Late litters depleted of α-tocopherol on the other hand show a significantly later maturation rate ($\alpha<.05$) than early litters in this group. Furthermore, litter group (a) of this group is significantly slower in maturing than α-tocopherol nematodes (α-Toc./α-Toc.). Litter group (b) is significantly slower in maturing than either the control or the α-tocopherol groups.

These results suggest that the presence of α-tocopherol during embryogenesis has a significant effect on maturation rate. The advantage of this experimental design is that the only difference

Table 3a

Growth and Maturation of Early and Late Litters as Influenced by α-Tocopherol Supplementation and Depletion to their Parents Before the Onset of their Reproduction Period

Media of Origin of Parent	Transfer[1] media	Stage	F_1 →	Mean Age (Hours) ± S.D. a I	a II	a III	b VI	b VII	b VIII
control / (C/C)	control	S5		132±16	144±16	144±16**	132±16	132±16	144±16**
control / (C/C)	control	S8		228±16	240±16	216±16**	240±16	240±16	256±16**
α-Toc. / (α-T/α-T)	α-Toc.	S5		108±16	108±16	96±16*	96±16	108±16	108±16*
α-Toc. / (α-T/α-T)	α-Toc.	S8		168±16	192±16	168±16*	192±16	168±16	192±16*
control / (C/α-T)	α-Toc.	S5		120±16	144±16	132±16**	120±16	132±16	144±16**
control / (C/α-T)	α-Toc.	S8		228±24	216±16	216±16** ***	180±16	192±16	206±16*
α-Toc. / (α-T/C)	control	S5		132±16	144±16	156±16** ***	168±16	168±16	NT* **
α-Toc. / (α-T/C)	control	S8		192±16	216±16	216±16** ***	228±16	262±24	NT**

[1]Media in which development took place.

Table 3b

Growth and Maturation of Progeny (F_2) of Early and Late Litter Groups

Media of Origin of Parent	Transfer media	Stage	F_1 →	I(aI)			VI(bVI)		
			F_2 →	I	II	III	I	II	III
				Mean Age (Hours) ± S.D.					
Control / (C/α-T)	α-Toc.	S5		132±16	120±16	132±16** ***	108±16	96±16	108±16*
Control / (C/α-T)	α-Toc.	S8		192±24	206±16	206±16*	180±24	180±24	192±16*
α-Toc. / (α-T/C)	Control	S5		144±33	144±16	144±16**	132±16	144±24	132±16**
α-Toc. / (α-T/C)	Control	S8		216±16	192±24	192±16**	206±16	206±16	206±16* ***

* $\alpha < .05$ as compared to respective control litter group and stage (Mann-Whitney U-test).

** $\alpha < .05$ as compared to respective α-Tocopherol litter group and stage.

*** $\alpha < .05$ for comparison between litters groups.

between these litter groups is the length of time that these embryos are exposed to the experimental media. Both litter groups were exposed to α-tocopherol via the mother, but this does not exclude the possibility of different embryonic stages having different capacities for absorbing this lipid molecule.

The effect of α-tocopherol on different embryonic stages was further tested by observing the maturation rate of the progeny of the embryos mentioned above. aI and bVI represent progeny from F_1 litter groups I and VI respectively. Table 3b shows that both litter groups of the α-tocopherol supplemented group (c/α-toc.), have a significantly faster maturation rate ($\alpha<.05$), by the time they get to the S_8 stage than does the control group. These results suggest that in early litter groups, it takes approximately one generation before the effects of α-tocopherol supplementation on maturation are observed. Late litter groups on the other hand are faster in showing these effects.

α-Tocopherol depletion in these litter groups appear to have an immediate effect on their growth rate and maturation, although a difference is observed between litter groups (a, b) in the F_1 generation.

It can be suggested from these findings that

α-tocopherol may be required in the early part of embryogenesis to have a significant effect on these embryos growth and maturation.

Photomicrographs showing the different stages of embryogenesis and larvae development are shown in Appendix III.

Effects of α-Tocopherol on Reproduction

The experiments in this section were designed to evaluate the effects of α-tocopherol on the reproductive capacity of nematodes. Three culture conditions were used in this section. Nematodes were maintained : (i) in mass cultures of more than 12 worms, (ii) as single fertilized females, and (iii) as individually mated pairs (see Materials and Methods for details). The advantages and disadvantages of these three culture methods will be discussed below.

Various parameters of fertilization were selected in the attempt to study the effects of α-tocopherol on the different mechanisms involved in reproduction.

Effect of α-Tocopherol on the Onset and Length of the Reproductive Period

The first two parameters used to evaluate the effect of α-tocopherol were the time of onset of the reproductive period, and the length of the reproductive period. To decrease the experimental error that may be found in any individual culture condition, all three procedures were used to study these parameters, and comparisons of the results were made.

Mass culture. The advantage of a mass culture experiment is that the large number of nematodes

ensures the probability of mating. The major disadvantage is the loss of some of the parents, when being separated from their offspring. Table 4 shows data on the length of the reproductive period in a mass culture. At least twelve nematodes were used in each dish to ensure an adequate male to female ratio. This was necessary, as it is difficult to differentiate nematodes by sex before they mature.

The length of the reproductive period was calculated as the interval between the time the first larvae appeared (onset) to the time larvae were no longer present. This was accomplished by removing parents from their offspring every twenty-four hours.

Comparison of control-cultured nematodes with those cultured in α-tocopherol shows that the control group started reproduction approximately two days later than the α-tocopherol group. A one-way ANOVA performed on this data indicated a significant difference between onsets of the two groups ($\alpha<.05$).

Single culture. In this procedure, females were isolated from mass cultures every twenty-four hours, from the fourth to the ninth day of their life cycles. This procedure provided information concerning the earliest mating time, the length of the period between fertilization and birth of progeny (length of

Table 4

The Effect of α-Tocopherol on the Onset and Length of the Reproductive Period in Mass Cultured *Turbatrix aceti*

Experiment	Media	# of Parents at Beginning of Experiment	# of Parents Lost at End of Experiment	Onset of Rep. Period (Days)	Age at End of Rep. Period (Days)	Length of Rep. Period (Days)
A	Control	13	4	9	15	6
B	Control	14	4	9	15	6
C	Control	12	6	12	16	4
D	Control	12	0	10	16	6
$\bar{X}$±S.D.(Sx^2)				10±1.4(1.5)	15.5±5.7	5.5±1(.75)
A	α-Toc.	12	4	9	16	7
B	α-Toc.	12	2	8	15	7
C	α-Toc.	12	6	8	14	6
D	α-Toc.	12	4	7	16	9
$\bar{X}$±S.D.(Sx^2)				8±.7(.5)	15.25±.9	7.25±1(1.1)
One way ANOVA				$F_{(1,6)}=6^*$	N.S	$F_{(1,6)}=4.74$
Paired t-Test Control vs. α-Toc.				$t_{(3)}=2.19^*$	N.S	$t_{(3)}=3.65^*$

* $\alpha<.05$

females. Data is presented in this way to show that α-tocopherol-treated nematodes go through a maximum growth period approximately 24 hours earlier than control nematodes. Females were used for these experiments because their change in size is more obvious than that of the males. Non-parametric test statistics performed on these values (Sign test) , indicate that α-tocopherol nematodes grow significantly faster ($\alpha<.05$) than control nematodes.

Similar observations were made on females whose parents (F_0) were supplemented with α-tocopherol just prior to the onset of their reproductive period. The effects of α-tocopherol were evaluated on the growth and development of their offspring (F_1 and F_2). In this experiment, α-tocopherol was present during the development and growth of the reproductive organs of the first generation (F_1), but not during their early embryogenesis. In the second generation (F_2) both somatic and germ cells were formed in α-tocopherol supplemented media.

It should be noted from Figure 5 that the most distinct difference in size between the F_0 and the F_1 and F_2 generations is observed between 72 and 120 hours. All three groups eventually reach the same size. Once again student t-tests were performed to

embryonic development) and the influence of α-tocopherol on these events.

Table 5 shows that while only ten per cent of control nematodes became fertilized by day seven of their life cycle, forty per cent of the α-tocopherol females became fertilized by day six of their life cycle. A one-way ANOVA indicates that there is a significant difference between the mean onset of the control and the α-tocopherol cultured females ($\alpha<.05$). No females isolated on day nine for the α-tocopherol group were tested because most of them were well into their reproductive period by then. The onset results may be affected by the fact that some females had already started reproduction at the time they were isolated.

Figure 9 shows the difference in the time of fertilization between control and α-tocopherol-cultured nematodes. It also demonstrates that the reproductive period starts earlier in the α-tocopherol group than in the control group, and that control nematodes do not achieve the fecundity observed in α-tocopherol nematodes.

The foregoing information proved useful in the design of later experiments. In the next experiment (Table 6), the isolation of the females was done on

Table 5

The % Females Reproducing are Used in Evaluating the Time of Fertilization in Control and α-Tocopherol Cultured Nematodes

Media	Age at Isolation (days)	Females Reproducing (%)	Mean Onset of Rep. Period±S.D. (days)	Test Statistic
Control	4	0	-	
	5	0	-	
	6	0	-	
	7	10	8.0 ± 0	
	8	20	9.5 ± .52	
	9	40	9.5 ± .52	
	10	55	11.1 ± .96	
$\bar{X}(Sx^2)$			9.6 (4.26)	
				$F_{(1,75)} = 38.2^*$
α-Toc	4	0		
	5	0		
	6	40	7.5 ± .52	
	7	71	7.2 ± .48	
	8	80	8.1 ± .35	
	9	N.T		
$\bar{X}(Sx^2)$			7.6 (.14)	

* $\alpha < .05$

(n = 48)

FIGURE 9. Graph illustrating the difference in time of fertilization between control and α-tocopherol cultured nematodes, as measured by the percentage of females which reproduced after isolation from mass cultures on different days after sexual maturation. (n= 30)

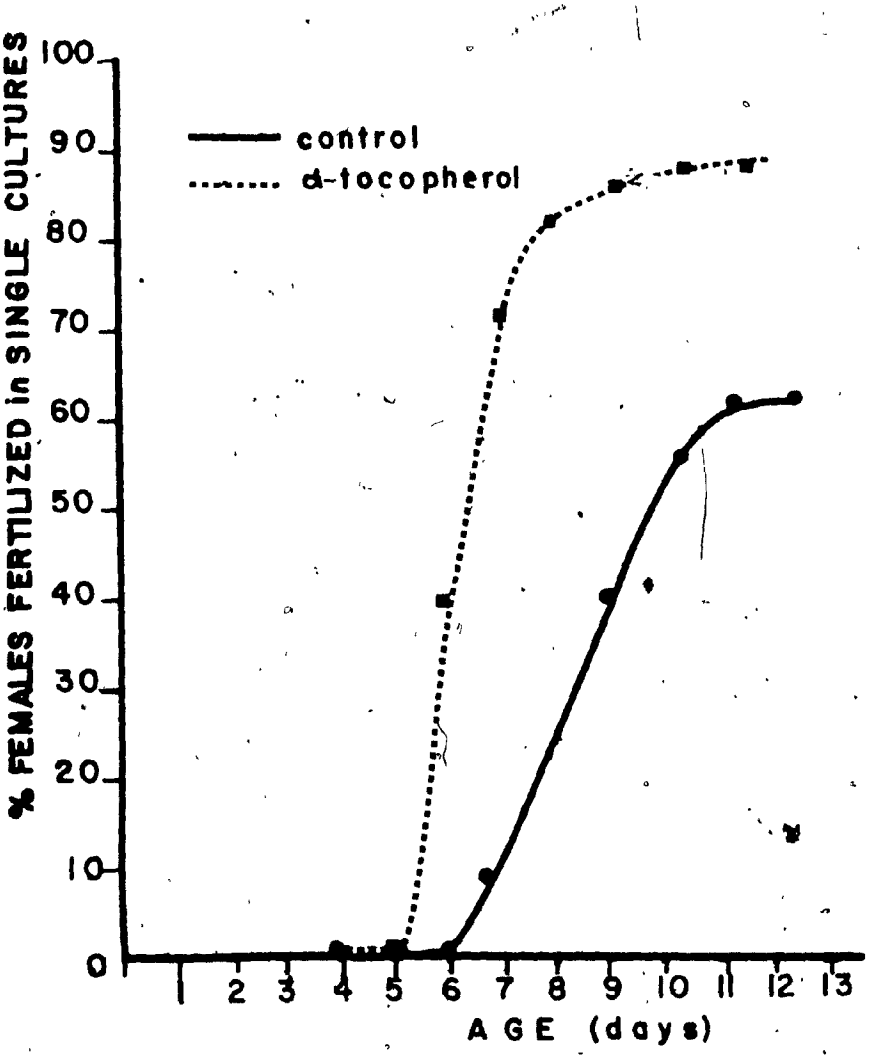

% FEMALES FERTILIZED in SINGLE CULTURES
100
90
80
70
60
50
40
30
20
10
0
control
d-tocopherol
1 2 3 4 5 6 7 8 9 10 11 12 13
AGE (days)

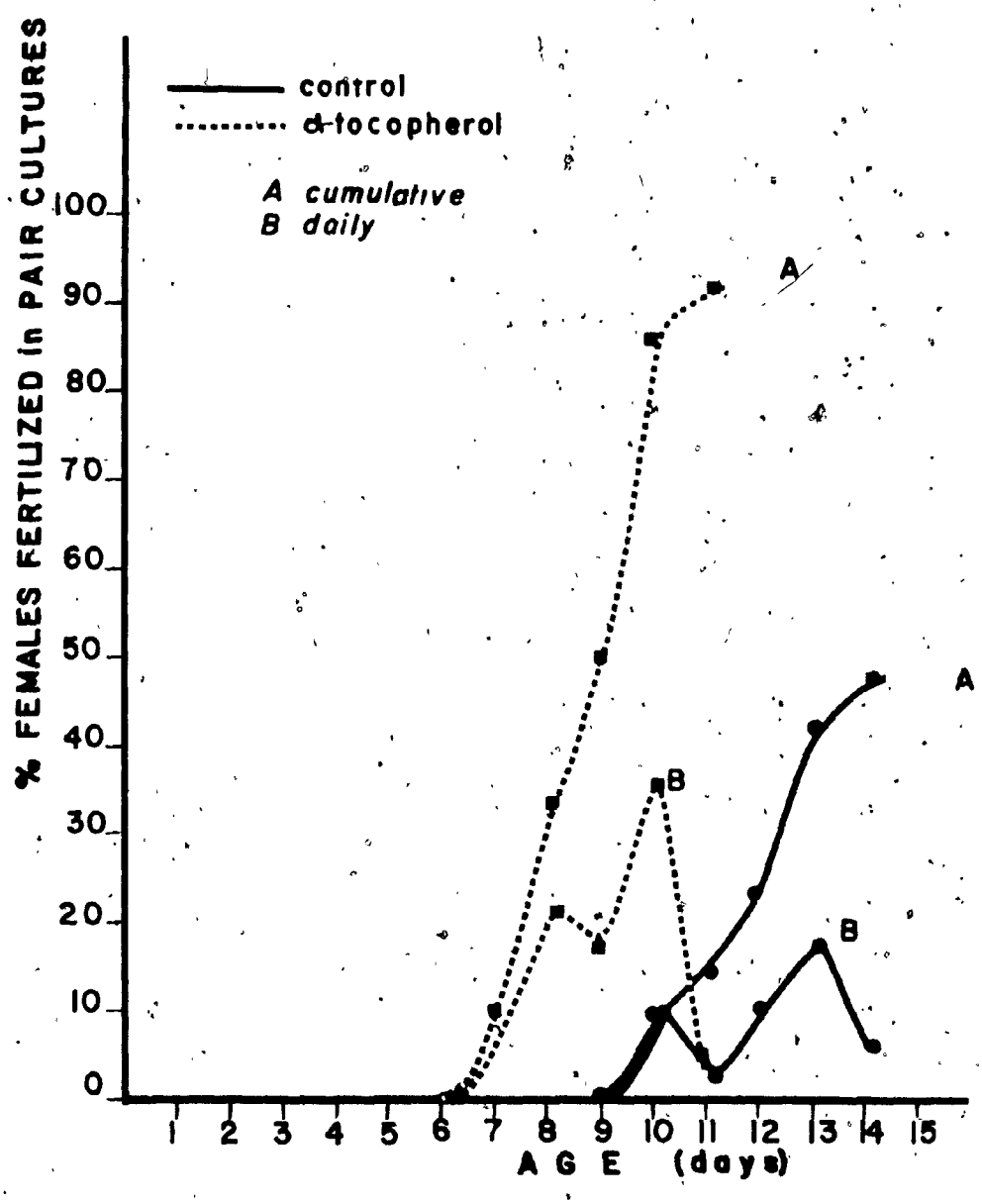
Table 6

Time Period Between Fertilization and Onset of Reproductive Period in Control and α-Tocopherol Cultured Nematodes

Media	Day of Isolation from Mass Culture	Females Reproducing (%)	Day of Onset of Rep. Period	Mean Onset of Rep. Period ± S.D. (days)	Mean Length of Rep. Period ± S.D. (days)
		(n = 30)			
Control	8	20	9		
		20	10	10.8 ± 1.4	7.8 ± 1.3
		40	11	(1.7)	
		20	13		
		(n = 28)			
α-Toc.	7	14	7	8.68 ± 1.0	8.0 ± 0.8
		42	8	(1.17)	
		14	9		
		28	10		
One-way ANOVA				$F_{(1,57)} = 43^*$	N.S
Student-t test Control vs. α-Toc.				$t_{(56)} = 6.29^*$	

* $\alpha < .05$

FIGURE 10. Graph illustrating the difference in the percentage of females which were fertilized after having been individually mated in control and α-tocopherol media. (n= 24)


control
d-tocopherol
A cumulative
B daily
% FEMALES FERTILIZED in PAIR CULTURES
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
AGE (days)
A
A
B
B

day eight for the control group, and on day seven for the α-tocopherol group. The onset of reproduction (determined as the time new-born larvae appear) was observed. Once again, the mean onset of reproduction proved to be significantly earlier in α-tocopherol females than in control females ($\alpha<.05$). No significant difference was observed between the lengths of the reproductive periods. The length of time between the time of isolation and the birth of progeny was also observed. For twenty per cent of control nematodes, there were five days between isolation and reproduction, whereas in no case was there more than three days between these two events for α-tocopherol females. This may mean that some females had not started oogenesis at the time of mating, or that females can store immature sperm until they mature. It may also indicate that the gestation period is longer for control nematodes than for α-tocopherol nematodes.

Table 7 contains data on the mean onset of reproduction and the mean length of the reproductive period with respect to two variables. These variables are the α-tocopherol treatment, and the culture method. Values for single cultures, as described above, are compared with the results from individually mated pair cultures.

Table 7

Comparison of Mating Times and Length of Reproductive Period in Single and Pair Cultures of Control and α-Tocopherol Supplemented Nematodes

Culture Type	Experiment	Mean Onset of Rep. Period ± S.D. (days)		Mean Length of Rep. Period ± S.D. (days)		Mann-Whitney U-test Control vs. α-Toc.	
		Control	α-Tocopherol	Control	α-Tocopherol	Onset	Length
Single	A	10.80±1.4	9.1±1.0 *	7.8±1.3	8.0±0.8		
	B	8.60±1.1	7.1±0.5 *	8.0±0.9	9.0±1.0 *		
	C	10.25±0.5	7.8±0.5 *	6.5±1.9	7.5±0.4 *	$U_{(4,4)}=15$**	$U_{(4,4)}=15.5$**
	D	9.80±1.5	7.4±0.5 *	6.8±1.0	7.8±0.8 *		
Pair	A	12.50±2.7	8.8±1.3 *	4.8±0.8	7.0±0.1 *		
	B	14.00±3.0	9.6±0.9 *	3.0±1.2	8.5±0.9 *		
	C	12.00±1.2	10.5±1.4 *	4.5±1.5	7.2±1.7 *	$U_{(4,4)}=16$**	$U_{(4,4)}=16$**
	D	11.10±1.3	8.8±1.9 *	4.5±1.4	7.8±1.4 *		
Mann-Whitney U-Test Single vs Pair		$U_{(4,4)}=16$**	$U_{(4,4)}=14$	$U_{(4,4)}=16$**	$U_{(4,4)}=13$		

** $\alpha<.05$

* $\alpha<.05$ for student t-test comparing α-Tocopherol with respective control.

Each single culture represents 30 females.

Each individually mated culture represents 24 pairs.

Individually mated pairs. It is useful to consider the advantages of pair cultures over single cultures. Pair cultures can be used to evaluate the length of the reproductive period, and to test the reproductive capacity of individual males. Single cultures, on the other hand, are more accurate for evaluating the female's reproduction, since the male's reproductive capacity is not a limiting factor. Table 7 shows the difference between these two culture methods using two reproductive parameters.

A non-parametric test (Mann Whitney U-test), comparing the mean onsets obtained in single cultures with those in paired cultures, indicates that there is a significantly larger difference between culture methods in control animals than in α-tocopherol-cultured animals ($\alpha<.05$). Comparisons of culture methods as to mean length of reproduction gave similar results. Student *t*-tests also show that every mean onset obtained for α-tocopherol-cultured nematodes is significantly earlier ($\alpha<.05$) than the respective control value. Comparisons between control and α-tocopherol cultures indicate a significantly longer reproductive period in individually mated α-tocopherol cultures than in the individually mated control cultures. Comparisons with respect to this parameter in single cultured

females do not show a significant difference between control and α-tocopherol groups.

Mean number of offspring

The next parameter used to evaluate the effects of α - tocopherol was the number of live progeny produced. Two culture conditions were used for these studies: single females, and paired cultures. The number of live offspring produced in each 24 hour period was counted as the parents were transferred to new culture dishes (see Materials and Methods for details). Dead offspring were noted, but not counted.

Single culture. Table 8 shows the mean number of offspring per female isolated from mass cultures at different ages. The variation in the number of offspring produced by the α-tocopherol cultured groups is probably due to the loss of offspring born before the isolation of the female. Progeny counts may therefore be inaccurate where the females were isolated after day nine. Control nematodes, however, show a peak progeny production on the tenth or eleventh day after birth. A visual representation of these findings is shown in Figure 11. A clearer representation of the mean number of offspring per female may be obtained by grouping the means according to the onset of reproduction of the female, and the day of isolation of the female.

Table 8

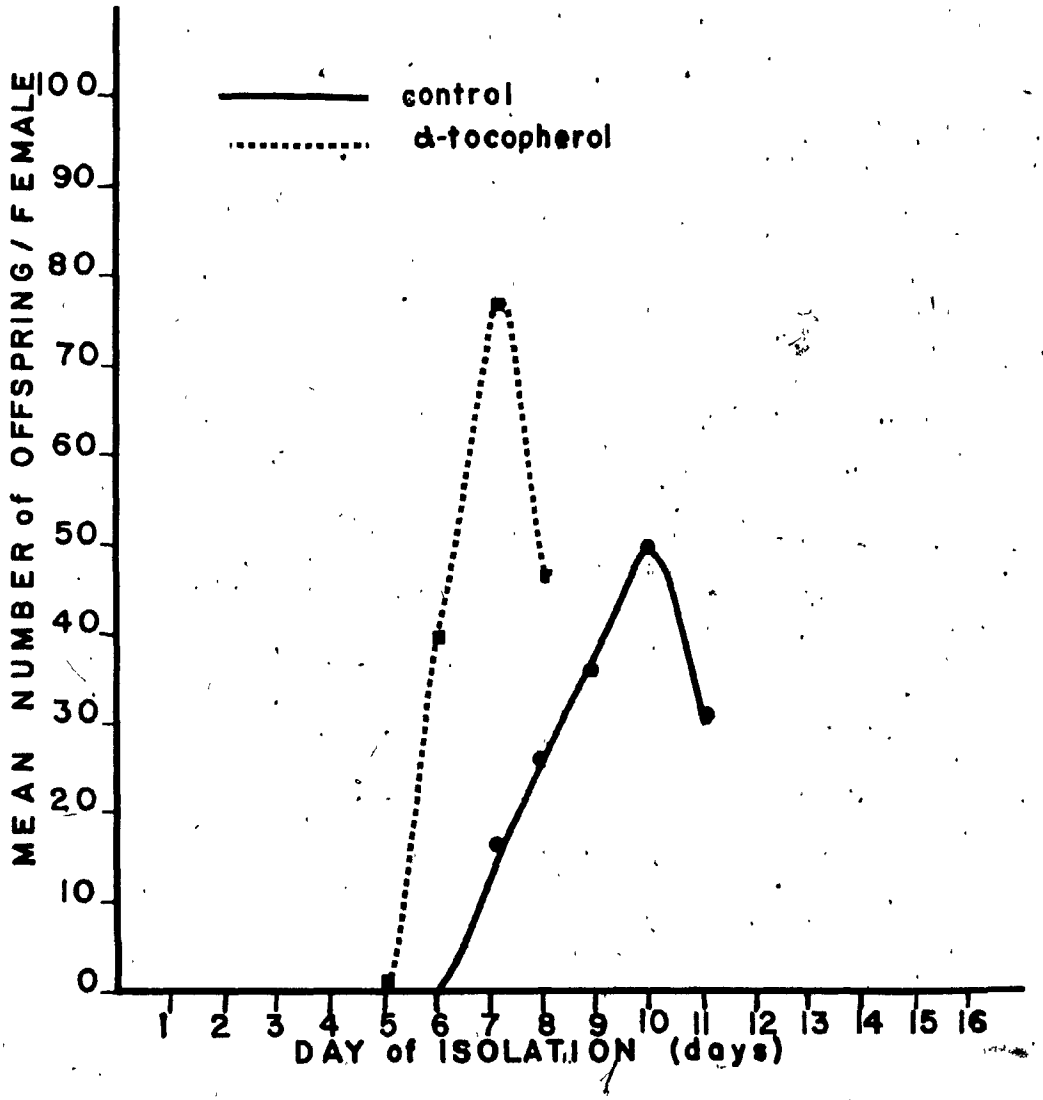
Mean # of Live Offspring Produced by Control and α-Tocopherol Cultured Females

Experiment	Age at the[1] time of Isolation from Mass Culture (days)	Mean # of Live Offspring ± S.D./Female		Mean Length of Rep. Period ± S.D. (days)	
		Control	α-Tocopherol	Control	α-Tocopherol
A	6	0	41.75±37.3	0	5.5 ± 1.5
B	7	16.50 ± 2.5	74.75± 7.3	4.0±1.0	7.8 ± 1.0
C	8	25.14 ± 14.0	46.20± 9.2	3.5±1.0	6.0 ± 0.5
D	9	36.20 ± 15.0	N.T	4.5±0.5	-
E	10	47.90 ± 21.0	N.T	6.5±1.5	-
F	11	48.00 ± 24.0	N.T	6.4±2.0	-

[1] age at time female isolated from mass culture.

N.T = not tested
(n=48)

FIGURE 11. Graph illustrating the difference in the mean number of offspring produced per female, between control and α-tocopherol cultured females isolated from mass cultures at different days after sexual maturation. (n=48)


control
d-tocopherol
MEAN NUMBER of OFFSPRING / FEMALE
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
DAY of ISOLATION (days)

An example of this may be seen in Figure 12. Control nematodes show a peak on the tenth day, whereas α-tocopherol females show peak offspring production on the seventh day. These results indicate that the state of maturity at the time of isolation does indeed affect the number of progeny produced.

Pair cultures. The variability with respect to offspring production that exists for single females, possibly due to the variation in the time of isolation, is reduced in pair cultures. Nontheless single cultures have their advantages over pair cultures in the evaluation of this parameter. Table 9 shows a comparison of these two culture procedures. Single females were isolated from mass cultures immediately after the first progeny appeared. Paired cultures were individually mated in the usual manner. Replicate experiments to determine total number of offspring were carried out for each culture method.

Student t-tests performed to compare α-tocopherol cultured nematodes with their respective controls, indicate that most α-tocopherol groups produce a significantly larger number of progeny ($\alpha<.05$) than the control groups, regardless of the type of culture used. A Mann Whitney U-test also demonstrates this significant difference. No significant difference

Table 9

Comparison of Mean # of Live Offspring in Pair and Single Cultures of Control and α-Tocopherol Treated Nematodes

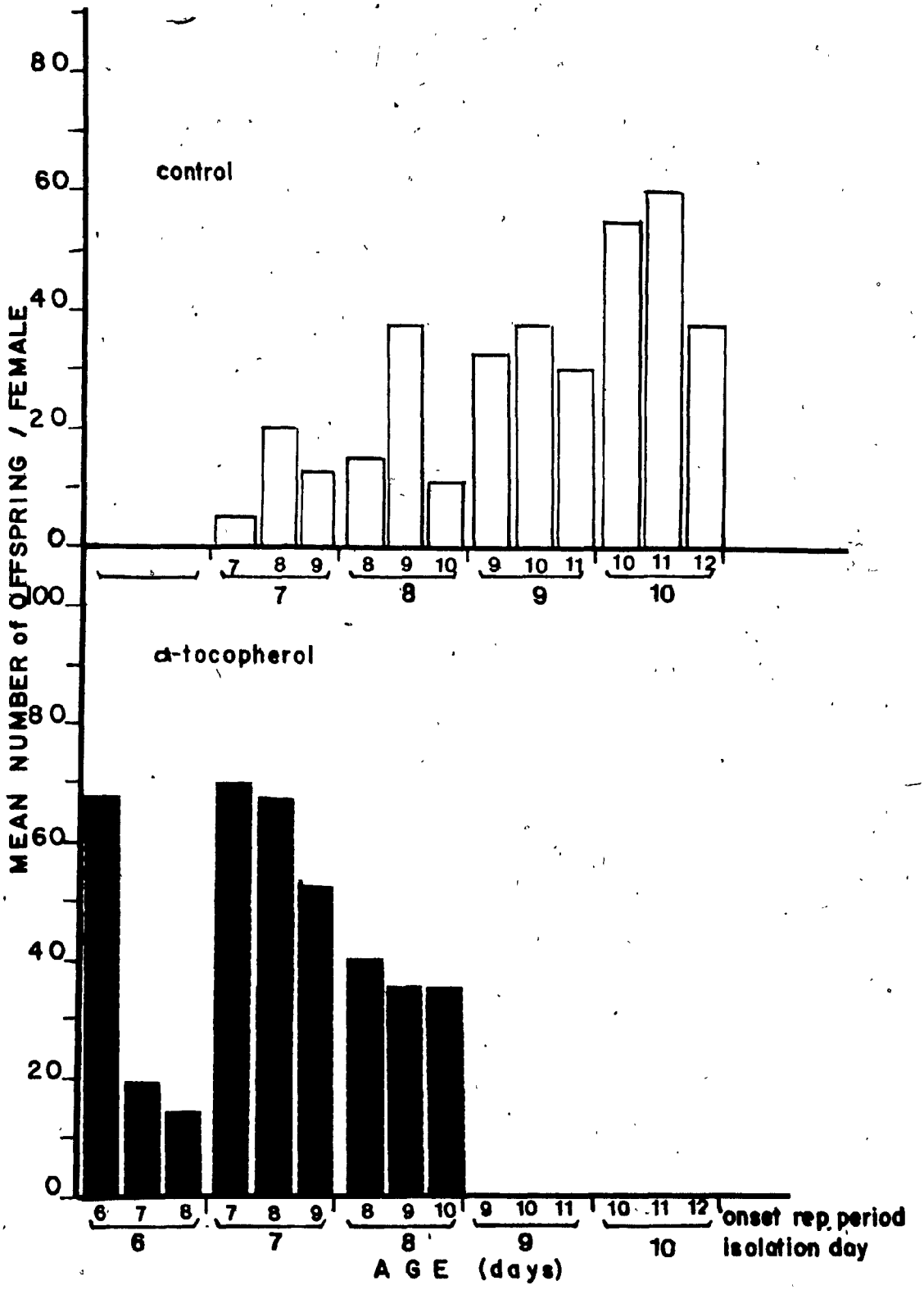
Experiment	Type of Culture	Mean # of Live Offspring ± S.D.		Statistical Comparison between Control/ α-Tocopherol	
		Control	α-Tocopherol	Student t-Test	Mann-Whitney U-Test
A	Single	42.0±17	71.0±17	6.7*	
B	Single	59.0±20	75.3±13	$t_{(30)}$ 3.7*	$U_{(3,3)} = 9*$
C	Single	67.8±29	75.0±17	1.31	
A	Paired	41.0±23	87.3±20	7.4*	
B	Paired	23.0± 7	91.3±40	8.2*	
C	Paired	36.0±13	67.5±36	$t_{(24)}$ 3.9*	$U_{(5,5)}=25*$
D	Paired	35.0±20	74.0±20	8.8*	
E	Paired	38.0±21	60.0±32	2.8*	
Mann-Whitney U-Test Comparing Single/Paired		$U_{(3,5)}=15*$	N.S		

* $\alpha < .05$

Each single culture represents 30 females.

Each individually mated culture represents 24 pairs.

FIGURE 12. Histogram illustrating the mean number of offspring produced by control and α-tocopherol females isolated from mass cultures at different days after sexual maturation and starting their reproductive period on different days after isolation.


control
α-tocopherol
MEAN NUMBER of OFFSPRING / FEMALE
80
60
40
20
0
6 7 8
7 8 9
8 9 10
9 10 11
10 11 12
6
7
8
9
10
onset rep. period
isolation day
AGE (days)

was observed between culture methods in α-tocopherol cultured nematodes.

Figure 13 shows the difference in reproductive rates between single and individually paired cultures. Reproductive rate was defined as a mean number of offspring per female or pair, per day. It is worthy of notice that α-tocopherol females seem to produce a higher number of offspring on the first day of their reproductive period than control females. A more accurate comparison of total number of offspring per pair is obtained by examining the distribution of total offspring produced among pairs presented in Figure 14.

The Effects of α-Tocopherol Supplementation During Various Stages of Development

The experiments in this section were designed to determine the age at which α-tocopherol has the greatest influence on reproduction, and thus to elucidate some of the mechanisms involved. As mentioned in the Materials and Methods, nematodes of control origin were transferred to α-tocopherol-supplemented media during the more rapid phases of growth. The same parameters of reproduction used above were examined.

FIGURE 13. Histogram illustrating the difference in reproductive rate (offsprings / female / day) between control and α-tocopherol females isolated from mass cultures just prior to the onset of reproduction (single) and females mated individually(pairs).

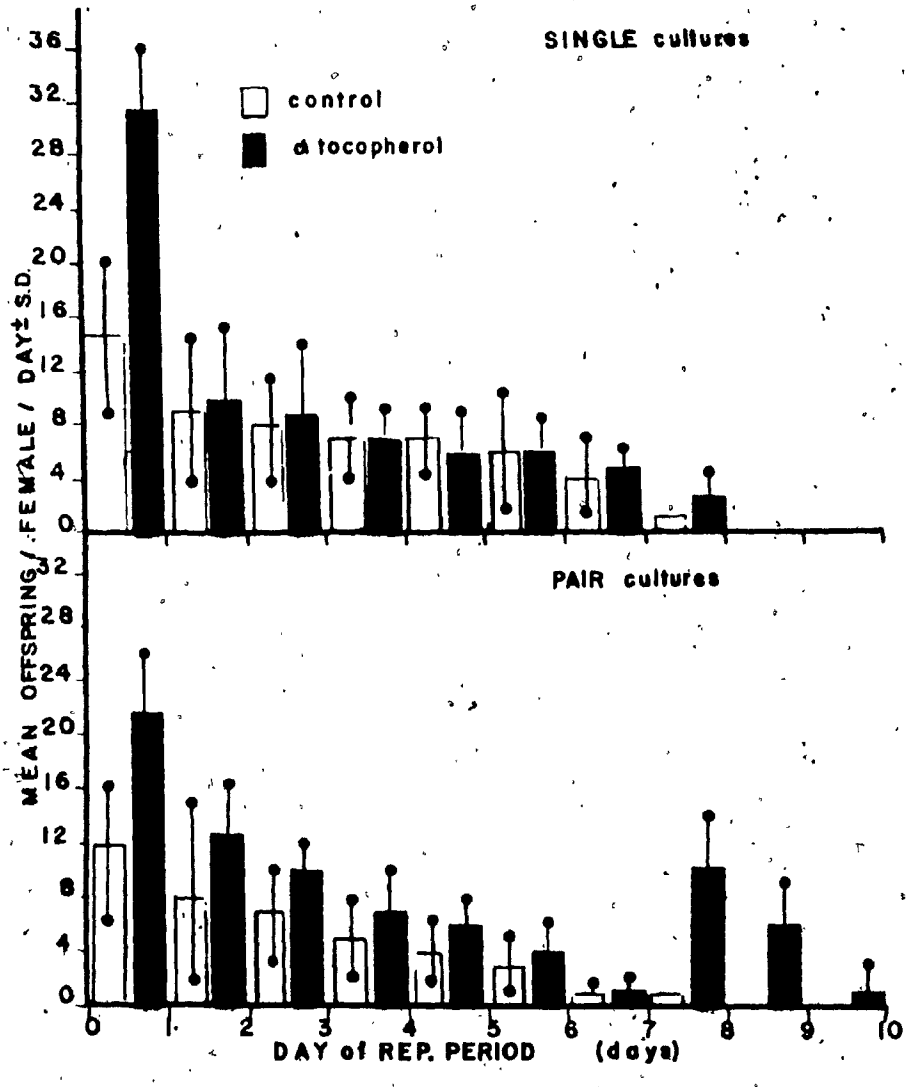

SINGLE cultures
control
d tocopherol
PAIR cultures
MEAN OFFSPRING / FEMALE / DAY ± S.D.
DAY of REP. PERIOD (days)
0
4
8
12
16
20
24
28
32
36
0
1
2
3
4
5
6
7
8
9
10

FIGURE 14. Histogram illustrating the difference in the distribution of number of offspring produced between control and α-tocopherol individually mated pairs. (n= 24x3)

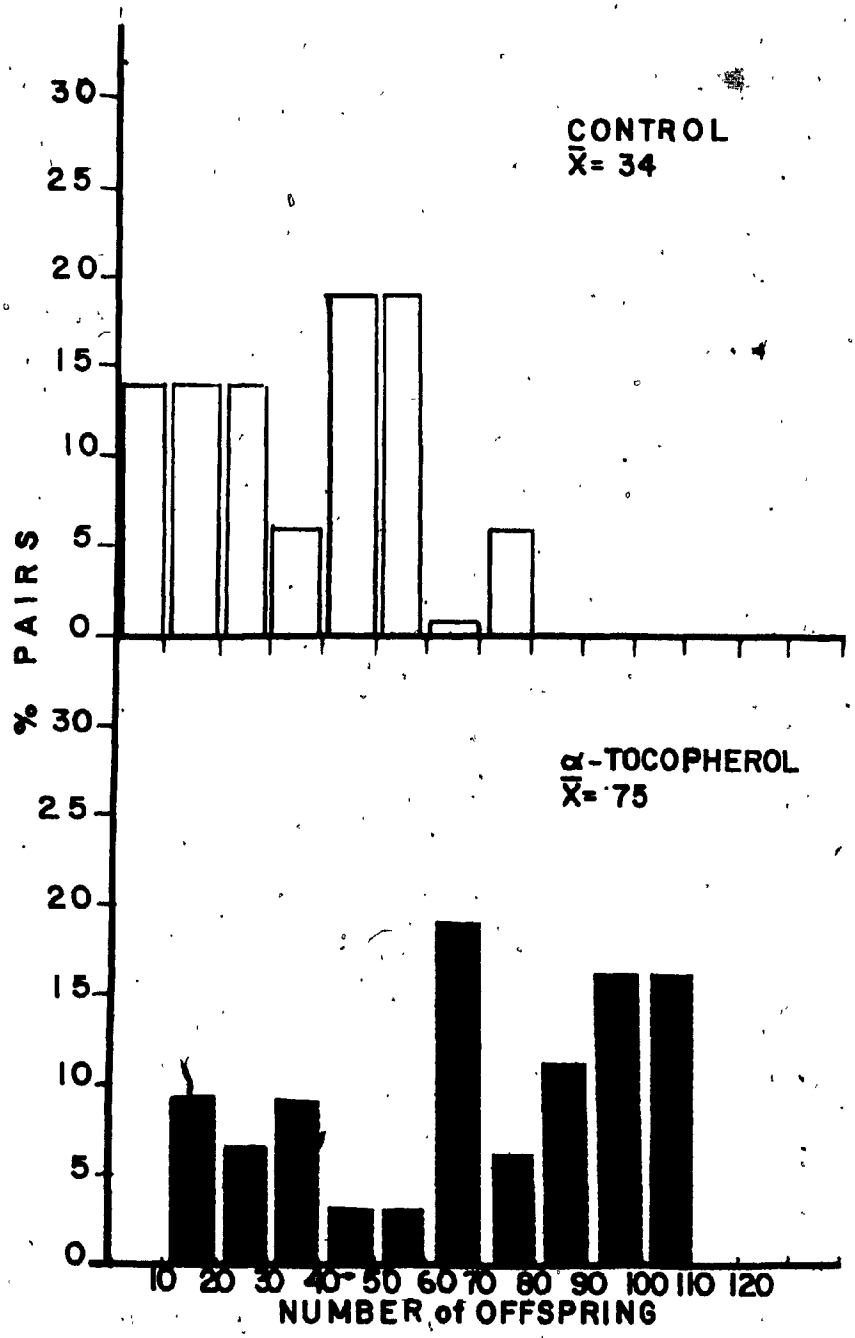

CONTROL
X̄= 34
α-TOCOPHEROL
X̄= 75
% PAIRS
0
5
10
15
20
25
30
10 20 30 40 50 60 70 80 90 100 110 120
NUMBER of OFFSPRING

Table 10 shows the differences in reproductive capacity among nematodes in paired cultures supplemented with α-tocopherol at successive stages of development. When α-tocopherol was added before birth, it caused the mean onset of the reproductive period to occur somewhat earlier than in control nematodes. When α-tocopherol was added at a later stage, there was no significant effect.

The results in Table 10 also indicate a decreased reproductive capacity (percentage fertilized) in nematodes supplemented with α-tocopherol between 72 and 96 hours after birth. This difference is largely due to the high percentage of nematodes that did not mature (dauerlarvae) which appeared in these groups. Except for these two groups, nematodes supplemented with α-tocopherol appear to have a higher per cent fertilized as compared to the control group. This is particularly obvious in the group of nematodes supplemented with α-tocopherol after 96 hours of age. It is interesting to note that dauerlarvae supplemented with α-tocopherol later than 96 hours after birth all eventually matured. This total recuperation was not observed in dauerlarvae of control origin supplemented with α-tocopherol at an earlier age. The possibility of a difference in absorbtion capacity should be considered.

Table 10

The Effect of α-Tocopherol When Supplemented During Different Stages of Development

Origin of Nematodes	Age at Time of α-Toc. Supp. (hours)	n. (pairs)	Pairs Fertilized (%)	Dauer-larvae (%)	Mean Onset of Rep. Period±S.D. (days)	Mean Length of Rep. Period±S.D. (days)	Mean Total # of Offspring ± S.D. Per Pair
Control	-	24	41	8	12.3±1.4	4.0±1.8	38.0±21.0
α-toc.	-	24	50	8	*10.5±2.5	*6.0±0.8	*54.0±12.0
Control	0-6	15	46	12.5	11.0±2.0	*7.5±1.5	*69.0±12.0
Control	24-36	24	50	8	11.1±0.89	*8.0±1.0	*72.0± 9.8
Control	36-48	24	63	8	12.3±3.0	*7.6±1.2	50.5±40.0
Control	48-72	24	66	8	12.1±1.2	*6.5±1.5	44.5±10.0
Control	72-84	20	42	33	12.0±2.0	*7.0±1.5	46.1±30.0
Control	84-96	24	28	33	12.3±1.0	(*)5.6±1.2	59.9±49.0
Control	92-120	24	88	0	12.8±1.0	*7.4±3.0	57.2±22.5
Control	>120	24	80	0	13.0±1.0	5.1±1.4	58.5±38.0

* $\alpha < .05$ for Student t-test as compared to control

Table 10 also shows that the reproductive period was significantly lengthened for most groups to which α-tocopherol was added. The only group that shows a non-significant difference compared to the control, is the group supplemented after 120 hours.

The mean number of progeny produced also appears to be affected by the supplementation of α-tocopherol, the only significant increase however was observed in those nematodes supplemented before 36 hours of age.

The experiments shown on Table 11 were designed to determine how the addition of α-tocopherol to nematodes (F_0) would influence their progeny (F_1) or the next generation (F_2). In other words, how long does it take for the full effect of α-tocopherol to appear? F_0 nematodes were transferred to α-tocopherol media at one of two stages: just before their birth (BB) and just before the onset of their reproductive periods (BR). In the first case, the nematodes were transferred after their embryonic development (or, somatic cell differentiation) was completed, but before their reproductive organs had differentiated. In the second case, the F_0 nematodes were transferred prior to the onset of their reproductive period; hence the transfer was prior to embryogenesis of the F_1 nematodes. As the F_0 nematodes and their descendants developed, they

were examined for the influence of α-tocopherol on the percentage of females fertilized, the percentage of dauerlarvae produced, the time of onset of reproduction, the length of the reproductive period and the number of offspring produced.

Using two transfer times allowed such testing for four main types of nematodes: 1) BB-F_0 nematodes, which were transferred before the development of reproductive organs; 2) BR-F_0 nematodes, which were transferred after reproductive cells had differentiated, but before spermatogenesis and oogenesis had commenced; 3) BR-F_1 nematodes, in which the germ cells that produced the nematodes were formed in α-tocopherol, but the reproductive organs of their parents were formed in control media; and 4) BB-F_1, BB-F_2 and BR-F_2, in which the germ cells and the reproductive organs of the parents were formed in α-tocopherol.

Table 11 shows the reproductive characteristics of these groups, compared with nematodes of control origin transferred to control media (both before birth and before reproduction), and nematodes of α-tocopherol origin transferred to α-tocopherol in a similar manner. These results show that the percentage of pairs fertilized is always higher in α-tocopherol supplemented nematodes (F_0, F_1 and F_2), than in control nematodes.

Table 11

The Effect of Supplementing Turbatrix Aceti with α-Tocopherol Before Birth and Before the Onset of the Reproductive Period on their Reproductive Capacity and the Reproductive Capacity of their Progeny

Origin and Transfer Time	Age at Time of α-Toc. Supp. (hours)	n (pairs)	Pairs Fertilized (%)	Dauer-larvae (%)	Mean Onset of Rep. Period±S.D. (days)	Mean Length of Rep. Period±S.D. (days)	Mean # of Offspring ± S.D. Per Pair Live	(dead)
Control[1]	-	30	75	4	**10.5±0.9	**5.0±1.4	45.8±33	+
Control[2]	-	24	55	8	**10.7±1.0	4.1±1.4	38.0±21	+
α-Toc.[1]	-	24	83	4	*8.8±0.8	*7.1±1.2	59.4±31	0
α-Toc.[2]	-	24	83	8	*8.6±1.0	*8.0±1.2	*62.1±30	0
F_0 Control (BB)	<0	24	81	8	**9.6±1.3	*6.5±1.7	*66.6±26	0
F_0 Control (BR)	96	24	100	0	**10.2±2.0	*7.0±2.6	49.6±31	+
F_1 Control (BB)	-	20	90	0	*8.9±0.6	*8.1±2.0	*72.8±27	0
F_1 Control (BR)	-	24	82	0	***9.6±1.6	*7.6±1.4	47.7±41	0
F_2 Control (BB)	-	22	100	0	*8.5±1.3	**5.6±2.3	46.0±31	0
F_2 Control (BR)	-	24	100	8	*8.6±1.0	**5.1±2.0	48.0±25	0

* $\alpha<.05$ for Student t-test as compared to respective control group
** $\alpha<.05$ for Student t-test as compared to respective α-tocopherol group
***$\alpha<.05$ for Student t-test comparing BB with respective BR
[1]controls for (BB)
[2]controls for (BR)

Once again, dauerlarvae appear to recuperate totally in groups supplemented with α-tocopherol after 96 hours of age. Supplementation before birth (BB-F_0) appears to cause the onset of reproduction to occur earlier than in nematodes supplemented after 96 hours of age (BR-F_0). This difference however, is not statistically significant ($\alpha > .05$). In both cases, the onset of reproduction is significantly later than that in nematodes of α-tocopherol origin ($\alpha < .05$). Significant differences in the time of onset of reproduction are observed after nematodes have been in α-tocopherol for one generation (F_1).

These results suggest that the presence of α-tocopherol before, and not after, embryogenesis is required for a significantly earlier onset to be observed, as compared with control nematodes. These results are in agreement with the maturation results shown in a previous section.

It is interesting to note, in the light of the above findings, that longer reproductive periods were observed in nematodes supplemented with α-tocopherol as late as 96 hours after birth (after reproductive organs have differentiated).

The mean total number of live offspring is somewhat affected by the length of the reproductive periods. Nematodes supplemented with α-tocopherol appear to

produce a higher number of live offspring than control groups. The large variance that exists among pairs with respect to this parameter makes it difficult to evaluate the significance of the difference (the evaluation of this parameter has been mentioned earlier). Figures 15 to 21 show a clearer representation of the differences between supplemented and non-supplemented nematodes with respect to this parameter.

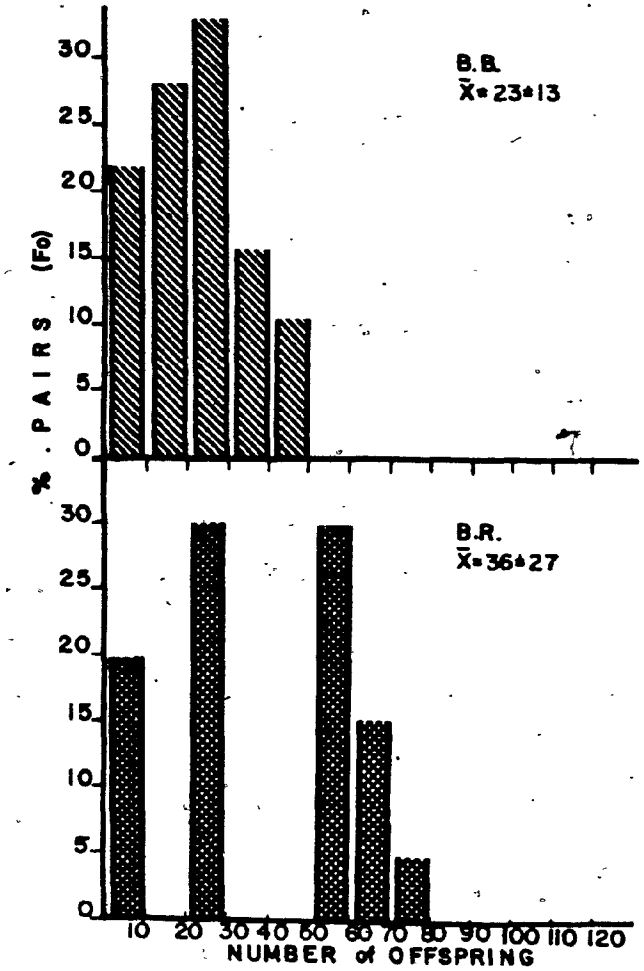
The Effects of Removing α-Tocopherol at Different Stages of Development

Table 12 shows the results of an experiment wherein nematodes of α-tocopherol origin were transferred out of the α-tocopherol medium, and into a control medium, at crucial stages of their development. The results of these experiments are compared with two controls: α-tocopherol nematodes transferred to α-tocopherol media, and control nematodes transferred to control media.

The data show that the percentage of the nematodes which matured that became fertilized is smaller in the groups which were removed from α-tocopherol to control media between 0 and 12 hours after birth, than in groups which were transferred from α-tocopherol to α-tocopherol media. The apparent decrease in

FIGURE 15. Histogram illustrating the difference in the distribution of offspring produced between pairs supplemented with α-tocopherol before birth (BB), and before the onset of reproduction (BR). (n-20/24).

FIGURE 16. Histogram illustrating the difference in the ditribution of offspring produced by progeny of nematodes supplemented with α-tocopherol before birth (BB), and before the onset of reproduction. (n-20/24).


B.B.
X̄=23±13
B.R.
X̄=36±27
% . PAIRS (Fo)
0
5
10
15
20
25
30
10 20 30 40 60 60 70 80 90 100 110 120
NUMBER of OFFSPRING

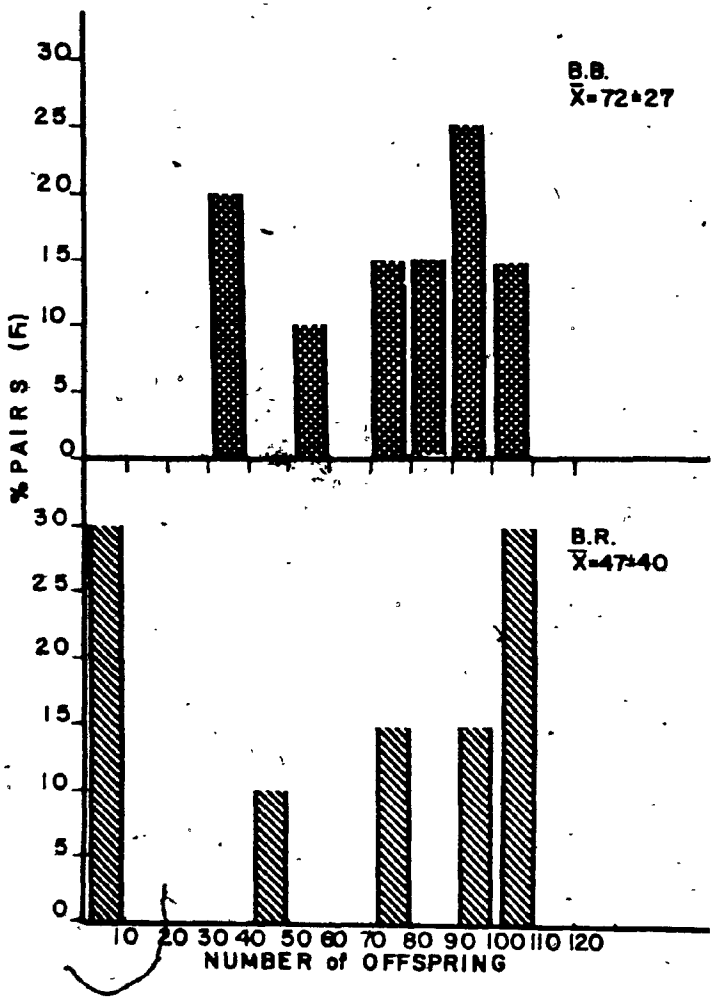

B.B.
X̄=72±27
B.R.
X̄=47±40
% PAIRS (F1)
0
5
10
15
20
25
30
10 20 30 40 50 60 70 80 90 100 110 120
NUMBER of OFFSPRING

Table 12

The Effect of the Removal of α-Tocopherol from The Nematodes Media During Different stages of Development on Reproduction

Origin of Nematodes	Age at Time of α-Tocopherol Depletion (hours)	Pairs Fertilized (%) (n = 30)	Dauer-larvae (%)	Mean Onset of Rep. Period±S.D. (days)	Mean Length of. Rep. Period±S.D. (days)	Mean Total # of Offspring ± S.D. per pair
Control	-	41	12	11.1±1.3	4.5±1.4	**38.0±21.0
α-Toc.	-	91	8	8.8±1.9	7.8±1.4	*73.0±28.0
α-Toc.	0-6	50	12	**13.1±2.0	**4.0±1.4	**22.0± 7.0
α-Toc.	6-12	61	4	*8.5±0.9	**4.1±1.0	**48.0±39.0
α-Toc.	24-48	66	33	*9.4±2.3	**4.6±1.1	**44.5±34.0
α-Toc.	48-72	80	20	*8.3±1.0	*6.5±1.5	*74.5±13.6
α-Toc.	72-96	81	8	*9.2±0.8	**6.2±1.5	*67.8±38.0
α-Toc.	96-120	90	8	*7.8±0.8	*5.7±1.5	*66.4±23.0
α-Toc.	>120	90	8	*9.5±0.8	**5.1±1.6	*60.0±20.0

* $\alpha < .05$ for Student t-test as comparison to control

**$\alpha < .05$ for Student t-test as comparison to α-tocopherol

fecundity in groups removed from α-tocopherol at 24-36 hours is due to an increase in dauerlarvae in this group.

Removal from α-tocopherol medium between 24-72 hours appears to significantly increase the number of dauerlarvae. Nematodes depleted before 24 hours, on the other hand do not appear to be affected in this way. It should be noted that nematodes of α-tocopherol origin are expected to be between their first and third molts between 24-72 hours (see Figure 3b)

The time of onset of the reproductive period did not appear to be affected by transfer from α-tocopherol to control media. The only group significantly affected was the group so transferred right after birth. One should note that previous experiments suggested that the onset of reproduction is not influenced by α-tocopherol supplementation for at least one generation.

All groups removed to control media from α-tocopherol media (except the group so removed in the 24-72 hours period) showed a significantly shorter reproductive period, compared with nematodes transferred from α-tocopherol media, to α-tocopherol media. Generally, it appears that the earlier the nematodes were removed from the α-tocopherol, the shorter the reproductive period was. This suggests that removal

of α-tocopherol affects the length of the reproductive period regardless of whether the removal is effected before or after reproductive cell formation. This observation is in accordance with the results of the supplementation experiments, which showed that significant lengthening of the reproductive period occurred even when the supplementation occurred in late or after gonadogenesis.

Nematodes removed from α-tocopherol between 0 and 30 hours had a significantly smaller number of live offspring than those maintained in α-tocopherol ($\alpha<.05$). It appears that the longer the nematodes were maintained in α-tocopherol media before transfer to control media, the greater the number of live offspring. This may be a direct result of the length of the reproductive period, noted above.

Table 13 shows the results of experiments designed to determine whether the effects of α-tocopherol are passed on to future generations by nematodes removed from α-tocopherol at one of two stages: before their birth (BB), and before the onset of their reproductive periods (72-96 hours) (BR). The same parameters were observed as in the previous experiments. The generation removed from α-tocopherol will be referred to as F_0, their progeny as F_1, and the third generation as F_2.

Table 13

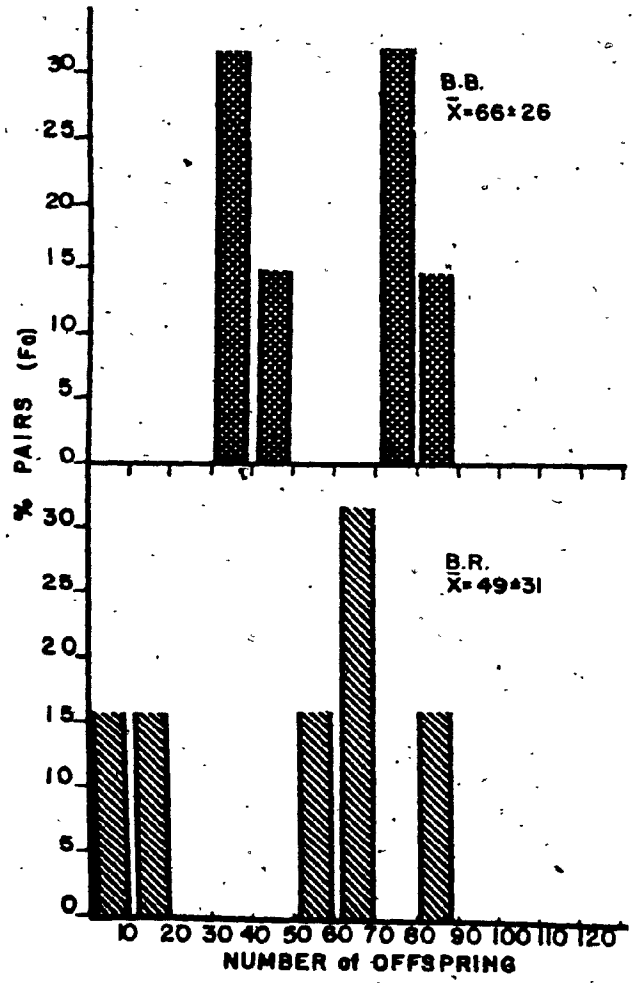
The Effect of Depleting Turbatrix Aceti of α-Tocopherol Before Birth and Before the Onset of the Reproductive Period on their Reproductive Capacity and the Reproductive Capacity of their Progeny

Origin and Transfer Time	Age at Time of α-Toc. depletion (hours)	n (pairs)	Pairs Fertilized (%)	Dauer-larvae (%)	Mean Onset of Rep. Period ± S.D. (days)	Mean Length of Rep. Period ± S.D. (days)	Mean Total #-of offspring ± S.D. per pair	
							live	(dead)
α-Toc.[1]	-	24	83	4.1	8.8±0.8	7.1±1.2	59.40±31.0	0
α-Toc.[2]	-	24	81	8	8.6±1.0	8.0±1.2	62.10±30.0	0
F_0 α-Toc. (BB)	<0	20	60	16	9.3±1.4	**4.1±1.5	**23.50±13.0	0
F_0 α-Toc. (BR)	72-96	24	80	20	9.2±1.0	**5.5±1.2	**36.00±27.0	+
F_1 α-Toc. (BB)	-	24	55	20	9.8±1.6	**4.4±1.9	**33.00±11.0	+
F_1 α-Toc. (BR)	-	26	44	40	**10.0±2.0	**3.5±1.0	**14.00± 7.7	+
F_2 α-Toc. (BB)	-	24	56	20	**10.6±1.1	**3.2±1.0	**22.75±14.0	+
F_2 α-Toc. (BR)	-	16	40	20	**11.5±1.5	**4.0±1.0	**19.50±19.0	+

** $\alpha < .05$ for student t-test as compared to respective α-tocopherol group.
[1] controls for BB groups
[2] controls for BR groups

FIGURE 17. Histogram illustrating the difference in the distribution of offspring produced between pairs of α-tocopherol origin removed from α-tocopherol medium before birth and before the onset of reproduction. (n-24/26).

FIGURE 18. Histogram illustrating the difference in the distribution of offspring produced by progeny of nematodes removed from α-tocopherol medium before birth and before the onset of reproduction. (n-24/26).


B.B.
X̄=66±26
B.R.
X̄=49±31
% PAIRS (Fo)
0
5
10
15
20
25
30
10 20 30 40 50 60 70 80 90 100 110 120
NUMBER of OFFSPRING

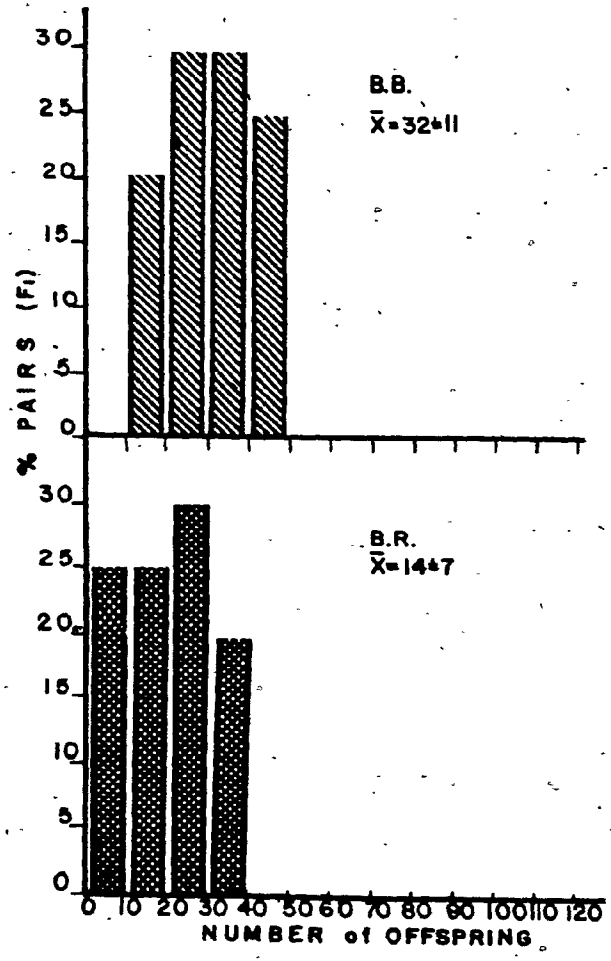

B.B.
X̄=32±11
B.R.
X̄=14±7
% PAIRS (Fi)
0
5
10
15
20
25
30
0 10 20 30 40 50 60 70 80 90 100 110 120
NUMBER of OFFSPRING

FIGURE 19. Histogram illustrating the difference in reproductive rate (# of offspring/ female / two day interval) between individually mated pairs cultured in control and α-tocopherol media.

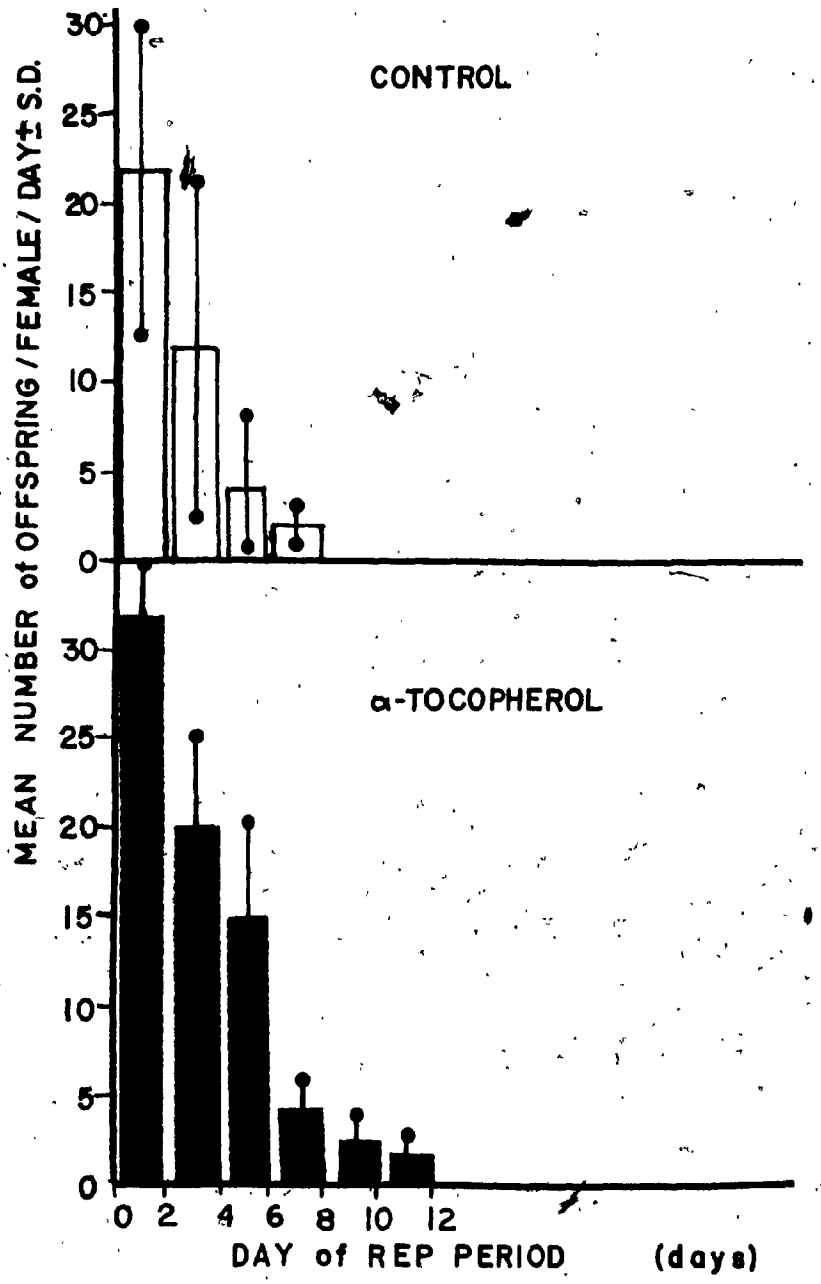

CONTROL
α-TOCOPHEROL
MEAN NUMBER of OFFSPRING / FEMALE / DAY ± S.D.
30
25
20
15
10
5
0
0 2 4 6 8 10 12
DAY of REP PERIOD (days)

FIGURE 20. Histogram illustrating the difference in reproductive rate between nematodes of control origin supplemented with α-tocopherol before birth (BB) and before the onset of reproduction (BR).

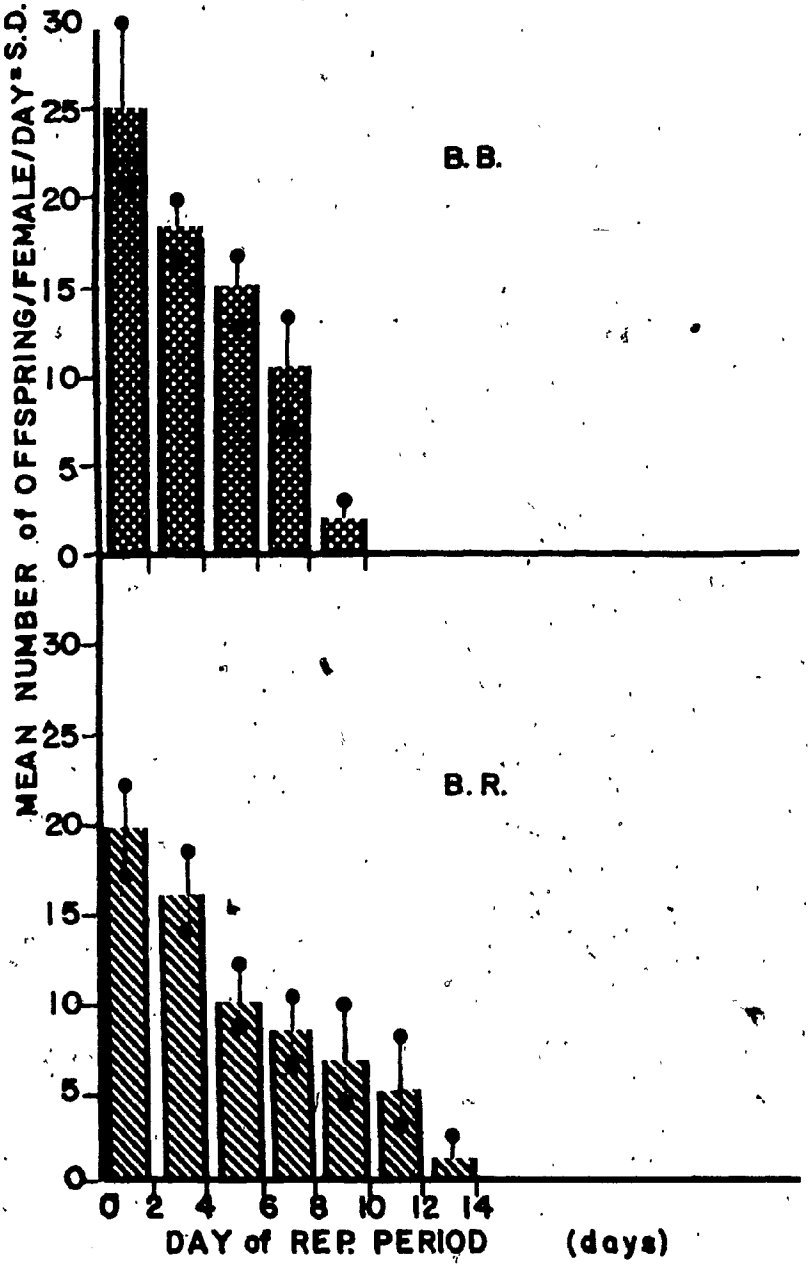
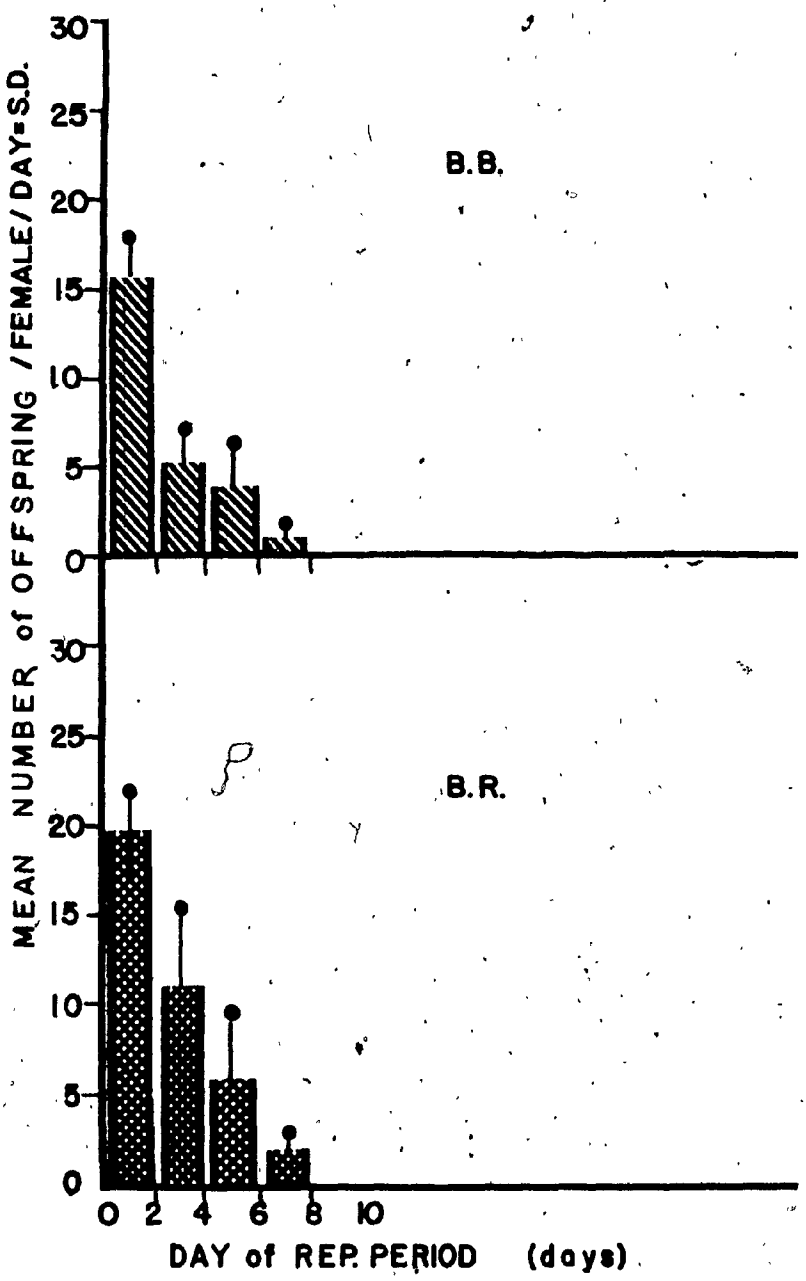

MEAN NUMBER of OFFSPRING/FEMALE/DAY ± S.D.
30
25
20
15
10
5
0
B.B.
B.R.
0 2 4 6 8 10 12 14
DAY of REP. PERIOD (days)

FIGURE 21. Histogram illustrating the difference in reproductive rate between nematodes of α-tocopherol origin removed from α-tocopherol medium before birth (BB) and before the onset of reproduction (BR).


MEAN NUMBER of OFFSPRING /FEMALE/ DAY±S.D.
30
25
20
15
10
5
0
B.B.
30
25
20
15
10
5
0
B.R.
0 2 4 6 8 10
DAY of REP. PERIOD (days)

A decreased percentage of mature pairs fertilized was observed in nematodes removed from α-tocopherol before birth, but this parameter was not affected in the F_0 BR group. It is interesting to note that the increased percentage of dauerlarvae observed in the F_0 generation persists into the next two generations as well.

The mean onset of reproduction took place slightly (but not significantly) later in the F_0 generation (both BB and BR) than in the group maintained in α-tocopherol. By the F_2 generation, the mean onset of reproduction was significantly later for all groups removed from α-tocopherol. After one generation, the time of onset for groups removed from α-tocopherol approached that for nematodes of control origin (Table 13). The mean length of the reproductive period became shorter in the groups removed from α-tocopherol than in groups maintained in α-tocopherol. This effect was noticeable as early as the F_0 generation. A significant decrease in the total number of offspring was also noticed following removal from α-tocopherol (Figures 17,18 and 21).

It may be noted that the reproductive capacities of the α-tocopherol-depleted nematodes observed after one or two generations appear to be significantly poorer than those of nematodes of control origin,

maintained in control media throughout (see Table 11). This may be explained by the fact that in order to obtain a large enough number of pairs of nematodes for a significant study, it was necessary to use progeny obtained from all litters. As has been explained elsewhere, later litters were usually not used for experimental purposes to avoid variables in reproduction that may arise from their early maturation. This difference is usually observed in control nematodes but not in α-tocopherol nematodes (Table 2), and hence we can infer that the poor reproductive capacity of the F_2 generation is a result of low fertility in late litters, and that these are no longer under the influence of α-tocopherol. The increasingly poor performance may also be attributed to a genetic variable. The presence or absence of dead larvae (indicated on Table 11 by a (+) or a (0), respectively) should also be noted. Dead larvae were not observed in the F_0 BB group. Dead larvae were observed in the F_0 BR group and in all F_1 and F_2 groups. These results indicate that the effect of α-tocopherol on reproduction that exist in nematodes grown in α-tocopherol medium is lost when these nematodes are removed from this medium.

The Differences Between the Effects of α-Tocopherol on Males and Females

In the next experiment (Table 14), males of α-tocopherol origin were mated with control females (A), and vice versa (B), by pairing them in control media between five and six days of age. The object was to determine differences between the effects of α-tocopherol on males and females. There were also three control groups: control males and control females in control media (1), α-tocopherol males and α-tocopherol females in α-tocopherol media (2) and α-tocopherol males and α-tocopherol females in control media (3).

While onset of reproduction was significantly earlier in each of A and B than in control, there is no significant difference between A and B. The length of the reproductive period for B appears to have been significantly shorter than that for A ($\alpha<.05$). Group A appears to show a significantly longer reproductive period than control 1 ($\alpha<.05$). However, the fact that group A also appears to have a significantly longer reproductive period than control 3, together with the degree of variance observed in the length of the reproductive period among replicate experiments (see page 85) casts some doubt upon the

Table 14

The Reproductive Capacity of α-Tocopherol Males Mated to Control Females was Compared to the Reproductive Capacity of α-Tocopherol Females Mated to Control Males

Group	Origin of Male	Origin of Female	Experimental Media	n (pairs)	Pairs Fertilized (%)	Mean Onset of Rep. Period ± S.D. (days)	Mean Length of Rep. Period ± S.D. (days)	Mean # live offspring ± S.D.
1	Control	Control	Control	72	65	**10.5±0.9	**5.0±1.0	**38± 7
2	α-Toc.	α-Toc.	α-Toc.	72	83	*8.9±1.0	*8.0±1.0	*72±15
3	α-Toc.	α-Toc.	Control	30	80	*8.4±1.0	**5.2±2.0	**47±16
A	α-Toc.	Control	Control	30	77	*7.7±0.4	***6.3±0.5	***32±12
B	Control	α-Toc.	Control	30	50	*8.0±1.0	***4.2±0.83	***23± 9
Test statistic comparing A with B						N.S.	$t_{(36)}=9.2$	$t_{(36}=2.64$

* $\alpha<.05$ for Student t-test compared with group 1

** $\alpha<.05$ for Student t-test compared with group 2

*** $\alpha<.05$ for Student t-test compared with group 3

true significance of these figures. The fact that control 2 had a significantly longer reproductive period than control 3 suggests that the experimental medium used is usually more important than the medium of origin, for this parameter.

The number of live progeny produced was somewhat higher in group A than in group B. However, both values were significantly lower than that in control 3. The fact that control 3 nematodes had significantly more offspring than control 1 nematodes confirms that the origin of the nematodes is quite important in evaluating this parameter. The significantly lower number of progeny in control 3 than in control 2 shows that the experimental media also plays an important role here. The length of the reproductive period will also influence the number of live progeny.

The only parameter which showed an apparently significant difference between A and B groups (and which was unaffected by the experimental medium) was the percentage of pairs which were fertilized. The results suggest that male vitality was more affected by α-tocopherol than was female vitality. This proposition is not inconsistent with the lack of a difference between groups A and B with respect to the other parameters. In drawing those results, pairs

FIGURE 22. Histogram illustrating the difference in offspring distribution between control males mated with α-tocopherol females (A) and control females mated with α-tocopherol males (B). (n=30)

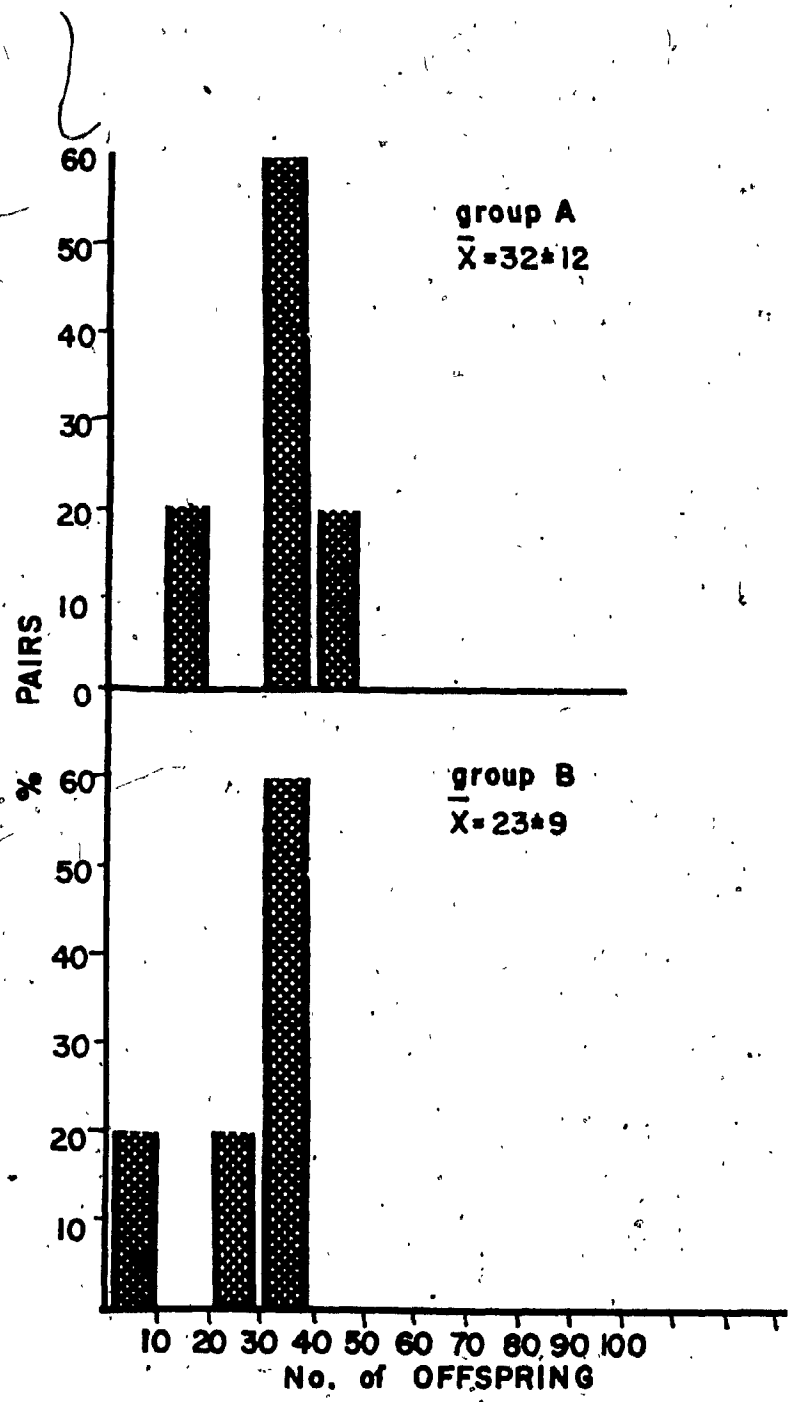
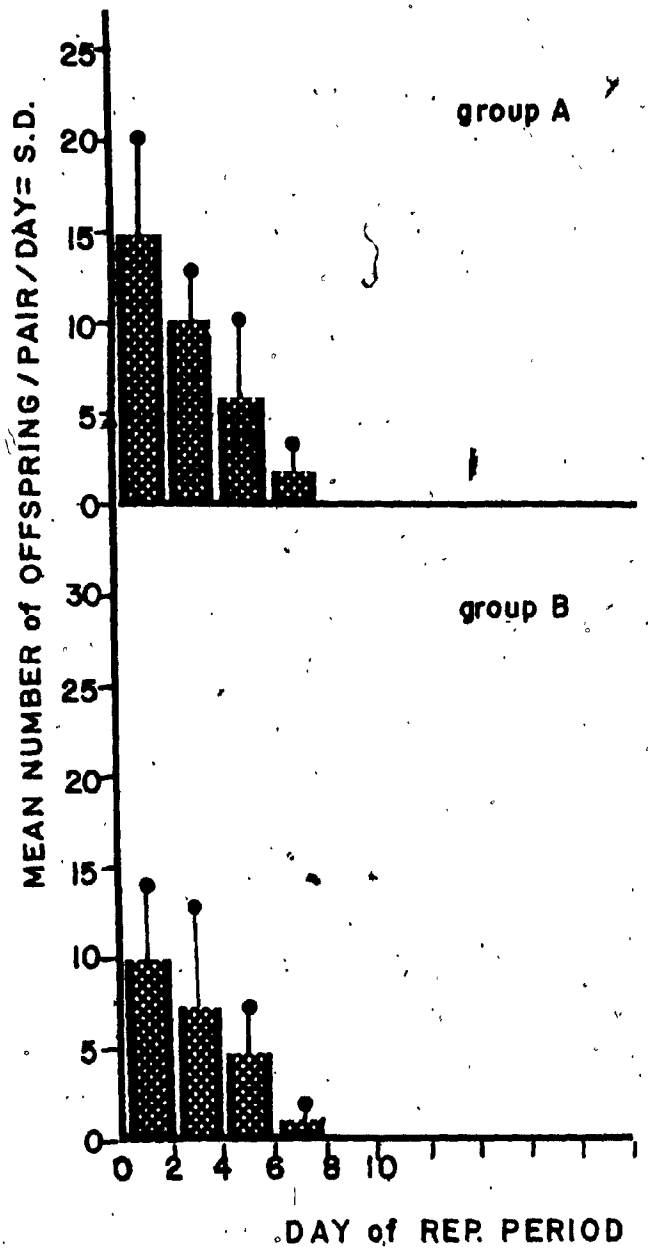

group A
$\bar{X}$=32±12
group B
$\bar{X}$=23±9
% PAIRS
60
50
40
30
20
10
0
10 20 30 40 50 60 70 80 90 100
No. of OFFSPRING

FIGURE 23. Histogram illustrating the difference in the reproductive rate of individually mated pairs, where control females were mated with α-tocopherol males (A), and vice versa (B).


group A
group B
MEAN NUMBER of OFFSPRING / PAIR / DAY = S.D.
25
20
15
10
5
0
30
25
20
15
10
5
0
0 2 4 6 8 10
DAY of REP. PERIOD

where no fertilization occurred were necessarily excluded in calculating mean onset, length of reproduction and number of offspring.

The results in this section do not let one draw any significant conclusions as to whether α-tocopherol has a greater direct effect on the reproductive system of the male or female of the species Turbatrix aceti. They do suggest, however, that the male's vitality is somewhat more affected than that of the female.

The Effect of α-Tocopherol on the Reproductive Capacity of Senescing Parents and their Progeny

The effect of parental senescence on the reproductive capacity of their offspring has been a subject of intense research, by other investigators. In the experiments described below, the effect of senescence on the reproductive capacity of nematodes of control and α-tocopherol origin was examined. Table 15, shows the reproductive capacity of nematodes (40 per group, in mass cultures of 10 each) allowed to mate at different times of their life cycles. These nematodes were isolated before their reproductive periods began, and were put into mass cultures at different ages. The results indicate that virgin control nematodes were able to commence reproduction at a later age than were α-tocopherol nematodes

Table 15

The Effect of α-Tocopherol on the Reproductive Capacity of Nematodes of Increasing Ages Mated in Mass Cultures

Type of Nematode	Experiment	Parents Age at Time of Mating (days)	Parents Age at Onset of Rep. Period (days)	Parents Age at End of Rep. Period (days)	Length of Rep. Period (days)
Control	A	7	9	20	11
	B	9	13	20	7
	C	13	14	20	6
	D	14	16	21	5
	E	15	17	21	4
	F	17	21	23	2
	G	19.20	22.5	23	1
	H	22	N.F	-	-
	I	25	N.F	-	-
α-Toc.	A	7	7.5	22	14
	B	9	10	21	11
	C	13	14	19	5
	D	14	15.5	20.5	5.5
	E	15	17	21	6
	F	17	N.F	-	-
	G	19.20	N.F	-	-
	H	22	N.F	-	-
	I	25	N.F	-	-

Each experiment represents 4 mass cultures of 10 nematodes (n = 40).

N.F = non-fertilized

(19 and 15 days, respectively). The ability of control nematodes to reproduce at a later age than α-tocopherol nematodes is probably related to their later onset of reproduction (later maturation). Furthermore, these data indicates that the longest reproductive period is achieved when nematodes are mated at seven days of age. Control nematodes mated at seven days of age have a reproductive period of eleven days, while α-tocopherol-cultured nematodes have a maximum reproductive period of 14 days. The length of the reproductive period decreased as the age at which the nematodes were mated increased.

The experiment shown on Table 16 was designed with the aid of the data on Table 15. In this experiment, older virgin females were mated, in pair cultures, with sexually mature males, and vice versa. 20 pairs were mated per group. The results suggest that control females maintained their reproductive capacity (although low) until day 20. Control males, on the other hand, were unable to successfully fertilize on day 13. The results also show that control 16 day old females mated with 4 day old control males started to reproduce when the females were 20 days old. Thirteen day old females mated with four day old males started to reproduce at day

Table 16

Difference in Reproductive Capacity Between Senescing Males and Females as Influenced by α-Tocopherol

Type of Nematode	Age at Mating (days) Male	Age at Mating (days) Female	Age at Onset of Rep. Period (days) Male	Age at Onset of Rep. Period (days) Female	Pairs Fertilized (%)	Mean Length of Rep. Period ± S.D. (days)	Mean # of Offspring ± S.D. per/pair	Presence of Dead Larvae
Control	13	13			0	-	-	
	4	13	8.5	17	25	2.6 ± 0.8	10.5 ± 5.0	+
	13	4	-	-	0	-	-	
	16	16	-	-	0	-	-	
	4	16	8	20	16	2.2 ± 0.5	9.7 ± 2.7	+
	16	4	-	-	0	-	-	
α-Toc.	13	13	-	-	0	-	-	
	4	13	8	17	50	3.5 ± 0.9	14.0 ± 6.6	0
	13	4	17	7	25	2.8 ± 0.4	12.5 ± 6.9	0
	16	16	-	-	0	-	-	
	4	16	-	-	0	-	-	
	16	4	-	-	0	-	-	

(n=24)

17. In the reverse situation, where old males were mated at 13 and 16 days of age, to young females, no successful mating was achieved.

In the α-tocopherol groups, the length of the reproductive period was shorter than in control nematodes in females, but longer in males. In these groups, both 17 day old males and females were able to reproduce to a certain extent. 20 day old females were unable to reproduce, however, even when mated with young males. It is interesting to note that where both males and females were 13 days old no reproduction took place, in either control or α-tocopherol.

If these results are compared with the results for reproductive capacity obtained in experiments dealing with young nematodes, a decrease in reproductive capacity is observed in all parameters for both α-tocopherol and control nematodes. The significant decrease in total offspring, and the presence of dead larvae in both groups should be noted. The percentage of nematodes fertilized is also notably lower in both control and α-tocopherol groups, compared with young nematodes.

The reproductive capacity of the progeny of these nematodes (F_1 and F_2) was not studied, due to the difficulty in obtaining large enough numbers for

statistical significance and reliability. General observations indicate that the control group had very late-maturing F_1 nematodes, and very few F_2 nematodes were produced. This finding is in agreement with results obtained by Beguet and his co-workers in a study performed with C. elegans (1972). The α-tocopherol group showed little difficulty in maturation, and the number of F_2 offspring was comparable to that produced by young nematodes. This indicates that the F_1 generation was probably relatively normal.

It is interesting to note that females reproducing late in their reproductive age had a very short life span, usually dying about two days after reproduction, in both control and α-tocopherol groups. The low number of females actually reproducing makes this observation nonsignificant, however.

In a previous section, the maturation rate of early litters (from young parents, I, II, III) was determined and compared with the maturation rate of later progeny (from older parents; litters VII, VIII, IX), in both control and α-tocopherol cultures. The results indicate that α-tocopherol can cause longer production of normal progeny. For example, progeny in group c of control-cultured parents were unable to

reproduce. Progeny of control parents in group b had a somewhat reduced reproductive capacity, compared with the F_1 generation in group a. A significant reduction in the mean number of progeny, as well as a reduction in the length of the reproductive period is observed in group b, as compared with group a, for control nematodes. Nematodes cultured in α-tocopherol appear to maintain normal reproduction up to litter XII.

The results on Table 17 also indicate a slight decrease in reproductive capacity in α-tocopherol litter groups c, d and e, compared with a and b. Student t-tests comparing α-tocopherol groups a, c, d and e with litter group a indeed indicate a significant decrease in reproductive capacity in groups c, d and e ($\alpha<.05$). The reproductive capacity of the F_2 generation was not analyzed in detail, but observations indicated that they matured normally in this group.

These results show that (1) α-tocopherol ensures normal reproductive capacity in late litters, to a significant extent. (2) α-tocopherol does not, however, lengthen the reproductive period of late-mating nematodes.

Table 17

The Effect of α-Tocopherol on the Reproductive Capacity of Late Litters in Pair Cultures

Media	Age of Parents	Day of Rep. Period	Litter (F_1)	Litter Group	n (pairs)	Dead F_1 Nematodes by Rep. Age (%)	% Fertilized	Mean Onset of Rep. Period±S.D. (days)	Mean Length of Rep. Period± S.D. (days)	Mean # of Offspring±S.D. (F_2)
Control	8	1	I							
	9	2	II							
	10	3	III							
	11	4	IV	a	30	4.0	53	10.50±0.9	5.0±1.4	38.0±12[1]
	12	5	V							
	13	6	VI							
	14	7	VII	b	20	35	30	*12.50±1.5	*3.5±1.5	*14.1±10[1]
	15	8	VIII							
	16	9	IX	c	18	24	0	-	-	-
	17	10	X							
α-Toc.	7	1	I							
	8	2	II							
	9	3	III							
	10	4	IV	a	24	0	87	7.80±7.6	8.5±0.9	75.5±20[1]
	11	5	V							
	12	6	VI							
	13	7	VII	b	24	0	91	8.60±1.0	8.0±1.2	67.5±20[1]
	14	8	VIII							
	15	9	IX	c	20	5	75	*9.00±1.0	*6.5±1.9	46.5±20[1]
	16	10	X							
	17	11	XI							
	18	12	XII	d	20	0	75	*9.75±0.8	7.2±1.7	*54.3±10[1]
	19	13	XIII							
	20	14	XIV	e	10[0]	10	60	*9.60±0.9	*5.1±1.2	*44.5±15[1]

[1]=Normal F_2 [2]=Abnormal F_2 [0]=Difficult to obtain large no.

* $\alpha < .05$ for student t-test comparing respective litter group with litter group a.

Photomicrographs were taken in an attempt to record changes in non-reproducing and senescing parents, and to determine differences between control and α-tocopherol cultured nematodes. A number of these photomicrographs are shown in Appendix III.

The most interesting observation made from these photographs was the presence of lipid-like patricles surounding the nucleus of the germ cells in α-tocopherol cultured nematodes.

It should be noted that these particles are not present in the germ cells of control cultured nematodes.

The Effect of α-Tocopherol on Longevity

The following set of experiments deals with the effects of α-tocopherol supplementation on the life-span of Turbatrix aceti. The survivorship of nematodes of α-tocopherol origin was compared with that of nematodes cultured in control media (non-treated). Figure 24 shows the mean survivorship curves obtained from three replicate experiments. To examine the statistical significance of these survivorship values, the data were analyzed in various ways.

(a) The per cent survivorship curves obtained from the means of 3 replicate experiments show that nematodes cultured in control media have an increased incidence of mortality between zero and thirty days of age, as compared with α-tocopherol-cultured nematodes. Control nematodes show a steady increase in mortality after 72 days of age. α-Tocopherol-supplemented Turbatrix aceti show a more gradual increase in mortality after 72 days. Furthermore, these curves show a maximum longevity of 112 days for control cultures and 120 days for α-tocopherol-supplemented cultures. Student t-tests comparing corresponding sets of points show that the only statistically significant differences are observed at both extremes of the curves.

FIGURE 24. Survival curves for control and α-tocopherol cultured nematodes. Each curve represents three replicate experiemnts. *=α<.05 (n = 48 x 3)

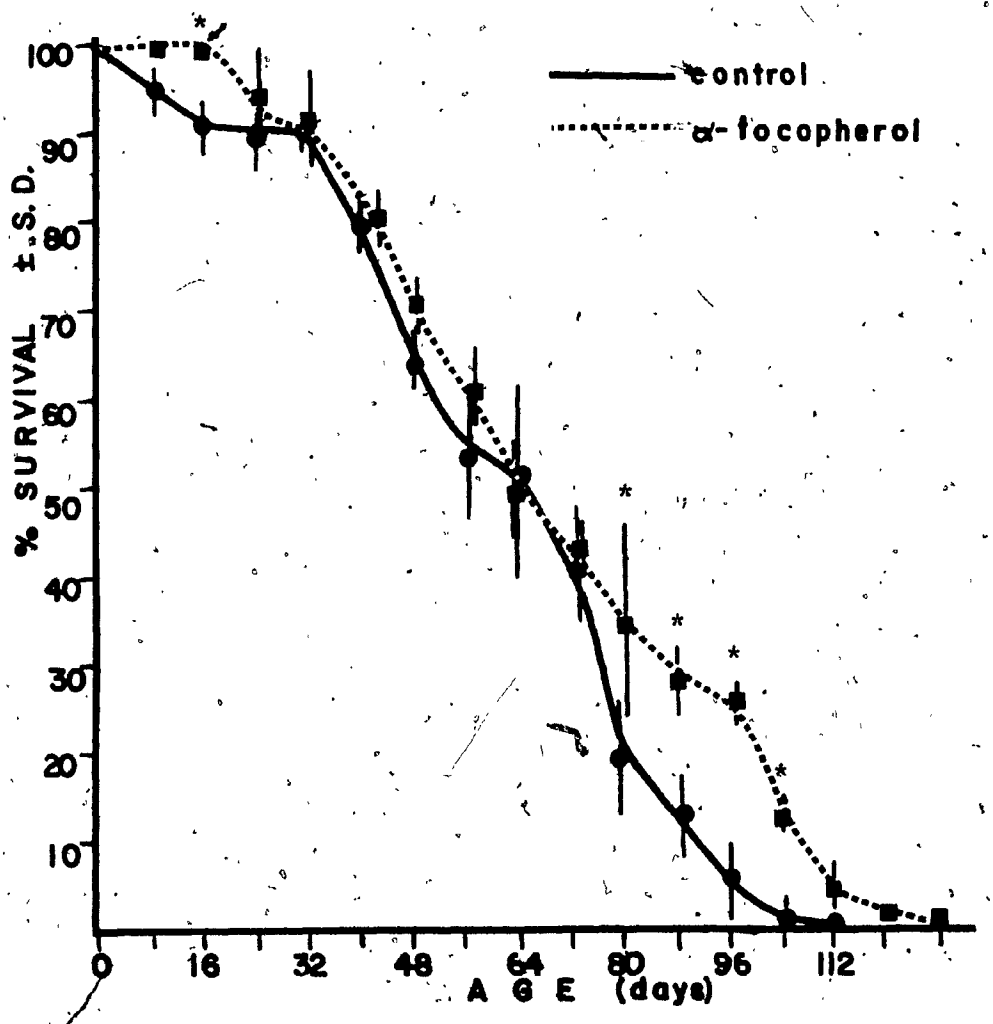

control
α- tocopherol
% SURVIVAL ± S.D.
100
90
80
70
60
50
40
30
20
10
0
16
32
48
64
80
96
112
AGE (days)
*

FIGURE 25. Survival curves for control and α-tocopherol cultured nematodes. (n=3x48)

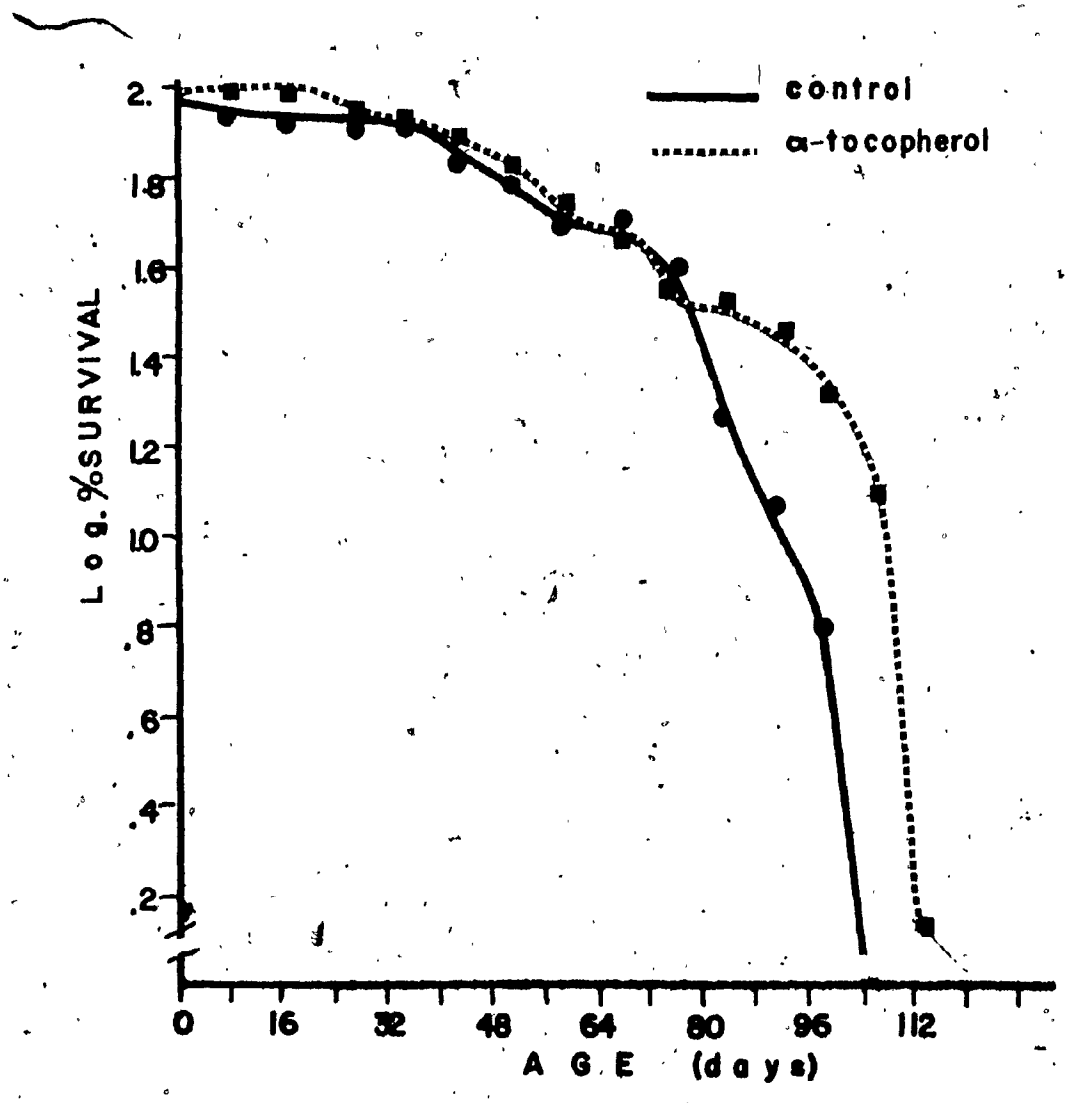

control
α-tocopherol
Log. %SURVIVAL
2.
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0
16
32
48
64
80
96
112
AGE (days)

FIGURE 26. Curves representing the death rate of control and α-tocopherol cultured nematodes. The regression lines were obtained from figure 25.

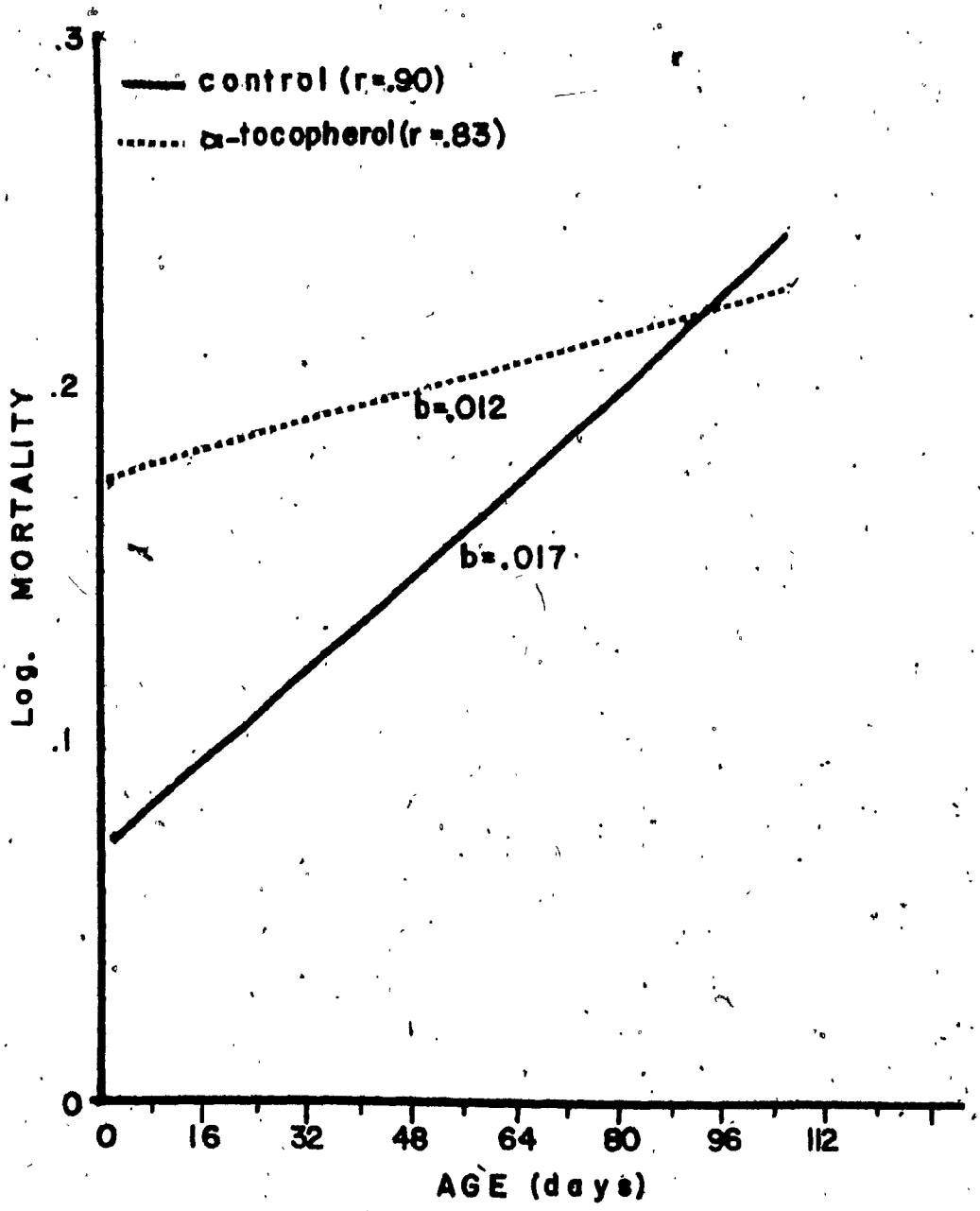
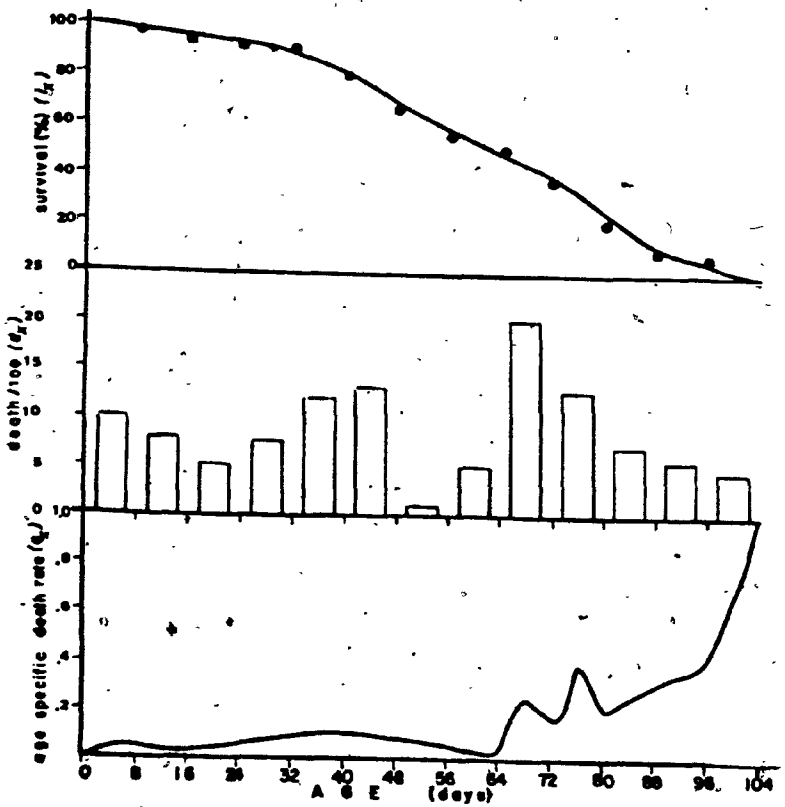
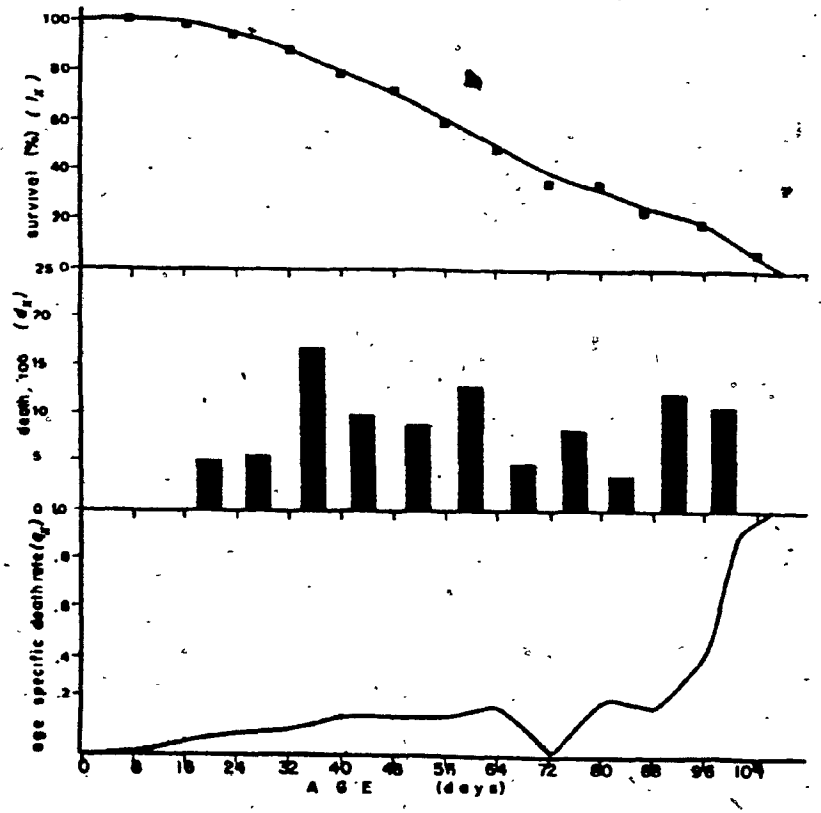

.3
.2
.1
0
control (r=.90)
α-tocopherol (r=.83)
b=.012
b=.017
Log. MORTALITY
0
16
32
48
64
80
96
112
AGE (days)

FIGURE 27a. Survival curve, distribution of ages at death and age specific death rate for control cultured nematodes.

FICURE 27b. Survival curve, distiribution of ages at death and age specific death rate for α-tocopherol cultured nematodes.


survival (%) (l_x)
death /100 (d_x)
age specific death rate (q_x)
A G E (days)


survival (%) (l_x)
death /100 (d_x)
age specific death rate (q_x)
A G E (days)

(b) The percent survivorship, as plotted on a log scale, is shown in Figure 25. Data presented in this way allow for a clearer representation of the difference between the curves. The 50% survivorship data obtained from these curves indicate that 50% of control-cultured nematodes live to 90 days, whereas 50% of α-tocopherol nematodes live to 105 days. A paired t-test comparing these curves results in $t = 2.71$ ($\alpha < .05$). Student t-tests have been used by Gershon (1972) in evaluating similar data.

(c) A more accurate evaluation of the survivorship of these groups can be obtained from the summary of the statistics of survival and death in relation to age (Table 18). The symbols used in Table 18 are those which are conventionally used in the study of animal populations. For every age interval "x", the life table gives "l_x", or the number of animals alive at the beginning of that period. The third column, "d_x", gives the number of animals dying during each age interval. The age-specific death rate, a more accurate way of measuring the rate of death, is given in column "q_x". This parameter shows the porportion of animals alive at the beginning of the age interval that die during that interval. The further expectation of life was obtained from column: "L_x" and "T_x". "L_x"

is the average number of animals alive during the age interval "x". "T_x" is the total number of animals alive at the beginning of the age interval. The further expectation of life at the beginning of the age interval "x" is "e_x". A discussion of these parameters may be found in Lamb (1977).

Although "maximum life span" is a term often used, it is not very precise, since it only shows the age that may be achieved by some of the animals in a population. The column "e_x", on the other hand, gives the average expectation of life of all the individuals in the population. The results then indicate that the average expectation of life for control-cultured nematodes is 58 days, whereas the same value to α-tocopherol-cultured nematodes is equal to 67 days (Table 18 & 19). Furthermore, a graphical representation of the results obtained from the life tables showed that the number of animals dying during each age interval "d_x" in control nematodes appears to increase steadily between sixteen and seventy-two days, with a maximum between sixty-four and seventy-two days. While this is fairly typical of a population with mortality concentrated in old age (Lamb, 1977), the number of deaths occurring between zero and sixteen days does not appear to fit the normal situation. A high death rate

Table 18

Life Table of Nematodes Cultured in Control Medium

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	3	.03	98.5	726	58.12
8-16	97	5	.05	94.5	628.4	51.79
16-24	92	2	.02	91	533.9	46.34
24-32	90	0	0	90	442.9	39.36
32-40	90	10	.11	85	352.9	31.28
40-48	80	16	.2	72	267.9	26.7
48-56	64	9	.14	59.5	195.9	24.37
56-64	55	3	.05	53.4	136.4	19.78
64-72	52	13	.25	44	83	12.76
72-80	39	19	.48	16	39	8.0
80-88	20	8	.40	9	23	9.2
88-96	12	6	.50	8.5	14	9.3
96-104	6	3.5	.58	4.25	5.5	7.33
104-112	2.5	2.5	1.0	1.25	1.25	4.0
112-120	0	-	-	-	-	-

Table 19

Life Table of Nematodes Cultured in α-Tocopherol Medium

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	0	0	100	839	67.12
8-16	100	0	0	100	739	59.12
16-24	100	7.5	.075	96	639	51.12
24-32	92.5	2.5	.027	91	543	46.96
32-40	90	7.5	.083	86.25	452	40.12
40-48	82.5	12.5	.151	76.25	265.7	35.39
48-56	70	10	.142	65	289.5	33.02
56-64	60	10	.166	55	224.5	29.86
64-72	50	10	.200	45	169.5	27.04
72-80	40	5	.125	37.5	124.5	24.8
80-88	35	8	.228	31	87	19.88
88-96	27	2	.074	26	56	16.59
96-104	25	12.5	.5	18.75	30	9.6
104-112	12.5	10	.80	7.5	11.25	7.2
112-120	2.5	0	0	2.5	3.75	12
120-128	2.5	2.5	1	1.25	1.25	4
128-136	0	-	-	-	-	-

in newborn animals is frequently present in animal populations, and is often caused by congenital abnormalities, or environmental factors. α-Tocopherol-supplemented nematodes show a more constant rate of deaths per unit time. These life tables further indicate that the average number of nematodes alive after seventy-two days in control groups was sixteen, while the average number in α-tocopherol groups was 37.5. These data, in conjunction with the rest of the data shown in the life tables, suggest that α-tocopherol delays processes that lead to the death of Turbatrix aceti.

d) A regression analysis was performed in order to compare the mortality rate (Δ survivorship/Δ t) of control-cultured nematodes with that of α-tocopherol nematodes (Figure 26). A comparison between the slopes (Stanley, 1963) of the curves shown in Figure 26 indicates that there is no significant difference ($\alpha > .05$).

e) To further emphasize the very similar sections of the survivorship curves shown in Figure 24 , weighted means were calculated for each of 3 replicate experiments ($\Sigma \frac{l_x . x}{x}$). Means calculated in this way put a stronger weight on the secton of the curve dealing with a larger number of animals. The results obtained are shown in Table 20 . An analysis of variance

comparing both sets of means results in an F value equal to 20.60 ($\alpha<.05$). These results indicate that the weighted means obtained from the curves of control nematodes are significantly lower than those obtained from α-tocopherol-cultured Turbatrix aceti (paired t-tests comparing replicate survivorship curves resulted in non-significant t-values ($\alpha>.05$) among groups).

(f) In addition to the weighted means, Table 20 shows the mean life span (± S.D.) calculated from the total populations used in the three replicate experiments. The test statistics obtained from these means indicate that the mean life span of control-cultured nematodes is significantly shorter than the mean life span of α-tocopherol-cultured nematodes ($\alpha<.05$).

(g) To examine the dip in the survivorship curves observed between 0 and 16 days in control-cultured nematodes, a closer analysis of this section of the curve was carried out. The survivorship of control and α-tocopherol-supplemented nematodes was followed every two days from the 3rd to the 18th day of their life cycle. Figures 28 a and b show the survivorship for four experiments in which nematodes were isolated at four different times after birth.

An analysis of variance between the slopes (Ralph & Sokal, 1969) obtained from the regression lines of

Table 20

The Effects of Supplemented α-Tocopherol on the Survivorship of Turbatrix Aceti Are Shown by the Weighted Mean of Three Replicate Experiments. Mean Life Spans Were Calculated for the Total Population

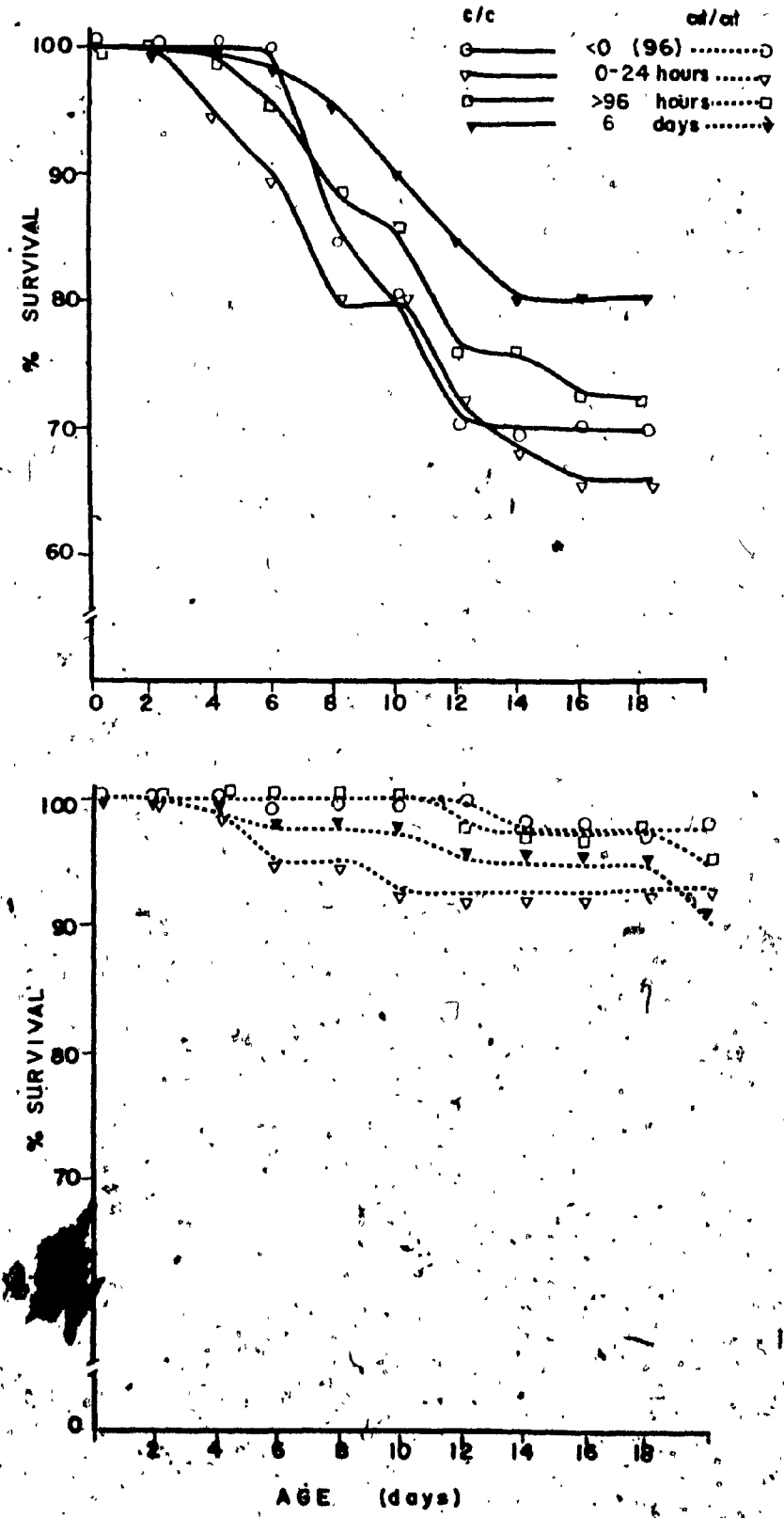
Experiment	Experimental Media	Weighted Mean $(\Sigma \frac{l_x \cdot x}{x})$		Mean Life Span±S.D. (days)	
A	Control	37.65			
B	Control	36.80		45.40 ± 29.6	
C	Control	38.83			
$\bar{X}$ ± S.D.		37.76±1.0	$F_{(1,4)}=20.60^*$		
A	α-Toc.	41,49	$t_{(2)}=6.4^*$		$t_{(4)}=3.60^*$
B	α-Toc.	40.19		60.68 ± 30.6	
C	α-Toc.	41.00			
$\bar{X}$ ± S.D.		40.89±.65			

* $\alpha < .05$

Each replicate experiment represents 48 nematodes (n = 48).

FIGURE 28a. Survival curves illustrating the difference in mortality between 0 and 20 days (early mortality) between control nematodes isolated on different days after birth. (n = 24)

FIGURE 28b. Survival curves illustrating the difference in mortality between 0 and 20 days (early mortality) between α-tocopherol cultured nematodes, isolated on different days after birth. (n = 24)


c/c
ot/ot
<0 (96)
0-24 hours
>96 hours
6 days
% SURVIVAL
100
90
80
70
60
0
2
4
6
8
10
12
14
16
18
AGE (days)

these points resulted in a significant F-value for comparisons among the curves obtained from control-cultured nematodes ($F_{(3,14)} = 10.39$, $\alpha<.05$), and a non-significant F-value among α-tocopherol curves ($\alpha>.05$). A two-way ANOVA test comparing the death rate (slope) between the two groups results in a significant F-value ($F_{(1,4)} = 12.82$, $\alpha<.05$). These results emphasize the previous findings that young nematodes have a significantly higher mortality in control nematodes than α-tocopherol-supplemented nematodes.

In previous experiments, we showed that α-tocopherol supplementation at various stages of development appears to affect the nematode's reproductive capacity in different ways. To further elucidate these findings, the survivorship of nematodes supplemented with and removed from α-tocopherol at various stages of their life span was monitored. The analysis of this data was approached in a manner similar to that used to evaluate the significant difference between the survivorship of control and α-tocopherol-cultured nematodes.

(a) Paired t-tests were performed to determine the significance of the differences between curves. Significant t-values ($\alpha<.05$) were only obtained in curves 29a and b. The abnormality in the α-tocopherol-supplemented curve shown in Figure 29a is difficult to

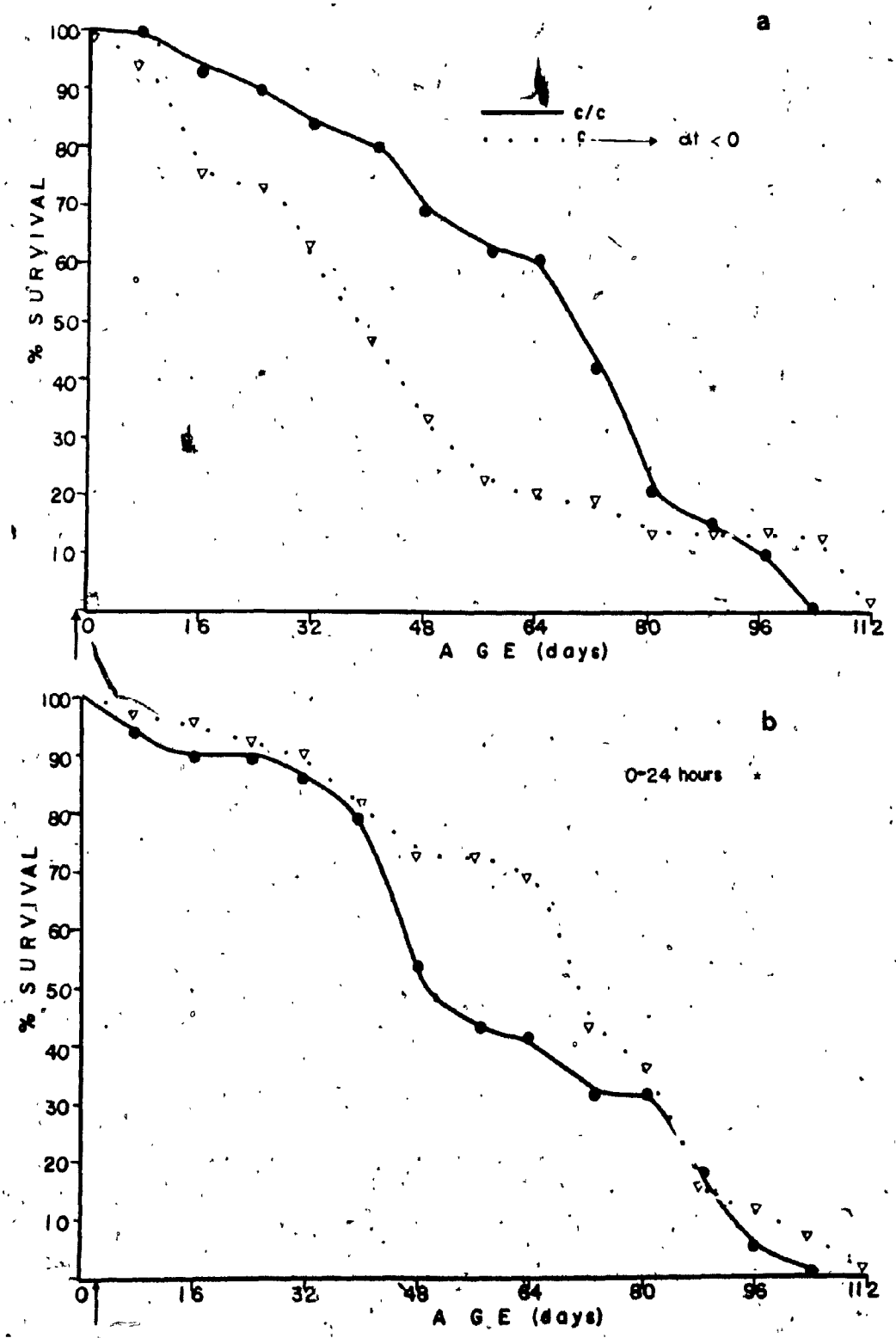
explain.

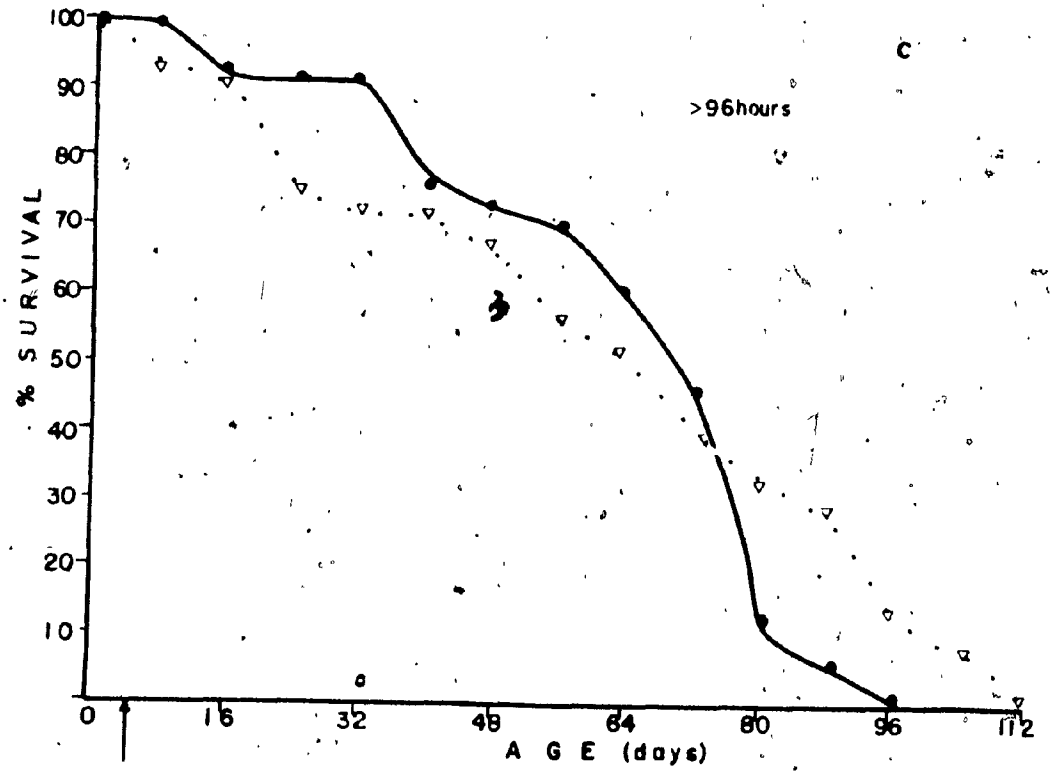
(b) Life tables for each curve give a more accurate and clearer understanding of the death rate throughout the life span of these nematodes (Tables 21 to 28). The life expectancy of these nematodes at time 0 is given in Table 35. It should be noted that the largest difference in life expectancy is observed between control nematodes and those supplemented with α-tocopherol between 0 and 24 hours.

(c) Weighted means calculated for each of the above curves resulted in very similar values for both the control and α-tocopherol-supplemented groups, with the exception of control nematodes supplemented with α-tocopherol before birth. It should be noted that none of these values are as high as those obtained from the life spans of nematodes cultured in α-tocopherol for generations (Table 20).

(d) The mean life spans obtained from groups of forty-eight nematodes show that the only significant difference is observed between control nematodes and those supplemented with α-tocopherol between zero and twenty-four hours of age (the significant difference observed in these groups may be exaggerated by the high mortality observed in the zero to twenty-four hour control group).

FIGURE 29. Survival curves for nematodes of control origin supplemented with α-tocopherol at different stages of development. (a) before birth; (b) 0-24 hours; (c) after 96 hours; and (d) after 30 days of age. * $\alpha < .05$ for paired t-test compared with control. (n=24x3)


a
c/c
ρ ⟶ at < 0
% SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
16
32
48
64
80
96
112
A G E (days)
b
0-24 hours
% SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
16
32
48
64
80
96
112
A G E (days)


c
>96hours
% SURVIVAL
100
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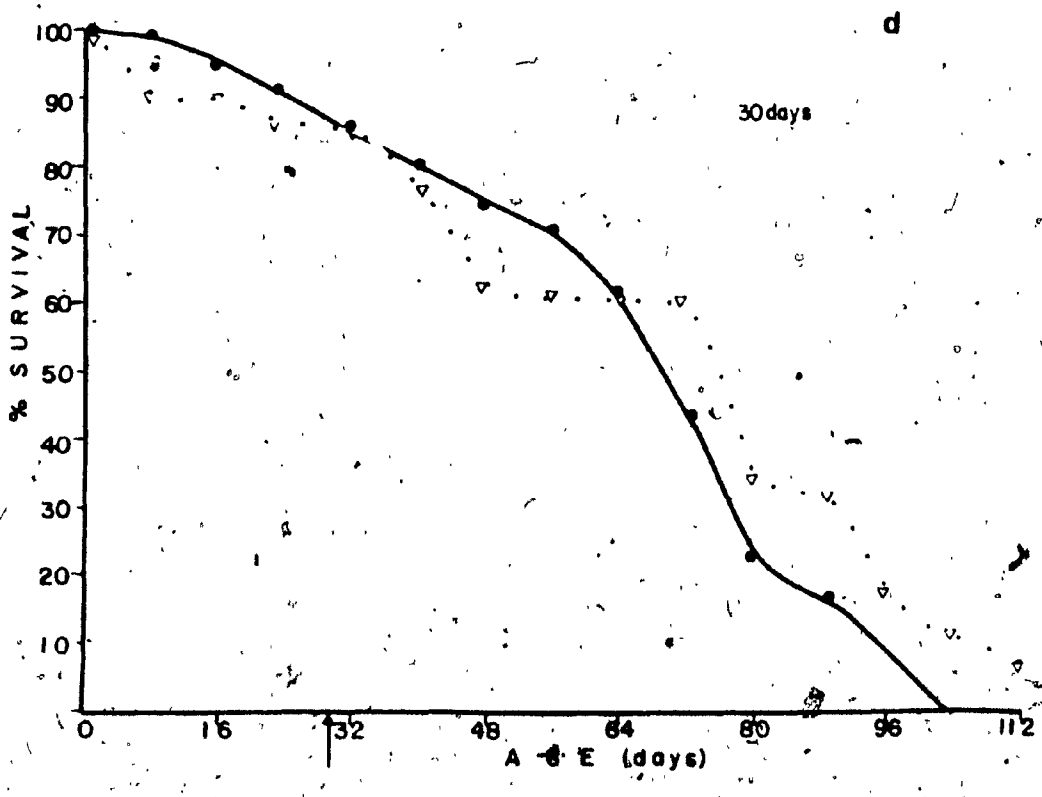
112
A G E (days)


d
30days
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100
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30
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64
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96
112
A G E (days)

Table 21

Life Table of Nematodes of Control Origin Transferred to Control Medium Before Birth

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	0	0	100	775	62.0
8-16	100	5	.05	97.5	675	54
16-24	95	3	.031	93.5	578	48.67
24-32	92	8	.086	88	484.5	42
32-40	84	4	.047	88	396.5	37.7
40-48	80	13	.162	73.5	314	31.4
48-56	67	5	.07	64.5	241.5	28.7
56-64	62	3	.04	60	177	22.83
64-72	59	19	.32	50	117	15.8
72-80	40	20	.5	30	67	13.4
80-88	20	5	.25	17.5	37	14.8
88-96	15	3	.2	13.5	19.5	10
96-104	12	12	1.0	6	6	4
104-112	0	-	-	-	-	-

Table 22

Life Table of Nematodes of Control Origin Transferred to α-Tocopherol Medium Before Birth

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	5	.05	100	527	42.16
8-16	95	18	.189	85	427	35.95
16-24	71	5	.07	75	342	35.53
24-32	72	13	.18	65.6	267	29.66
32-40	59	14	.23	62	201.5	27.25
40-48	45	14	.31	38	149.5	26.48
48-56	31	11	.35	26.5	111.5	28.64
56-64	20	0	.1	20	86	34.4
64-72	20	2	-	19	76	30.4
72-80	18	6	.33	15	57	25.33
80-88	12	0	-	12	42	28
88-96	12	4	.33	10	30	20
96-104	8	0	-	8	20	20
104-112	8	0	-	8	12	12
112-120	8	8	1.0	4	4	4
120-128	0	-	-	-	-	-

Table 23

Life Table of Nematodes of Control Origin Transferred to Control Medium Between 0-24 Hours After Birth

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	5	.05	97.5	707	56
8-16	95	5	.052	92.5	609	51.2
16-24	90	0	0	90	517	45.9
24-32	90	3	.03	88.5	427	37.9
32-40	87	7	.08	83.5	338.9	31
40-48	80	28	.35	66	255	25.5
48-56	52	12	.135	46	189	29
56-64	40	0	0	40	143	28.6
64-72	40	12	.25	35	103	20.6
72-80	30	0	0	30	68	18.1
80-88	30	15	.5	22.5	38	10.1
88-96	15	7	.46	11.5	15.5	8.2
96-104	8	8	1.0	4	4	4
104-112	0	-	-	-	-	-

Table 24

Life Table of Nematodes of Control Origin Transferred to α-Tocopherol Medium Between 0-24 Hours After Birth

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	3	.03	98.5	838	67.04
8-16	97	2	.02	96.5	740	61
16-24	95	3	.03	93.5	643.5	54.14
24-32	92	2	.04	91	555	48.2
32-40	90	10	.11	85	459	40.8
40-48	80	10	.125	75	374	37.4
48-56	70	0	0	70	299	34.1
56-64	70	3	.042	68.5	229	26.1
64-72	67	0	0	67	160	19.1
72-80	67	33	.49	51	93.5	11.1
80-88	35	23	.647	23.5	42.5	9.7
88-96	12	4	.33	10	19	12.6
96-104	8	3	.37	6.5	9	9
104-112	5	5	1.0	2.5	2.5	4
112-120	0	-	-	-	-	-

Table 25

Life Table of Nematodes of Control Origin Transferred to Control Medium After 72 Hours of Age

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	0	0	100	758	60.64
8-16	100	10	.1	95	658	52.6
16-24	90	0	0	90	563	50
24-32	90	0	0	90	743	42
32-40	90	15	.16	82.5	383.5	34
40-48	75	3	.04	73.5	301	32
48-56	72	5	.069	69.5	227.5	25
56-64	67	8	.119	63	158	18.8
64-72	59	14	.23	52	95	12.8
72-80	45	33	.72	28.5	43	7.6
80-88	12	4	.33	10.5	14.5	9.6
88-96	8	8	1.0	4	4	4
96-104	0	-	-	-	-	-

Table 26

Life Table of Nematodes of Control Origin Transferred to α-Tocopherol Medium After 72 Hours of Age

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	8	.08	.96	745	59.6
8-16	92	0	0	92	649	56.4
16-24	92	16	.173	84	557	48.4
24-32	76	5	.065	73.5	473	49.78
32-40	71	0	0	71	399.5	45
40-48	71	6	.085	68	328.5	36.9
48-56	65	10	.153	60	260.5	32
56-64	55	5	.09	52.5	200	29
64-72	50	13	.26	43.5	148	23.6
72-80	37	10	.27	32	104.5	22.4
80-88	27	0	0	27	72.5	21
88-96	27	15	.55	19.5	45.5	13.3
96-104	12	2	0	11	26	17.3
104-112	10	0	0	10	15	12
112-120	10	10	1.0	5	5	4
120-128	0	-	-	-	-	-

Table 27

Life Table of Control Nematodes Transferred to Control Medium at 30 Days of Age

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	10	.1	95	776	62.08
8-16	90	0	0	90	681	60.53
16-24	90	5	.05	87.5	591	52.53
24-32	85	0	0	85	503.5	47.36
32-40	85	10	.117	80	418.5	39.36
40-48	75	15	.2	67.5	338.5	36.05
49-56	60	10	.16	55	271	36.13
56-64	50	0	0	50	216	34.56
64-72	50	0	0	50	166	26.26
72-80	50	20	.4	40	166	18.56
80-88	30	0	0	30	76	20.26
88-96	30	15	.5	22.5	46	12.26
96-104	15	7	.46	11.5	23.5	12.26
104-112	8	0	0	8	12	12
112-120	8	8	1.0	4	4	8
120-128	0	-	-	-	-	-

Table 28

Life Table of Control Nematodes Supplemented with α-Tocopherol at 30 Days of Age

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	0	0	100	705	56
8-16	95	5	.05	97.5	605	48.4
16-24	90	5	.052	92.5	507.5	45.1
24-32	80	10	.11	85	450	36.8
32-40	75	5	.062	77.5	330	33
40-48	70	10	.066	72.5	252	26.9
48-56	60	20	.142	65	180	20.5
56-64	40	20	.33	50	115	15.3
64-72	20	5	.5	30	65	23.0
72-80	15	5	.25	17.5	35	14.0
80-88	10	10	.33	12.5	17.5	9.3
88-96	0	0	1.0	5	5	4.0

(e) The maximum life spans obtained from these curves indicate that nematodes supplemented with α-tocopherol during any stage of their life cycle showed higher maximum life spans than did control nematodes.

The variation in survivorship observed among control curves may lead to some scepticism over the significance of the difference between control and α-tocopherol--treated nematodes. It should be noted that nematodes cultured in similar media, and handled similarly, show no significant difference between their survivorship curves (Table 20). If nematodes cultured in similar media, but handled at different ages (twenty-four hours to ninety-six hours) show a decrease or increase in mortality, this variation is probably due to different levels of sensitivity to stress at different ages, it should be safe to assume that as long as the stressful situation is kept constant between control and α-tocopherol groups, comparisons to determine the effects of α-tocopherol at different stages should be valuable.

To summarize, then, it appears that supplementation with α-tocopherol is required as early as 0 to 24 hours after birth, to increase life expectancy and the mean life span of the population over that of control nematodes isolated between 0 and 24 hours. Maximum

life span, however, can be lengthened with α-tocopherol supplementation as late as 30 days of age. 30 day old Turbatrix aceti are past their reproductive age and have completed cell growth and development. Weighted means do not appear to be significantly affected by any α-tocopherol supplementation after birth. The increased weighted mean values obtained from nematodes of α-tocopherol origin (Table 36), are probably due to the low mortality rate between 0 and 16 days. Decreased mortality between 0 and 16 days is not observed in nematodes supplemented with α-tocopherol after birth.

The high mortality rate observed in control animals transferred between 0 and 24 hours may suggest, that α-tocopherol supplemented at this time prevents mortality induced by the excess stress caused by the handling of these larvae at this early age. If this is the case, it appears that α-tocopherol is required before birth to have a significant effect on longevity.

The possibility of a factor interfering with absorption at a very early age make it difficult to be conclusive about these results.

The effects of transferring α-tocopherol nematodes to control media at various stages of development have been evaluated with respect to reproduction. In the next section, we attempted to further elucidate the

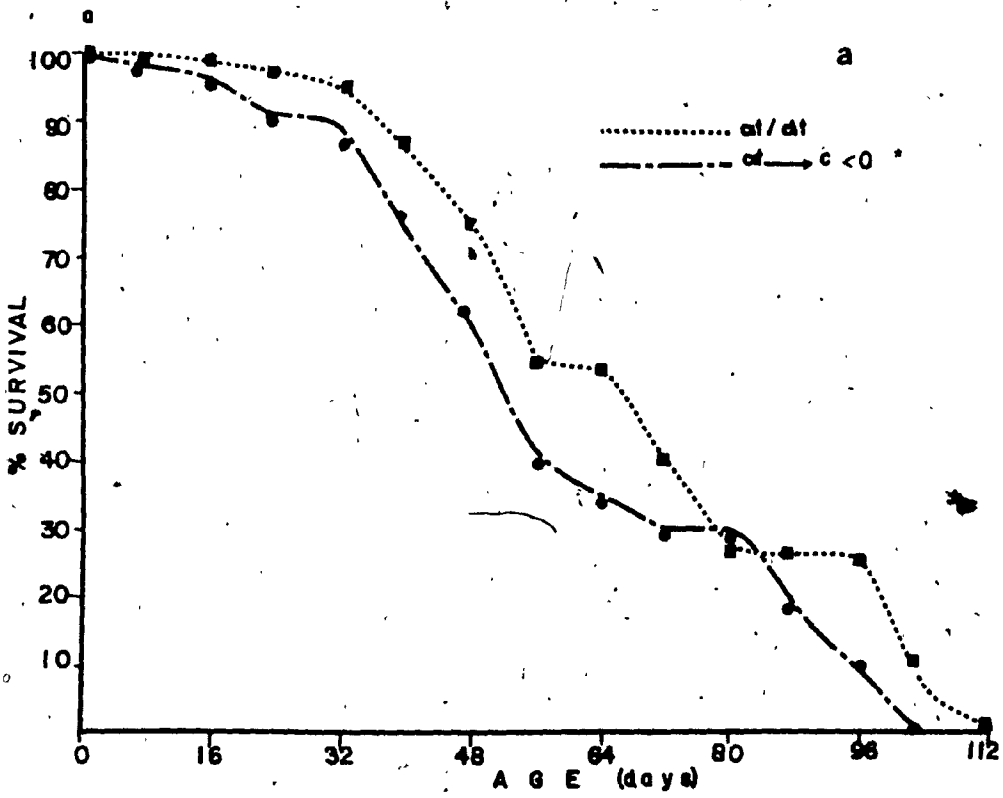
above findings, by looking at the survivorship of nematodes treated in this way. The curves obtained from monitoring the survivorship of these nematodes are shown in Figures 30 a, b and c. Data were analyzed in the usual way.

(a) Close observation of the survivorship curves indicates that α-tocopherol is required for at least twenty-four hours after birth to prevent early mortality. Nematodes of α-tocopherol origin, transferred to control media between zero and twenty--four hours, showed a high mortality rate between birth and sixteen days of age. Nematodes removed from α-tocopherol anytime before ninety-six hours of age had a shorter life span than groups maintained in α-tocopherol.

(b) Paired t-tests were performed between α-tocopherol-depleted curves and their respective α-tocopherol curves. According to these test statistics, the only significantly different curves are those shown in Figures 30a and b. This may suggest that α-tocopherol depletion before ninety-six hours significantly affects the survivorship of these nematodes, as compared with non-depleted nematodes.

(c) Weighted means, calculated in the usual fashion, indicate that nematodes removed from

FIGURE 30. Survival curves for nematodes of α-tocopherol origin removed from α-tocopherol medium at different stages of development. (a) before birth; (b) 0-24 hours; (c) after 96 hours and (d) after 30 days of age. * $\alpha < .05$ compared with nematodes of α-tocopherol origin transferred to α-tocopherol medium at different stages of development. (n= 24x3)


a
100
90
80
70
60
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% SURVIVAL
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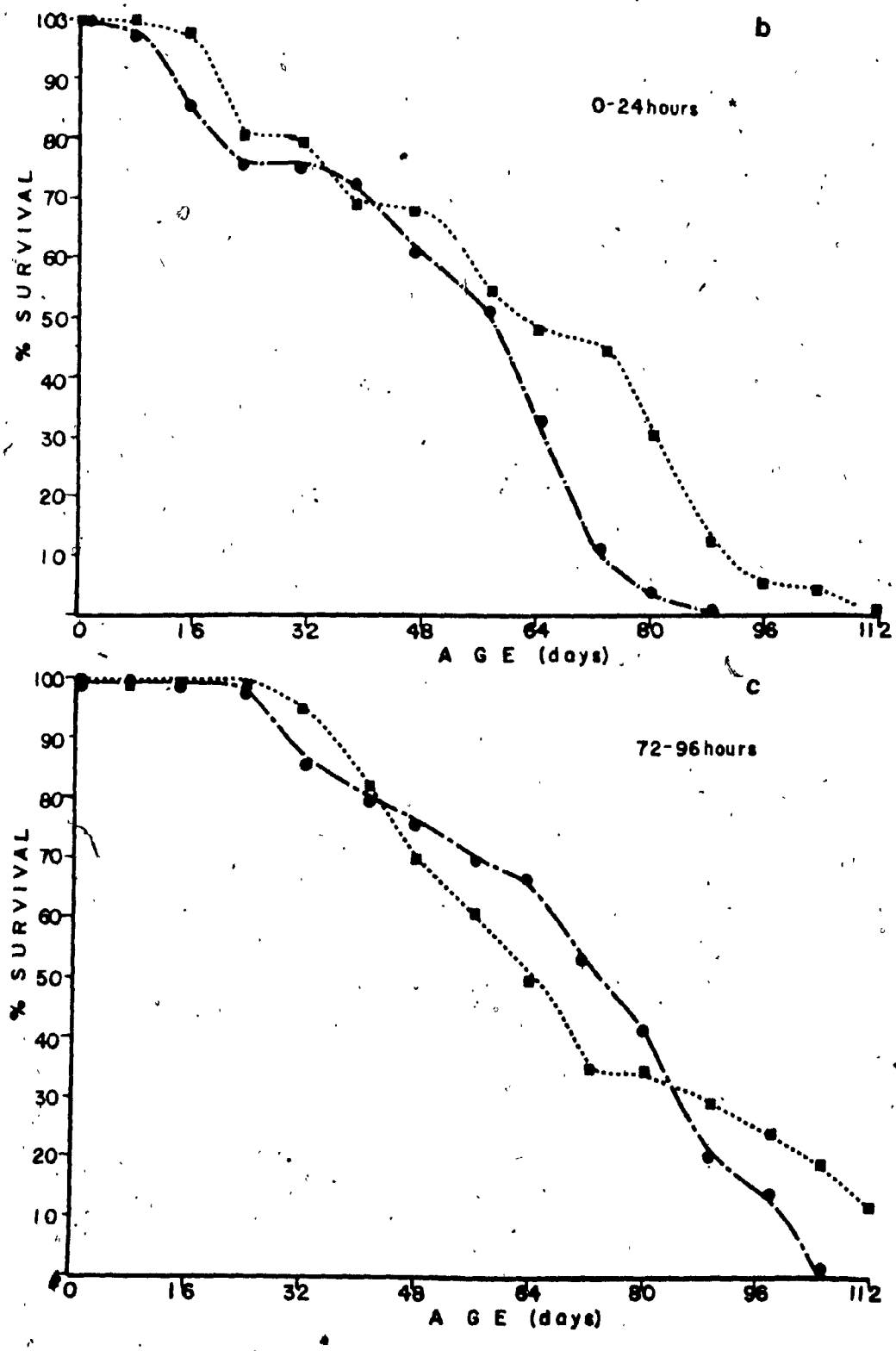
b
0-24 hours
% SURVIVAL
100
90
80
70
60
50
40
30
20
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0
16
32
48
64
80
96
112
A G E (days)
c
72-96 hours
% SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
16
32
48
64
80
96
112
A G E (days)

Table 29

Life Table of Nematodes of α-Tocopherol Origin Transferred to α-Tocopherol Medium Before Birth

x	l_x	q_x	d_x	L_x	T_x	e_x
0-8	100	0	0	100	835	66.8
8-16	100	0	0	100	735	58.8
16-24	100	5	.05	97.5	635	50.8
24-32	95	0	0	95	537.5	45.22
32-40	95	10	.10	90	442.5	37.22
40-48	85	10	.117	80	352.5	33.12
48-56	75	20	.26	65	272.5	29.01
56-64	55	0	-	55	207.5	30.1
64-72	55	15	.27	47.5	152.5	22.1
72-80	40	15	.375	32.5	105	21
80-88	25	0	-	25	72.5	23
88-96	25	0	-	25	47.5	15
96-104	25	20	.8	15	22.5	7.2
104-112	5	0	-	5	7.5	12
112-120	5	5	1.0	2.5	2.5	4.0
120-128	0	0	-	-	-	-

Table 30

Life Table of Nematodes of α-Tocopherol Origin Transferred to Control Medium Before Birth

x	l_x	q_x	d_x	L_x	T_x	e_x
0-8	100	0	0	100	739.2	59.12
8-16	100	5	.05	97.5	639.2	51.13
16-24	95	5	.052	92.5	541.7	45.55
24-32	90	0	-	90	449.2	39.9
32-40	90	15	.160	82.5	359.2	31.9
40-48	75	15	.2	67.5	276.7	29.44
48-56	60	20	.33	50	209	27.86
56-64	40	5	.125	37.5	159	31.8
64-72	35	5	.142	32.5	122.2	25.6
72-80	30	0	-	30	96	25.6
80-88	30	5	.166	27.5	60	16
88-96	25	15	.2	22.5	32.5	10.25
96-104	20	20	1.0	10	10	4
104-112	0	-	-	-	-	-

Table 31

Life Table of Nematodes of α-Tocopherol Origin Transferred to α-Tocopherol Medium between 0-24 Hours

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	0	0	100	826	66.08
8-16	100	0	0	100	726	58
16-24	100	8	.08	96	626	50.08
24-32	92	12	.13	86	530	46.08
32-40	80	0	0	80	444	44.4
40-48	80	15	.18	72.5	364	36.4
48-56	65	0	0	65	291.5	35.8
56-64	65	13	.2	58.5	226.5	27.81
64-72	52	7	.13	48.5	168	25.8
72-80	45	0	0	45	119.5	21.1
80-88	45	15	.33	37.5	74.5	13.15
88-96	30	18	.60	21	31	8.2
96-104	12	7	.58	8.5	16	10.6
104-112	5	0	0	5	7.5	12.0
112-120	5	5	1.0	2.5	2.5	4.0
120-128	0	-	-	-	-	-

Table 32

Life Table of Nematodes of α-Tocopherol Origin Transferred to Control Medium between 0-24 Hours

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	3	.03	98.5	676	54.08
8-16	97	12	.123	91	577.5	47.58
16-24	85	0	-	85	486.5	45.74
24-32	85	10	.117	80	401	37.74
32-40	75	0	0	75	321.5	34.26
40-48	75	5	.06	72.5	246.5	26.24
48-56	70	13	.186	63.5	174	19.88
56-64	57	10	.175	52	110.5	15.43
64-72	47	22	.468	36	58.5	9.87
72-80	25	15	.6	17.5	22.5	7.2
80-88	10	10	1.0	5	5	4.0
88-96	0	-	-	-	-	-

Table 33

Life Table of Nematodes of α-Tocopherol Origin Transferred to α-Tocopherol Medium After 72 Hours of Age

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	0	0	100	859	68.7
8-16	100	0	0	100	759	60.72
16-24	100	0	0.0	100	659	52.7
24-32	100	5	.05	97.5	559	44.72
32-40	95	10	.105	90	461	38.82
40-48	85	15	.176	77.5	371.5	34.91
48-56	70	10	.142	65	294	33.6
56-64	60	13	.12	53.5	229	30.5
64-72	47	12	.25	41	175.5	29.78
72-80	35	0	0.22	35	134.5	30.62
80-88	35	8	.22	31	99.5	22.74
88-96	27	2	.07	26	68.5	20.29
96-104	25	5	.2	22.5	42.5	13.44
104-112	20	10	.5	15	20	8
112-120	10	10	1.0	5	5	4
120-128	0	-	-	-	-	-

Table 34

Life Table of Nematodes of α-Tocopherol Origin Transferred to Control Medium After 72 Hours of Age

x	l_x	d_x	q_x	L_x	T_x	e_x
0-8	100	0	0	100	864	69.12
8-16	100	0	0	100	764	61.12
16-24	100	0	0	100	664	53.12
24-32	100	13	.03	88.5	564	45.12
32-40	87	2	.022	86	475.5	38.00
40-48	85	10	.117	80	389.5	36.61
48-56	75	5	.066	72.5	309.5	32.96
56-64	70	3	.042	68.5	237	27.08
64-72	67	17	.25	58.5	168.5	20.05
72-80	50	10	.20	45	110	17.6
80-88	40	20	.5	30	65	13
88-96	20	5	.25	17.5	35	14
96-104	15	5	.33	12.5	17.5	9.3
104-112	10	10	1.0	5	5	4
112-120	0	-	-	-	-	-
120-128						

α-tocopherol before 96 hours of age show a significant decrease in survivorship as compared with non-α-tocopherol-depleted Turbatrix aceti. Nematodes removed from α-tocopherol after 96 hours, on the other hand, are not affected with respect to this value.

(d) The life expectancy values at time 0 (e_x) are in accordance with the above results. Nematodes removed from α-tocopherol after 96 hours of age have a similar life expectancy to those not removed from α-tocopherol. The contrary is true for those removed from α-tocopherol before twenty-four hours.

(e) Student t-tests between the mean life spans indicate that nematodes depleted of α-tocopherol before 96 hours of age had a significantly lower mean life span than did non-depleted groups ($\alpha<.05$). Nematodes depleted of α-tocopherol after 96 hours of age showed mean life spans similar to those of α-tocopherol nematodes not so depleted.

(f) The maximum life spans appear to indicate a longer life span among nematodes not removed from α-tocopherol than among those which were removed at any time. Note that the maximum life span of nematodes removed from α-tocopherol after 96 hours is 104 days, while it is 120 days for the respective α-tocopherol group.

Table 35

The Effects on Survivorship of α-Tocopherol Supplementation at Various Stages of Development, are Shown by Mean Life Spans, Weighted Means and Life Expectancy

Origin of Nematode	Transfer Media	Age at Time of Transfer (days)	Mean Life Span ± S.D. (days)	Weighted Mean $(\Sigma \frac{l_x \cdot x}{x})$	Life Expectancy of Population at Time 0 (e_o) (days)	Maximum Life Span (days)
Control	Control	< 0	52.2±27	37.65	62.0	96
Control	α-Toc.	< 0	38.4±32	33.80	42.1	104
Control	Control	0-1	48.8±27	36.80	56.0	96
Control	α-Toc.	0-1	*60.9±27	37.73	67.0	104
Control	Control	> 4	50.6±27	38.80	60.6	88
Control	α-Toc.	> 4	54.6±32	37.70	59.6	104
Control	Control	30	52.3±22	38.50	56.4	96
Control	α-Toc.	30	55.2±34	37.60	62.0	112

* $\alpha < .05$ for Student t-test as compared to control

(n = 24 x 3)

α-tocopherol before 96 hours of age show a significant decrease in survivorship as compared with non-α-tocopherol-depleted Turbatrix aceti. Nematodes removed from α-tocopherol after 96 hours, on the other hand, are not affected with respect to this value.

(d) The life expectancy values at time $\dot{0}$ (e_x) are in accordance with the above results. Nematodes removed from α-tocopherol after 96 hours of age have a similar life expectancy to those not removed from α-tocopherol. The contrary is true for those removed from α-tocopherol before twenty-four hours.

(e) Student t-tests between the mean life spans indicate that nematodes depleted of α-tocopherol before 96 hours of age had a significantly lower mean life span than did non-depleted groups ($\alpha<.05$). Nematodes depleted of α-tocopherol after 96 hours of age showed mean life spans similar to those of α-tocopherol nematodes not so depleted.

(f) The maximum life spans appear to indicate a longer life span among nematodes not removed from α-tocopherol than among those which were removed at any time. Note that the maximum life span of nematodes removed from α-tocopherol after 96 hours is 104 days, while it is 120 days for the respective α-tocopherol group.

Table 36

The Effects on Survivorship of Removing α-Tocopherol from the Nematodes Media at Various Stages of Development, are Shown by Mean Life Spans, Weighted Mean and Life Expectancy

Origin of Nematode	Transfer Media	Age at Time of Transfer (hours)	Mean Life Span ± S.D. (days)	Weighted Mean $(\Sigma \frac{l_x \cdot x}{x})$	Life Expectancy of Population at Time O (e_o) (days)	Maximum Life Span (days)
α-Toc.	α-Toc.	<0	58.6 30	41.49	66.8	104
α-Toc.	Control	<0	**42.0 32	37.15	59.1	96
α-Toc.	α-Toc.	0-24	61.1 28	41.19	66.08	112
α-Toc.	Control	0-24	**41.8 29	35.38	54.08	88
α-Toc.	α-Toc.	> 96	64.6 28	41.00	68.70	120
α-Toc.	Control	> 96	65.4 24	40.97	69.12	104

** $\alpha < .05$ for student t-test as compared to α-tocopherol group.
(n = 24 x 3)

The results in this section indicate that α-tocopherol plays a role in decreasing mortality in young nematodes if present in their media until at least ninety-six hours after birth. The long term survivorship of Turbatrix aceti is not affected by α-tocopherol depletion after ninety-six hours of age. Removal from α-tocopherol within twenty-four hours after birth does significantly affect this nematode's life span. Maximum life span appears to be the only parameter which is affected by depletion at this stage.

Photomicrographs of aged and young nematodes are shown in Appendix III.

The Effect of α-Tocopherol on the Longevity of Irradiated Nematodes

The effects of α-tocopherol on the normal life span of Turbatrix aceti have been shown in a previous section. The next experiment was designed to test the effects of α-tocopherol where the normal life span has been experimentally modified. The aging process of irradiated nematodes was examined.

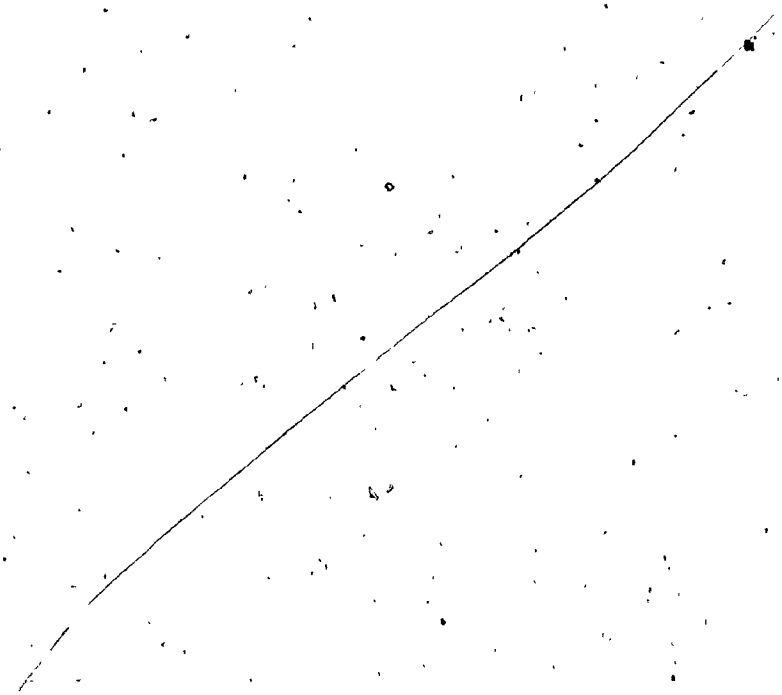
Previous experiments indicated that α-tocopherol decreases the incidence of early mortality due to stress. To further elucidate the function of α-tocopherol in this regard, the recovery of nematodes damaged with ^{60}Co gamma radiation was examined. An attempt was first made to determine whether α-tocopherol had any influence on reproduction following gamma irradiation. These experiments showed that radiation at the lowest dosage (10 kR) induced total sterility in both control and α-tocopherol-cultured nematodes, when administered before gonadogenesis was completed (96 hours in α-tocopherol nematodes, and 120 hours in control nematodes). Low dosage (10 kR) radiation administered after gonadogenesis did not prevent reproduction in either group. Post-gonadogenic irradiation at higher dosages caused total sterility in both of the groups. This may suggest

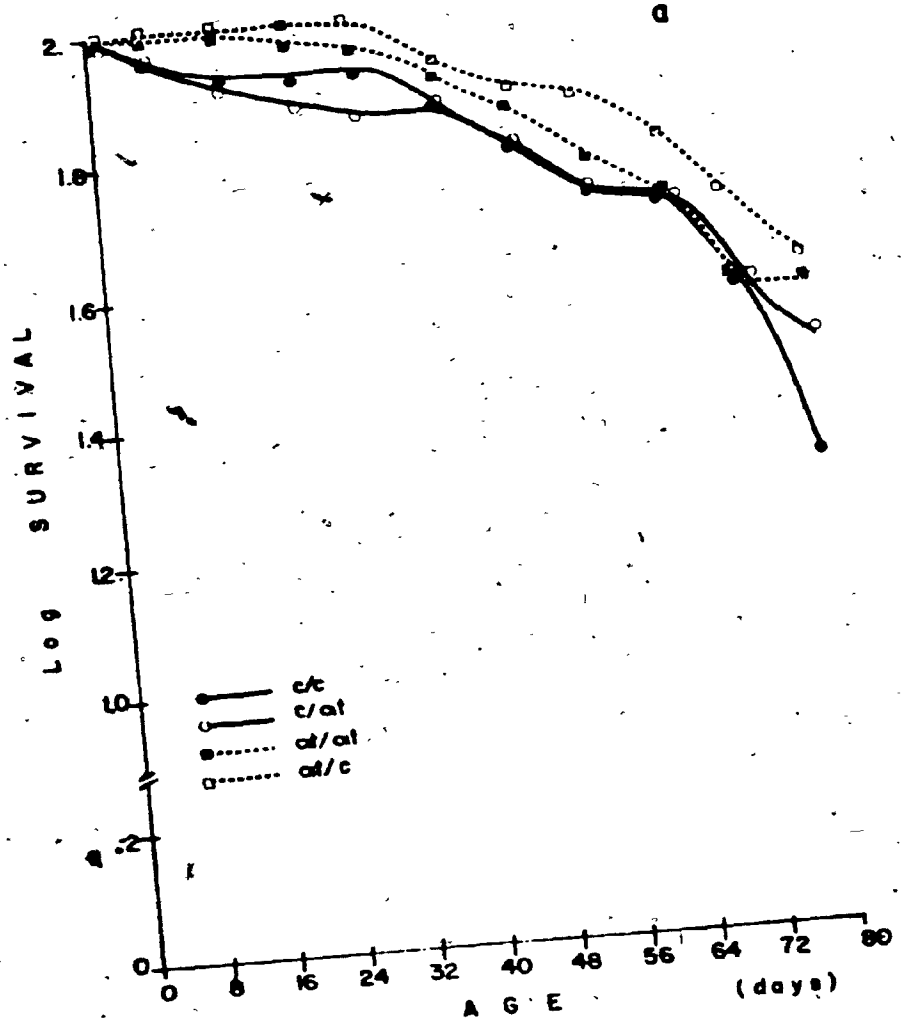
that this effect is dependent upon the developmental stage of the gonads. The morphology of the irradiated gonads was not studied.

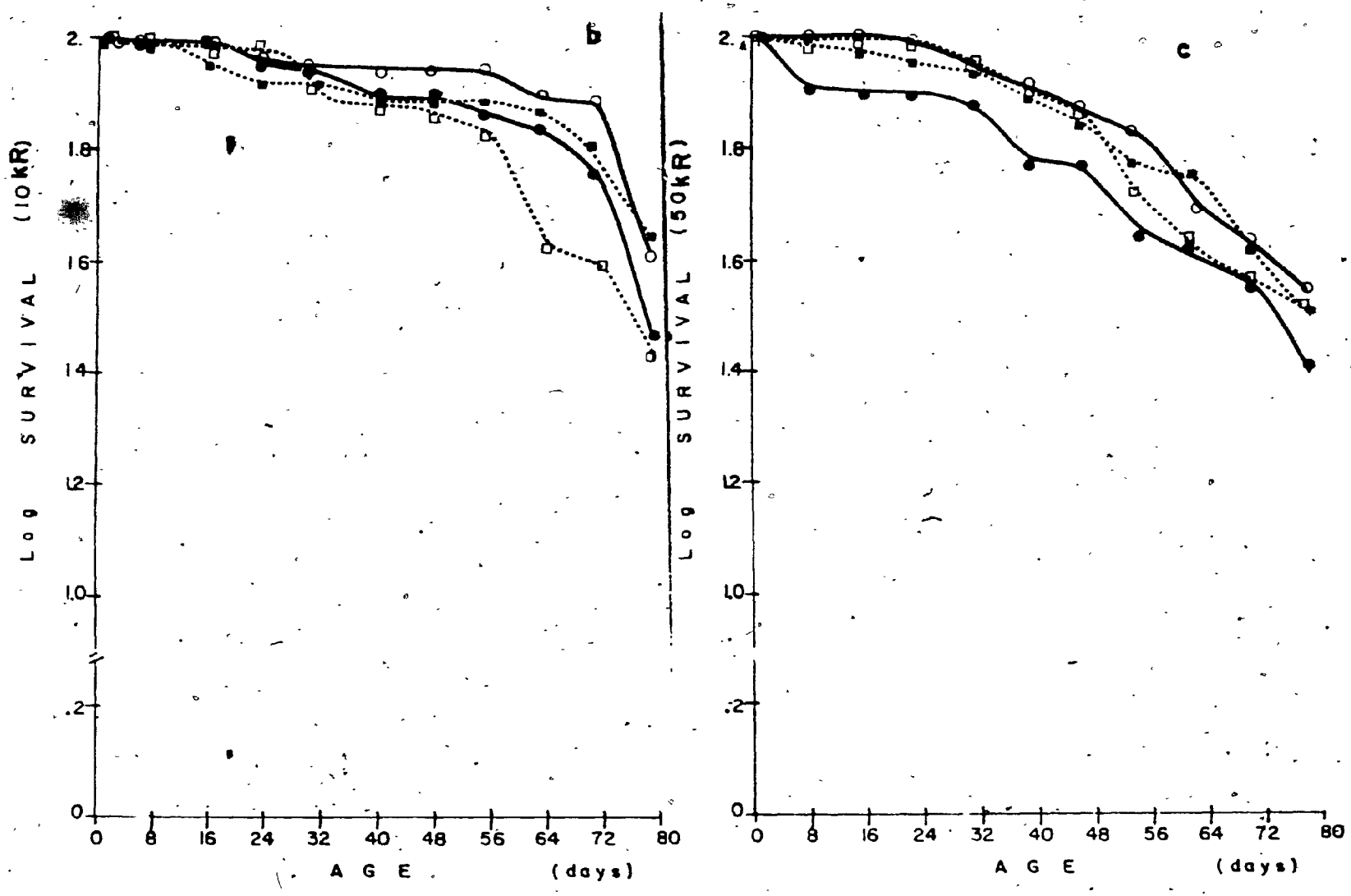
The next experiment, designed to determine the effects of irradiation on the life span of Turbatrix aceti, proved to be more suited to this organism. Survivorship was monitored for several dosages of radiation. Nematodes between ninety-six and hundred and twenty hours of age, were used to avoid any complications arising from handling during molting (see page 47). The age chosen also removed the problem of complication due to reproduction, as exposure took place after cell division (gonadogenesis) had ceased in both groups; and hence removed the possible complication of abnormal gonads.

Irradiated nematodes were treated in four different ways; nematodes of control and α-tocopherol origin were both transferred to control or to α-tocopherol media immediately after irradiation (these will sometimes be abbreviated thusly: α-Toc/α-Toc; α-Toc/C; C/α-Toc; C/C). A non-irradiated group was treated in a similar manner, to control for the stress induced by the different treatments. Figures 32 a to d show the survivorship of nematodes irradiated with dosages from 0 kR. to 100 kR. Each individual graph shows

FIGURE 31. Survival curves for nematodes of control and α-tocopherol origin exposed to a single acute dose of gamma rays, treated with α-tocopherol or control media after irradiation. (a) 0 R . ; (b) 10 kR . ; (c) 50 kR .; (d) 75kR . ; (e) 100kR .




a
Log SURVIVAL
2
1.8
1.6
1.4
1.2
1.0
.2
0
c/c
c/at
at/at
at/c
0
8
16
24
32
40
48
56
64
72
80
AGE
(days)


b
c
Log SURVIVAL (10kR)
Log SURVIVAL (50kR)
2.
1.8
1.6
1.4
1.2
1.0
.2
0
0 8 16 24 32 40 48 56 64 72 80
AGE (days)

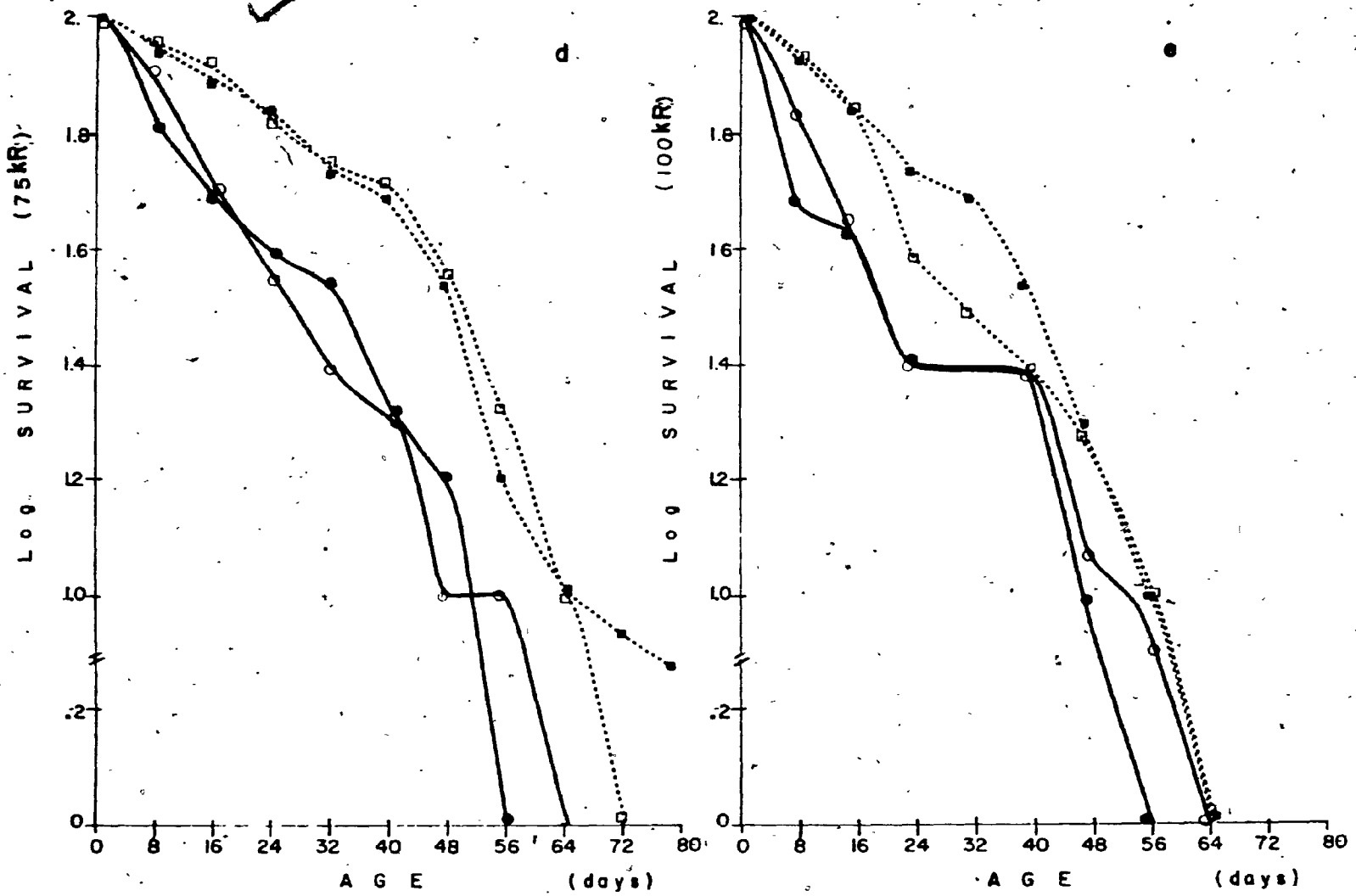
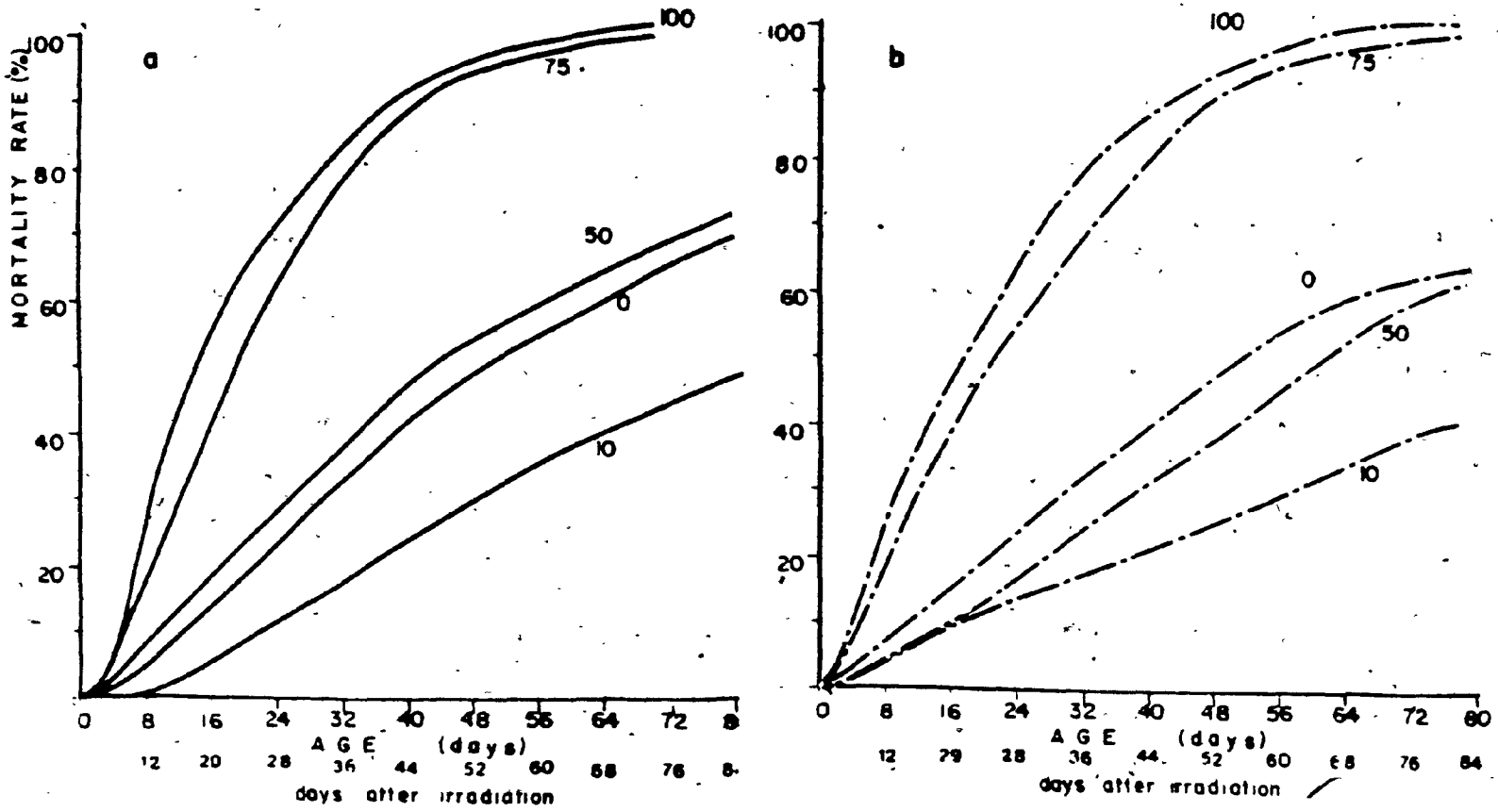
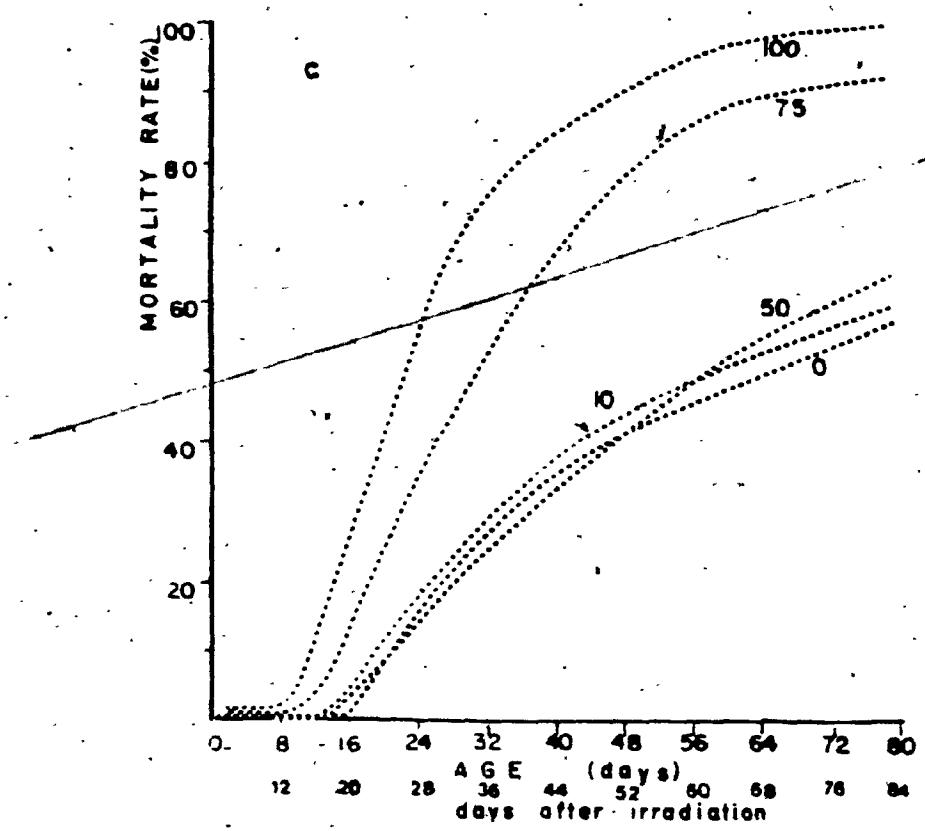
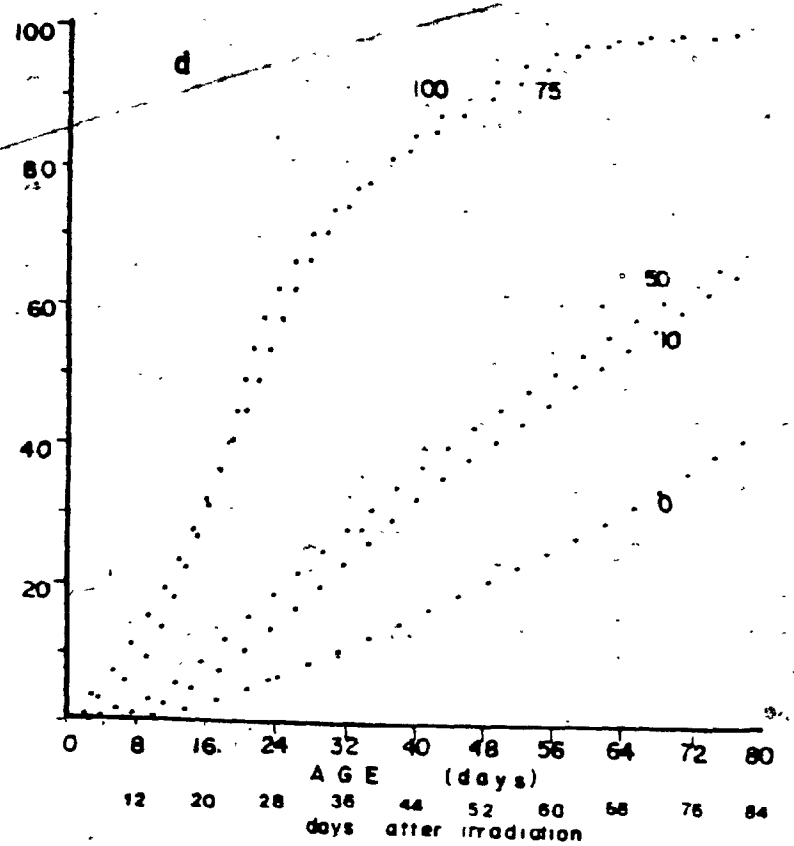

d
Log SURVIVAL (75kR)
Log SURVIVAL (100kR)
2.
1.8
1.6
1.4
1.2
1.0
.2
0
0 8 16 24 32 40 48 56 64 72 80
AGE (days)

FIGURE 32. Mortality rate from all causes for nematodes of control and α-tocopherol origin, exposed to various single acute doses of gamma rays on the third day of their life cycle. (a) nematodes of control origin treated with control medium after irradiation; (b) nematodes of control origin treated with α-tocopherol; (c) nematodes of α-tocopherol origin treated with α-tocopherol medium after irradiation; (d) nematodes of α-tocopherol origin treated with control medium after irradiation.


a
b
MORTALITY RATE (%)
100
80
60
40
20
0
8
16
24
32
40
48
56
64
72
80
AGE (days)
12
20
28
36
44
52
60
68
76
84
days after irradiation
100
75
50
0
10


c
MORTALITY RATE(%)
100
80
60
40
20
100
75
50
10
0
0 8 16 24 32 40 48 56 64 72 80
AGE (days)
12 20 28 36 44 52 60 68 76 84
days after irradiation


d
100
80
60
40
20
100
75
50
10
0
0 8 16 24 32 40 48 56 64 72 80
AGE (days)
12 20 28 36 44 52 60 68 76 84
days after irradiation

one of the four treatments noted above.

Low dosage irradiation (10 kR.) resulted in a lower mortality rate than that observed in non-irradiated nematodes. The results show a significant increase in the mortality rate in all treatment groups, when irradiated with dosages of 75 or 100 kR. Figures 31a to d, show the survivorship curves of these nematodes, grouped according to dosage. Paired t-tests to determine the significance of the difference between the curves obtained for each treatment are shown in Table 46 (significant values ($\alpha<.05$) obtained in this way are indicated by an asterisk, next to the weighted mean values obtained from the respective curves, on Table 37).

Table 37 shows the weighted means of each individual curve. It should be noted that the survival of these nematodes was only followed until day 80, thus the discrepancy between the weighted mean values obtained from these non-irradiated survivorship curves, and those shown in a previous section. A paired t-test comparing the weighted means between treatment groups further indicates that the only significant difference in the overall effect of gamma irradiation on survival is that observed between treatment C/Cand C/α-Toc. ($\alpha<.05$). The difference between control nematodes (C/C) and

Table 37

The Effect of Various Dosages of Gamma Irradiation on the Survivorship (Weighted Mean) of Nematodes Treated with α-Tocopherol Before and After Irradiation

Dosage (kR)	Treatment Before/After Irradiation [1]Weighted Mean (days)			
	C/C	α-Toc./α-Toc.	C/α-Toc.	α-Toc./C
0	35.76	36.50	38.24 **	36.80
10	38.12	41.10 *	35.80 **	40.10
25	38.16	40.69	37.73	37.36
35	36.48	38.96	35.13	34.28
50	31.56 **	37.10 *	36.17	36.94*
75	23.13**	22.27	26.46	27.00*
100	21.63**	23.23	22.00	26.70*

[1]Obtained from survivorship curves at respective dosage.

* $\alpha < .05$ for Student t-test comparing respective survival curve with C/C curve.

** $\alpha < .05$ for Student t-test comparing respective survival curve with α-Toc./α-Toc.

nematodes of α-tocopherol origin treated with α-tocopherol after irradiation (α-Toc. /α-Toc.), is of borderline significance (α=.07). This is shown in graphical from in Figure 33.

It should be noted that different treatments have different effects at different dosages of ^{60}Co. and that the significance obtained in comparing the weighted means for all dosages, is largly because three low and two high doses were used in these tests. Results from treatment with α-tocopherol after, rather than before, irradiation showed that low radiation dosages have a stimulating effect on survivorship. Nematodes of α-tocopherol origin, treated with control media after irradiation, on the other hand, do not appear to be stimulated in this way.

These results were further analyzed by computing the life expectancy of the whole population for each individual dosage and treatment. These values were obtained from life tables (see survival experiments) A graphical representation of these values is shown in Figure 34. It should be noted that these values are almost in agreement with the weighted means in Figure 33. In particular, it should be noted that Turbatrix aceti is very radioresistant. 75 kR. are required to induce a significant life-shortening effect in either of the

FIGURE 33. Curves illustrating the difference in dose response between nematodes treated with α-tocopherol before and or after irradiation, as measured by the weighted means obtained from the respective survival curves.

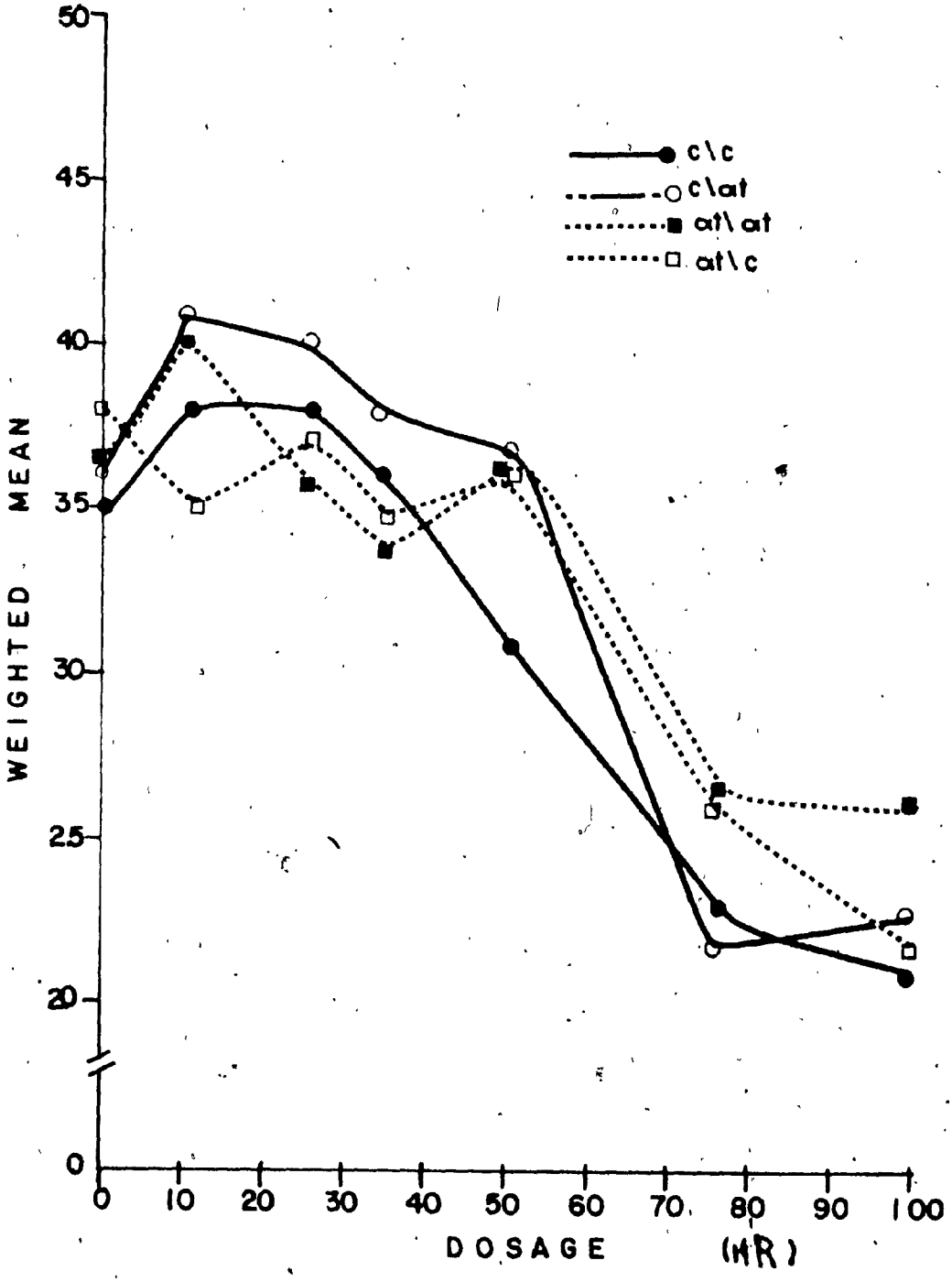

50
45
40
35
30
25
20
0
WEIGHTED MEAN
c\c
c\at
at\at
at\c
0
10
20
30
40
50
60
70
80
90
100
DOSAGE (HR)

FIGURE 34. Curves illustrating the difference in the response to different doses of gamma irradiation between nematodes treated with α-tocopherol before and or after irradiation, as measured by their life expectancy after exposure. Nematodes were irradiated at 3 days of age.

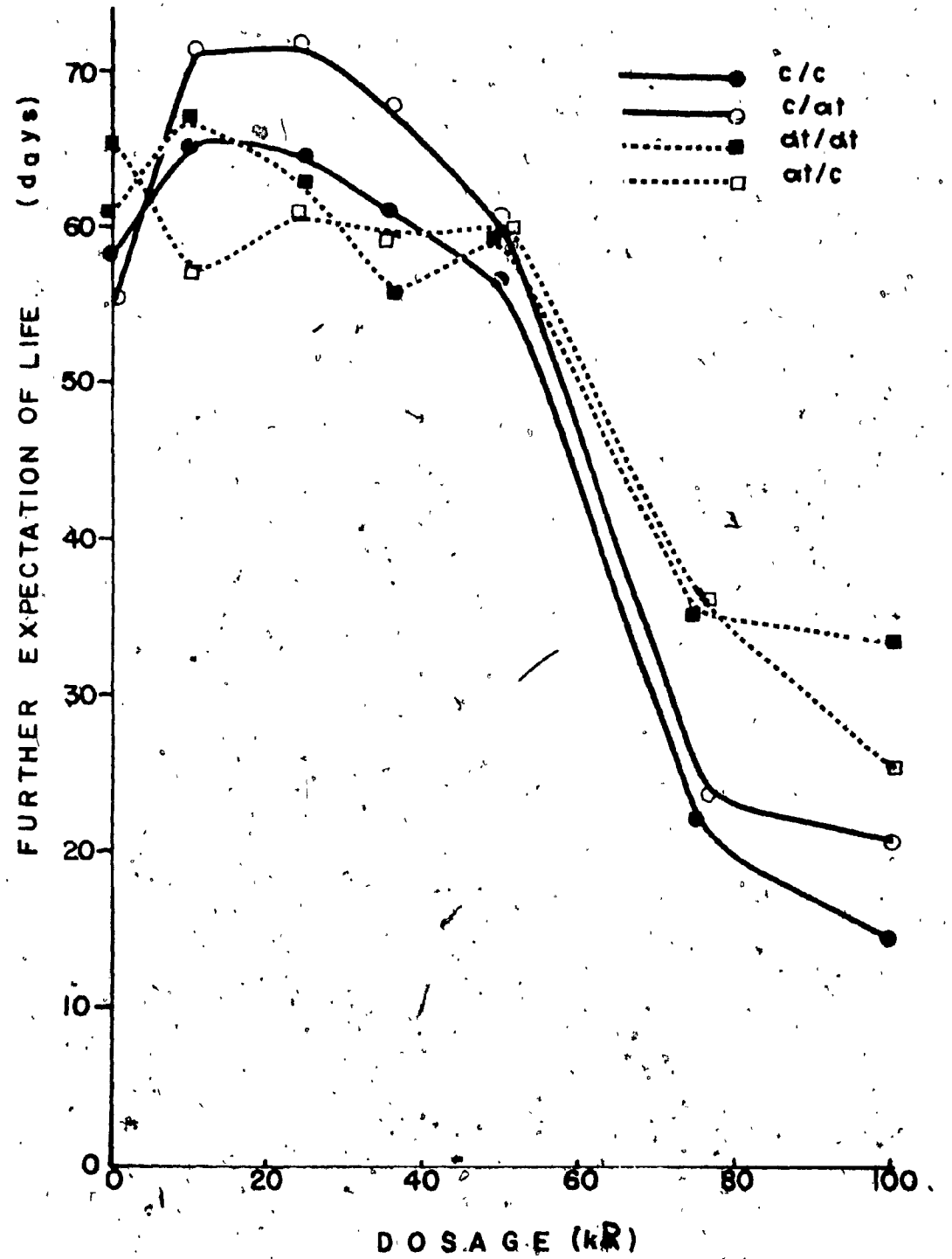
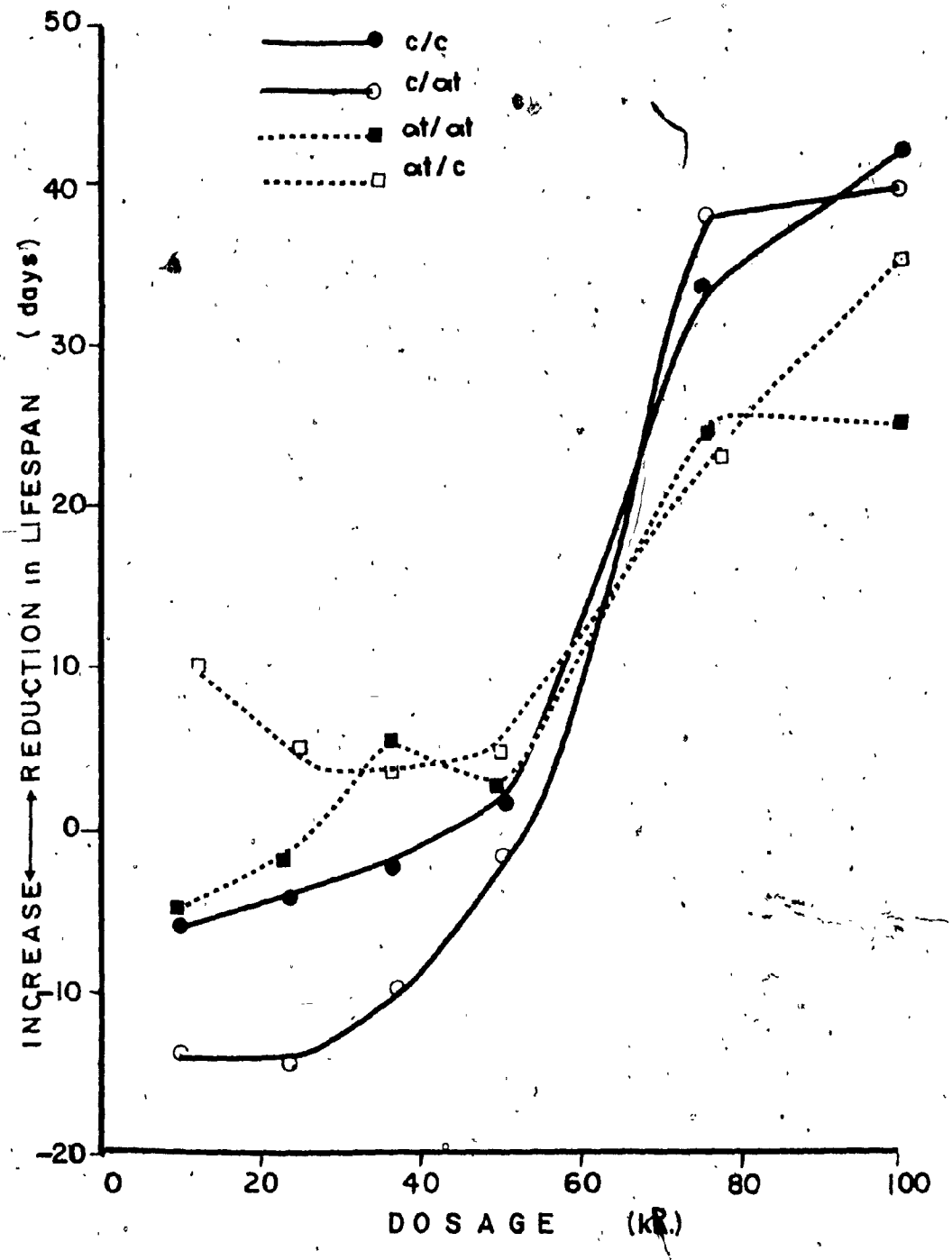

FURTHER EXPECTATION OF LIFE (days)
70
60
50
40
30
20
10
0
0
20
40
60
80
100
DOSAGE (kR)
c/c
c/at
at/at
at/c

FIGURE 35. Further expectation of life for nematodes treated with α-tocopherol before and or after exposure to high doses of gamma rays.

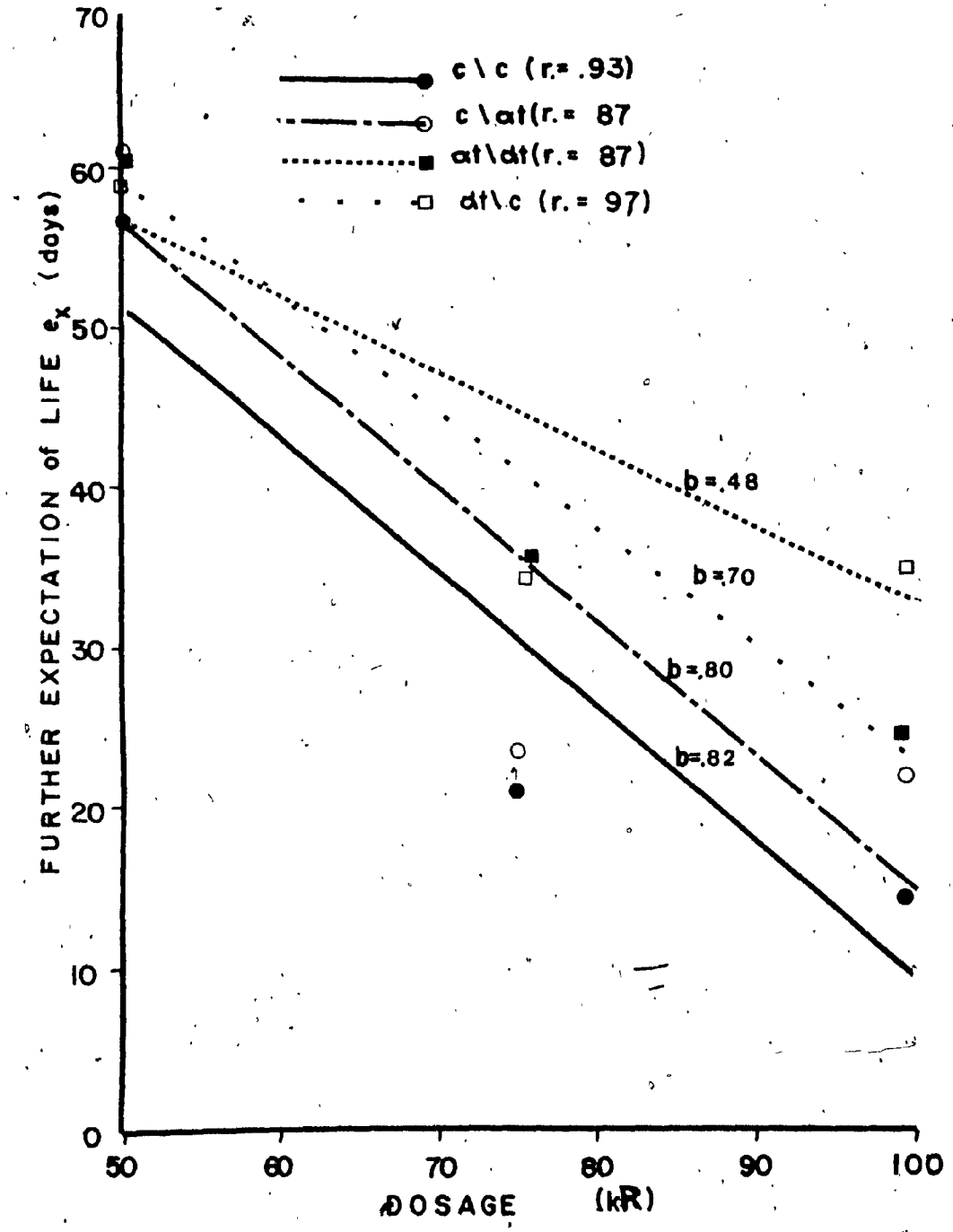

c/c
c/at
at/at
at/c
INCREASE ← REDUCTION in LIFESPAN (days)
50
40
30
20
10
0
-10
-20
0
20
40
60
80
100
DOSAGE (kR.)

four groups.

Figure 36 shows once again that low dosages of gamma radiation appear to stimulate an increase in this nematode's life span. This effect is emphasized when α-tocopherol supplementation is provided after radiation. The effect of low doses of radiation on nematodes of α-tocopherol origin are not clear.

α-Tocopherol treatment appears to play a different role at higher radiation dosages. Nematodes of α-tocopherol origin, (α-Toc./C and α-Toc./α-Toc.), are more resistant to higher radiation dosages than are nematodes of control origin, regardless of their treatment after irradiation. A graphical representation of these findings is seen in Figure 35. A regression analysis of the bottom half of curve 34 was performed in order to determine the statistical significance of the differences observed. This analysis showed that nematodes of α-tocopherol origin treated with α-tocopherol after irradiation had a significantly lower mortality rate than either of the groups of control origin. On the other hand, nematodes of α-tocopherol origin treated with control media after irradiation had a slight (but not significantly) lower mortality rate than groups of control origin. It should be noted that these regression lines only included three

FIGURE 36. Graph illustrating the difference in reduction in life span after exposure to different doses of gamma rays between different treatment groups. The reduction in life spans were obtained from the difference in life expectancy between irradiated and non-irradiated groups.

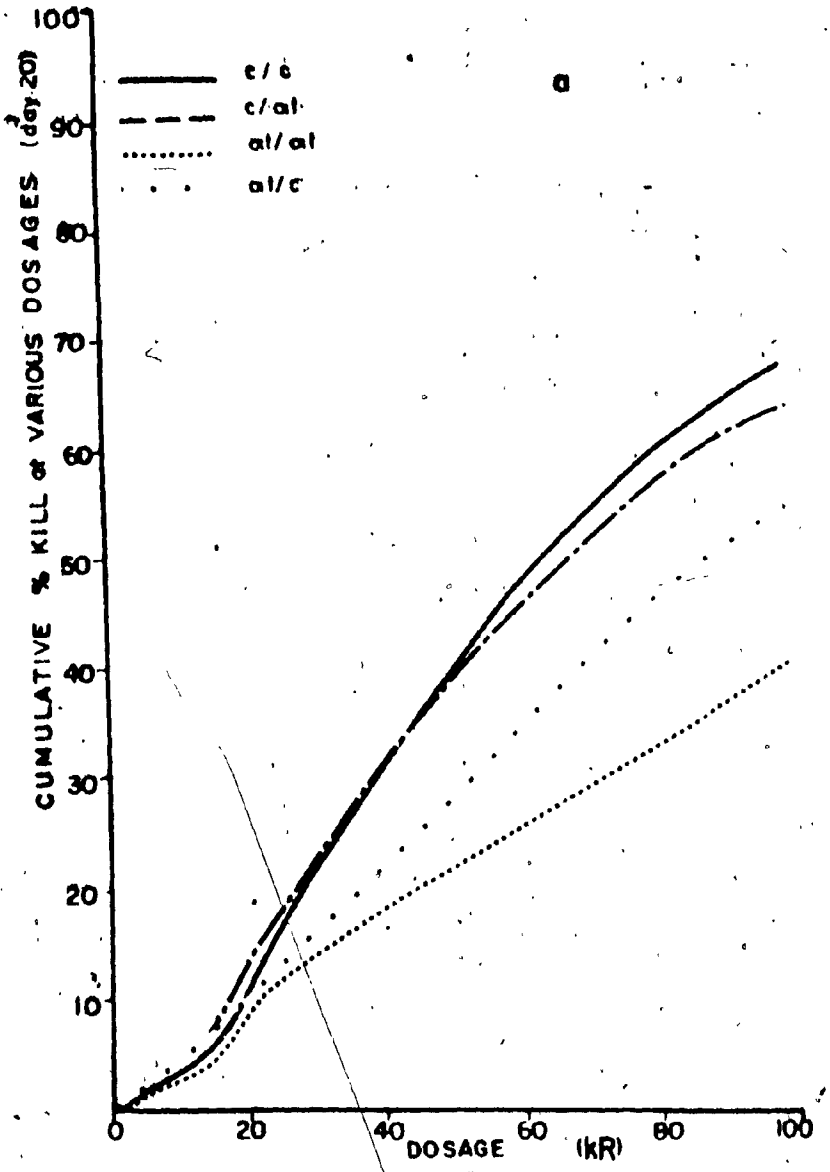

c \ c (r.= .93)
c \ at (r.= 87
at \ dt (r.= 87)
dt \ c (r.= 97)
b=.48
b=.70
b=.80
b=.82
FURTHER EXPECTATION of LIFE e_x (days)
DOSAGE (kR)
0
10
20
30
40
50
60
70
50
60
70
80
90
100

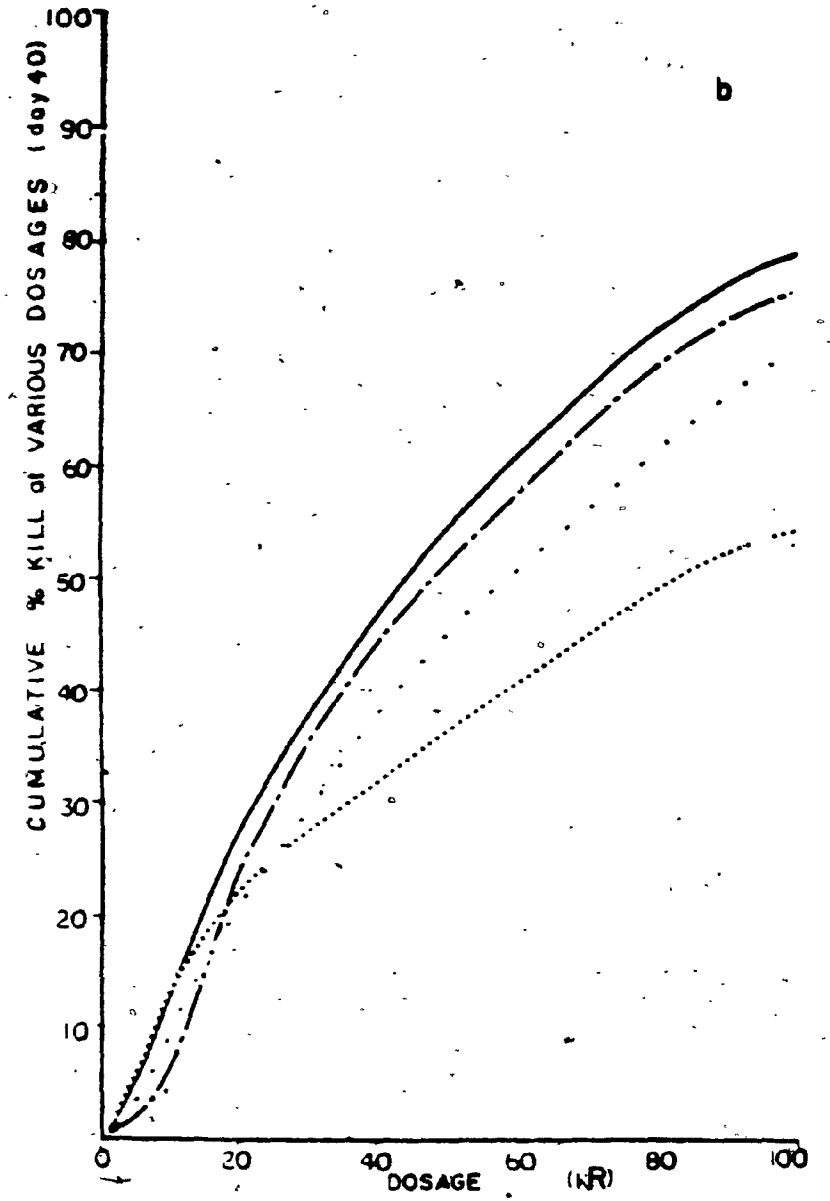
points. Observations at higher dosages of radiation would be required to follow this trend.

Figures 37 a to c show the dose response at three stages of this nematode's life cycle after irradiation (20, 40 and 60 days). The life shortening effect of ^{60}Co gamma irradiation is more obvious in the latter part of the life cycle than in the early part. Figures 38 a to c show the regression lines of these curves plotted on a probit scale. A probit analysis was performed to determine the LD_{50} values of these curves (Stanley, 1963). LD_{50} values obtained in this way were compared with LD_{50} values obtained directly from these curves. Values were within 2% of error in every case. Table 38 shows a summary of all the values obtained in the above calculations.

The test statistics for comparisons between LD_{50} values, and between the slopes of the curves, to evaluate the effects of different treatments at different stages of this nematode's life cycle, are shown in Table 47 . Observations made on the twentieth day of the life cycle showed that nematodes treated with α-tocopherol before and after irradiation were the least sensitive to the life-shortening effect induced by gamma radiation. Nematodes of control origin transferred to control media after irradiation (C/C) were the most

FIGURE 37. Curve illustrating the difference in dose response between nematodes of control and α-tocopherol origin treated with control or α-tocopherol media after exposure to a single acute dose of gamma rays, as measured by the number of nematodes killed on the (a) 20th day, (b) 40th day and on the 60th day (c).


c / c
c/al
al/al
al/c
a
CUMULATIVE % KILL at VARIOUS DOSAGES (day 20)
100
90
80
70
60
50
40
30
20
10
0
20
40
60
80
100
DOSAGE (kR)


b
CUMULATIVE % KILL at VARIOUS DOSAGES (day 40)
100
90
80
70
60
50
40
30
20
10
0
20
40
60
80
100
DOSAGE (kR)

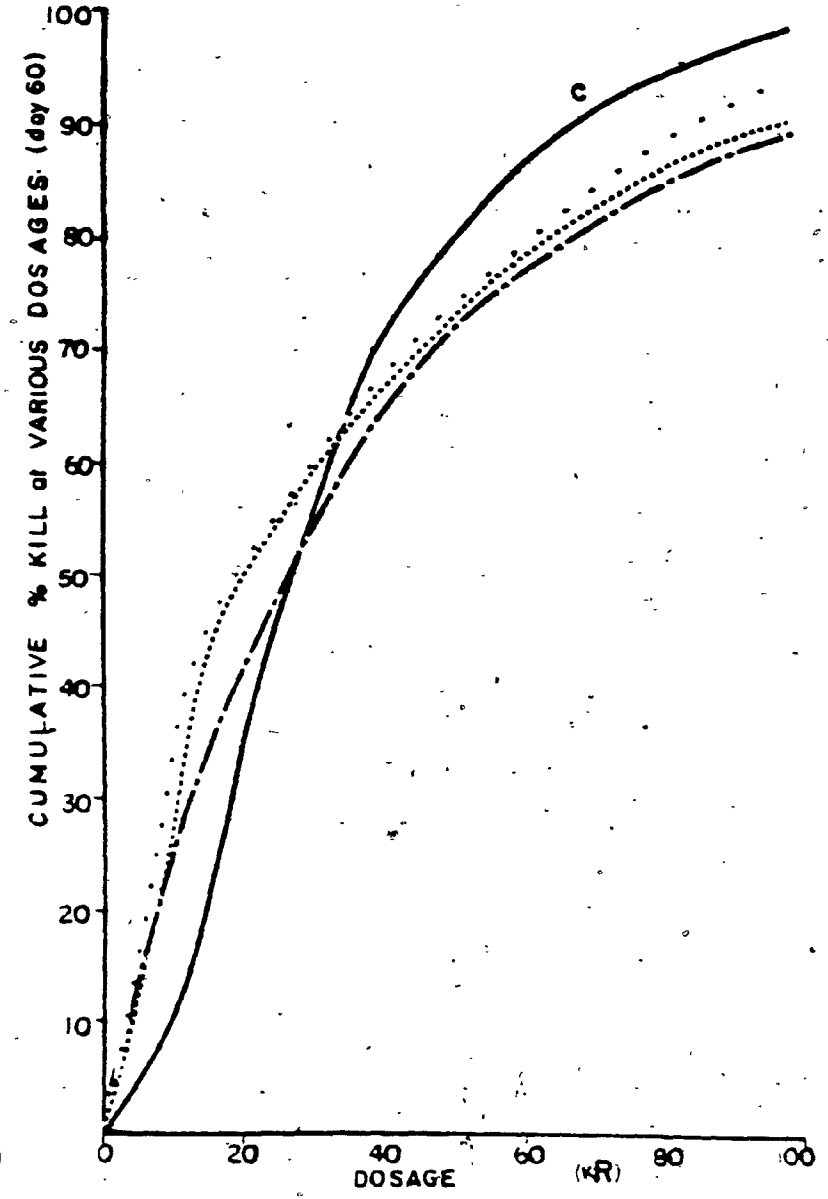

c
CUMULATIVE % KILL at VARIOUS DOSAGES (day 60)
100
90
80
70
60
50
40
30
20
10
0
20
40
60
80
100
DOSAGE (kR)

Table 38

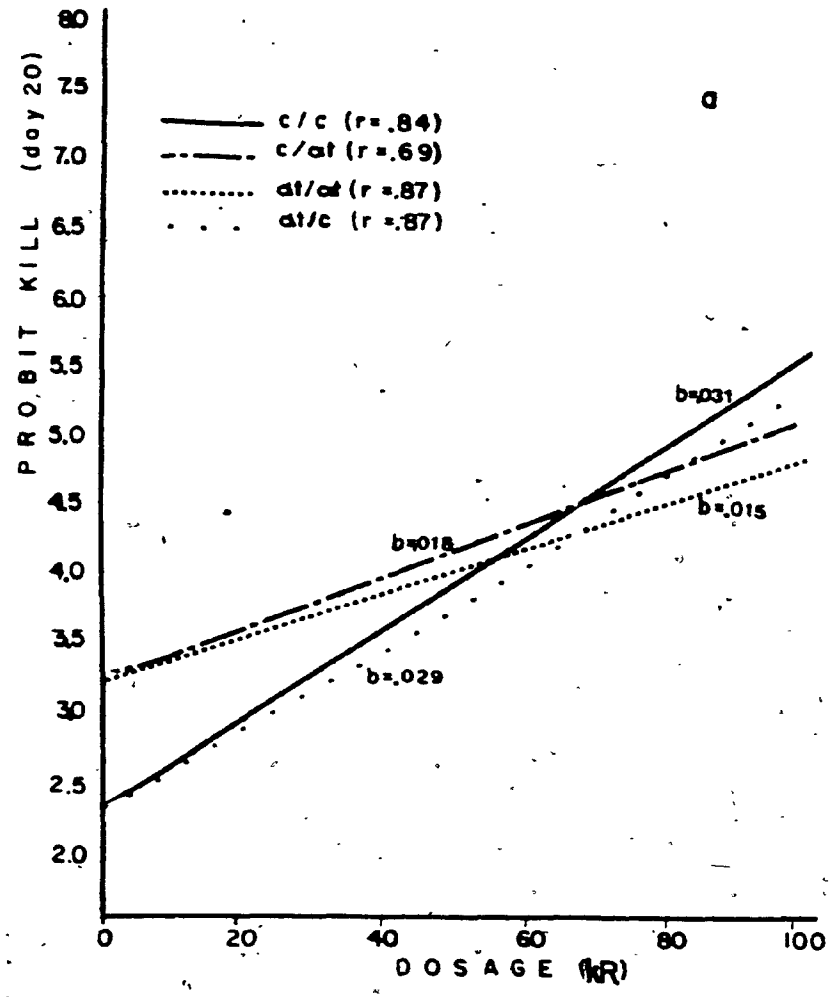
Summary of Probit Analysis Data Used to Do Test Statistic for Comparisons of Slopes and LD_{50} Values

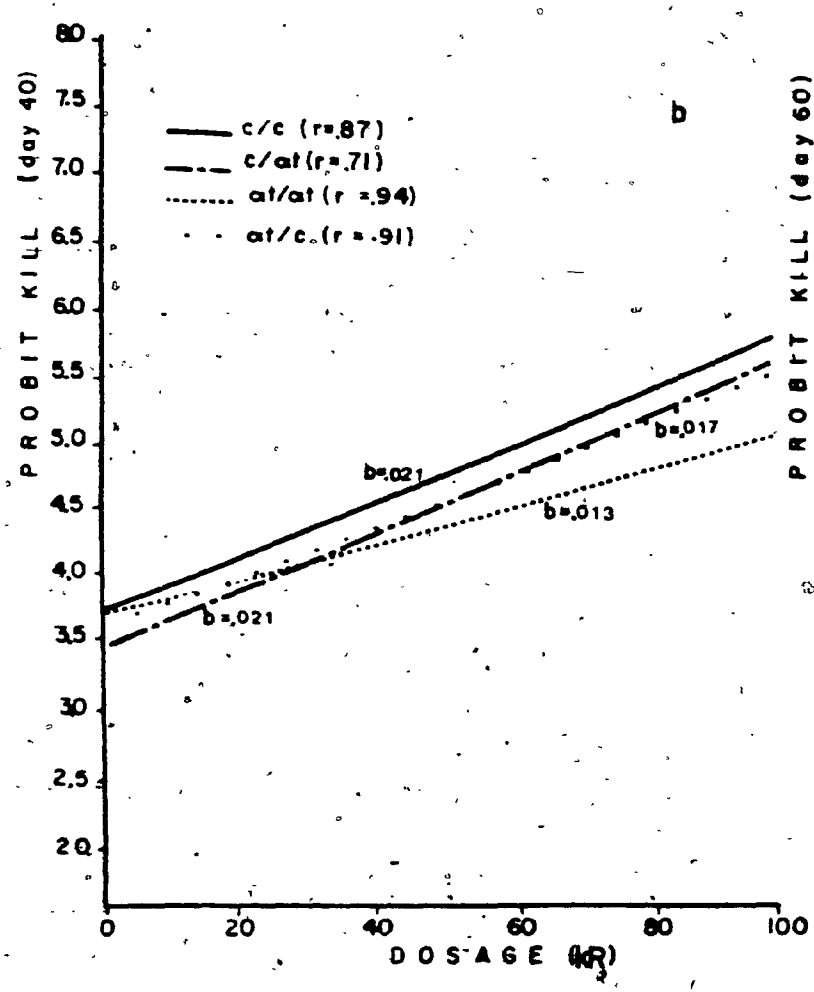
Treatment Before/After	Age at Time of Analysis (days)	$\bar{Y} \pm SE$	Corr. coeff. (r)	Slope (b)	$LD_{50} \pm SE$
Control/Control	20	3.8 ± 1.3	.84	.031	79 ± 1
α-Toc. / α-Toc.	20	3.8 ± 0.5	.87	.015	110 ± 10
Control/ α-Toc.	20	4.1 ± 0.9	.69	.018	91 ± 7
α-Toc. /Control	20	3.6 ± 1.1	.87	**.029	**88 ± 4
Control/Control	40	4.5 ± 0.8	.87	.021	**61 ± 4
α-Toc. / α-Toc.	40	4.3 ± 0.8	.94	.013	94 ± 10
Control/ α-Toc.	40	4.4 ± 0.9	.77	.021	69 ± 5
α-Toc. /Control	40	4.4 ± 0.6	.91	.017	74 ± 6
Control/Control	60	5.9 ± 1.8	.89	**.047	**21 ± 1
α-Toc. / α-Toc.	60	5.2 ± 0.7	.84	*.018	*30 ± 4
Control/ α-Toc.	60	5.0 ± 0.9	.83	.021	*40 ± 4
α-Toc. /Control	60	5.2 ± 0.6	.92	.016	28 ± 7

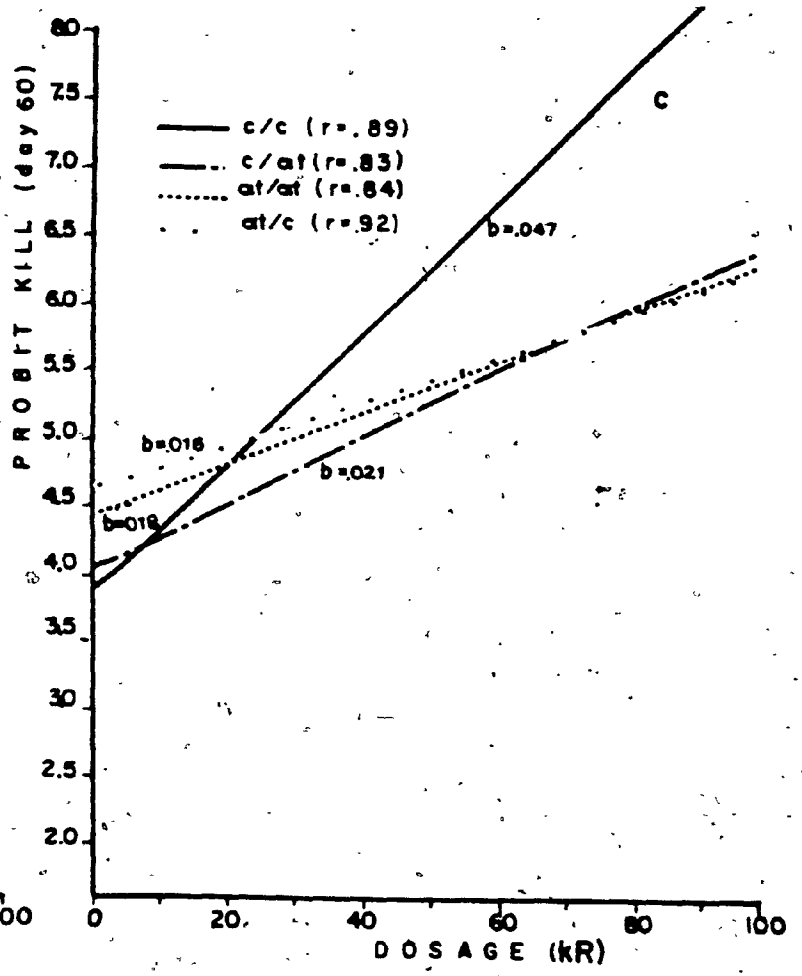
* $\alpha < .05$ compared with control/control

**$\alpha < .05$ compared with α-Toc./α-Toc. (test statistic shown in Appendix II)

FIGURE 38. Regression lines illustrating the difference in probit kill as a function of dosage between four treatment groups, as measured by the number of dead nematodes present on the (a) 20th, (b) 40th and 60th (c), day of their life cycle.


a
c / c (r = .84)
c/at (r = .69)
at/at (r = .87)
at/c (r = .87)
PROBIT KILL (day 20)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
b=.031
b=.018
b=.015
b=.029
0
20
40
60
80
100
DOSAGE (kR)


b
c/c (r=.87)
c/at (r=.71)
at/at (r=.94)
at/c (r=.91)
b=.021
b=.017
b=.013
b=.021
PROBIT KILL (day 40)
DOSAGE (kR)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
0
20
40
60
80
100


c
c/c (r=.89)
c/at (r=.83)
at/at (r=.84)
at/c (r=.92)
b=.047
b=.018
b=.018
b=.021
PROBIT KILL (day 60)
DOSAGE (kR)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
0
20
40
60
80
100

susceptible. Nematodes of the other two groups (C/α-Toc. and α-Toc./C) showed intermediate values.

Observations made on the fortieth day showed that groups treated with α-tocopherol before irradiation (α-Toc./α-Toc. and α-Toc/C) appear to have been more resistant to radiation damage than the C/α-Toc. group, treated with α-tocopherol only after irradiation. The only statistically significant difference observed, however, as between the LD_{50} values of non-treated nematodes (C/C) and nematodes treated with α-tocopherol both before and after irradiation (α-Toc./α-Toc.).

The pattern is similar, with respect to the slopes of the lines, by the 60th day of the life cycle. The only significantly higher mortality in response to increases in dosage was observed in untreated nematodes (C/C) ($\alpha<.05$). The only significant difference in LD_{50} values was that of nematodes of control origin treated with α-tocopherol after irradiation (C/α-Toc.), as compared with nematodes of control origin transferred to control media after irradiation (C/C).

In summary, the evidence presented in this section shows that α-tocopherol appears to have a protective effect against the life shortening effect that high dosages of gamma radiation have on Turbatrix aceti. Whether α-tocopherol treatment is

more important before or after irradiation appears to be dosage dependent.

Interaction of α-Tocopherol and HLE in the Nematodes Growth Media

The next experiment was designed to determine whether α-tocopherol can be used as a substitute, in whole or in part, for the essential nutrients supplied by heated liver extract (HLE) to promote optimum levels of growth and development of the species Turbatrix aceti. Preliminary experiments showed that 10 ml of HLE per 100 ml of medium is sufficient to maintain optimum growth and development.

Table 39 shows the generation time of nematodes depleted of HLE at different stages of development. Generation time is defined for these purposes as the time it takes the first generation to mature. Nematodes in a culture were considered mature as soon as the first progeny appeared (not mean generation time). The results show that when HLE was removed from control cultures before ninety-six hours of age, optimal growth and reproduction levels were not maintained. The removal of HLE from α-tocopherol cultures had no significant effect after seventy-two hours of age. These results suggest that the effects of the removal of HLE are dependent on the stage of gonadogenesis that

Table 39

The Effect of α-Tocopherol When HLE Has Been Removed at Various Stages of Development

Origin of Nematodes	Transfer Media	Nematode Age at Transfer (hours)	Generation[1] Time (days)	Estimated Number of F_1
Control	Control/No HLE	0-24	Non-Maturing	-
Control	Control/No HLE	24-48	Non-Maturing	-
Control	Control/No HLE	72	18	+
Control	Control/No HLE	96	12	+
α-Toc.	α-Toc./No HLE	0-24	Non-Maturing	-
α-Toc.	α-Toc./No HLE	24-48	13	+
α-Toc.	α-Toc./No HLE	72	11	++
α-Toc.	α-Toc./No HLE	96	8	++++

Inoculum = 20 larvae x 3 (n = 60)

[1]determined by presence of first progeny

these nematodes are going through at the time of HLE depletion.

Table 40 shows the effects of α-tocopherol on generation time and population growth, as measured by estimates made on day 21, using mass cultures grown in media depleted of HLE or in media with aged HLE. The results show that α-tocopherol promotes optimum growth and maturation in media with lower concentrations of HLE than would be required for optimum growth and maturation using control media.

Table 41 shows the results of the experiment where both control and α-tocopherol-supplemented media were compared, using varying concentrations of HLE. Maturation and fertility were tested in the usual ways, using pair cultures. Control groups showed a direct correlation between the ability to mature and the amount of HLE. The α-tocopherol group showed a similar correlation, but it was less pronounced, and was not apparent in the groups with higher concentration of HLE. α-Tocopherol-treated nematodes became fertilized in media supplemented with as little as one ml of HLE per hundred ml of medium. Control nematodes, on the other hand, were unable to reproduce in media supplemented with 2.5 ml of HLE.

Variation in the concentration of HLE has a

Table 40

The Effect of α-Tocopherol on the Maintenance of Continuous Populations in Media That is Aged and Depleted of HLE

Media	Age of Media at Onset of Experiment (days)	Amount of HLE/100 mls of Media (ml)	Generation[1] Time (days)	Estimated Population Increased on Day 21
Control	1	0	Non-Maturing	< +[†]
Control	1	1	Non-Maturing	< +[†]
Control	1	2.5	18	+
Control	1	5	12	++
Control	1	10	9.5	++++[†]
Control	30	10	13	+[†]
α-Toc.	1	0	Non-Maturing	+
α-Toc.	1	1	10.5	+++
α-Toc.	1	2.5	10.5	+++
α-Toc.	1	5	8.5	+++++
α-Toc.	1	10	7.5	+++++
α-Toc.	30	10	11	+++

Inoculum = 20x3 (n=60)

[1]determined by presence of first progeny

[2]+ = 10 100, ++ = 100 200, +++ = 200 500, ++++ = 1000 2000, +++++ = >2000

[†]Large number of dead nematodes

Table 41

The Effect of α-Tocopherol as a Possible Compliment to the Essential "Growth Factor" HLE, is Evaluated with Respect to Maturation and Reproductive Capacity in Pair Cultures

Basal Media	Amounts of HLE per 100/mls of media (mls)	n[1]	Dauer-larvae (%)	Mature Nematodes (%)	Pairs-Fertilized (%)	Mean Onset of Rep. Period±S.D. (days)	Mean Length of Rep. Period±S.D. (days)	Mean # of live offspring ± S.D. Per Pair
Control	1	34	41	23	0	-	-	-
Control	2.5	58	34	51	0	-	-	-
Control	5	70	11	85	62.5	11.8±2.0	4.2±1.7	*19.2±15
Control	10	96	8	83	70	11.1±1.3	4.5±1.4	45.5±15
α-Toc.	1	44	18	81	87.5	10.8±1.4	*4.4±0.5	*24.6± 5
α-Toc.	2.5	68	6.2	94	85	10.5±1.6	*4.4±1.7	*23.3±12
α-Toc.	5	96	5.5	94	80	9.0±1.1	6.3±1.1	67.3±12
α-Toc.	10	96	4	96	80	7.8±0.5	7.5±0.4	55.0±20

* $\alpha < .05$ as compared with non HLE depleted (10 mls)

n^1 represents total number of larvae per group (n/2 = pairs)

significant effect on the reproductive capacity of control-cultured nematodes. Nematodes in this group supplemented with less than five ml of HLE per hundred ml of media are unable to reproduce. Furthermore, nematodes supplemented with five ml of HLE produced fewer progeny, although the other parameters of reproduction were not affected.

Nematodes depleted of HLE in α-tocopherol media were affected to a lesser extent. Nematodes in this group supplemented with 2.5 ml of HLE or less showed a significantly shorter reproductive period and a lower mean number of progeny ($\alpha<.05$) as compared with α-tocopherol nematodes supplemented with 10 ml of HLE, but still showed the same incidence of fertilization. Nematodes supplemented with five ml of HLE showed a similar reproductive capacity to that observed in the group supplemented with 10 ml of HLE.

In the next experiment, the effects on reproductive capacity of addition of α-tocopherol on nematodes cultured in aged media was tested. Singly cultured nematodes were placed in either one day old medium, or thirty day old medium. Three replicate experiments were carried out in each medium, as shown in Table 42. The data in this table show that the age of the medium did have a significiant effect ($\alpha<.05$) on

Table 42

Effect of the Age of the Media on the Reproductive Capacity of Control and α-Tocopherol Singly Cultured Females

Age of Media (days)	n (pairs)	Mean Onset of Rep. Period ± S.D. (days)		Mean Length of Rep. Period ± S.D. (days)		Mann-Whitney U-Test Control vs. α-Toc.	
		Control	α-Tocopherol	Control	α-Tocopherol	Onset	Length
1-2	24	8.9±1.2	8.8±1.8	7.75±3.1	9.0±2.3		
		9.8±1.5	7.1±1.5*	7.00±2.0	9.0±1.0*	$U_{(3,3)}=9$**	$U_{(3,3)}=9$**
		10.2±1.5	7.4±1.5*	6.40±1.2	7.8±0.8*		
21	30	11.7±1.0	9.0±0.81*	5.50±1.2	5.0±1.4		
		10.7±0.5	9.0±0.63*	3.80±1.9	5.6±1.6*	$U_{(3,3)}=9$**	$U_{(3,3)}=8$
		12.4±1.0	7.2±1.8*	3.80±1.0	7.0±0.8*		
Mann-Whitney U-Test $U_{(3,3)}$ New vs. old media		9**	8	9**	9**		

** $\alpha<.05$

* $\alpha<.05$ for student t-test comparing α-Tocopherol with respective control.

the onset of the reproductive period of control nematodes. The mean onset for control nematodes was at 9.5 days in new media, but at 11.6 days in old media. There was no such difference when α-tocopherol was present in the media. Table 42 further shows that old media caused a significant shortening of the mean length of the reproductive period ($\alpha<.05$), regardless of the presence of α-tocopherol.

The Effect of Alternative Lipids on The Nematodes Growth and Reproduction

The next experiment tested the effects of other lipid-type substances in addition to, or instead of, α-tocopherol, on the population turnover in mass cultures. Cod liver oil (CLO) and immersion oil (IO) were chosen for these purposes because of their different nutritional properties. CLO has been shown to inhibit the effects of α-tocopherol. Although a beneficial effect by this lipid might suggest that α-tocopherol ensures the availability of nutrients, the main purpose of these experiments was to rule out the possibility that α-tocopherol acts merely as a lubricant preventing damage due to handling. Table 43 shows the estimated population increase twenty-one days after inoculating various culture

Table 43

The Effect of CLO and IO as Total or Partial Substitutes for α-Tocopherol on Population Increase and Generation Time

Origin of[1] Nematodes	Type and Concentration[2] Of Lipid in Experimental Media	Estimate of Population Increase on Day 21	Generation Time (days)
Control	Control	+++	9.5
α-Toc.	Control	+++	7.5
Control	α-Tocopherol (10^{-4})	+++++	9
Control	α-Tocopherol ($5x10^{-4}$)	++++	9
Control	CLO (10^{-4})	+	14
α-Toc.	CLO (10^{-4})	+	10
Control	α-Toc. ($5x10^{-5}$) + CLO ($5x10^{-5}$)	++	11
α-Toc.	α-Toc. ($5x10^{-5}$) + CLO ($5x10^{-5}$)	+++	9
Control	IO (10^{-4})	+	16
α-Toc.	IO (10^{-4})	+	11
Control	α-Toc. ($5x10^{-5}$) + IO ($5x10^{-5}$)	+++	12.5
α-Toc.	α-Toc. ($5x10^{-5}$) + IO ($5x10^{-5}$)	+++	9.5

Inoculum = 20 larvae x 3 (n = 60)

[1]Transferred to experimental media before birth

[2]In control media

flasks with twenty synchronized larvae. The results indicate that neither cod liver oil nor immersion oil, were able to maintain a continuous population when added to the control growth media at the concentration usually used for α-tocopherol. Both groups had a very low increase in population, as compared with the one observed in control media.

Supplementation of α-tocopherol growth medium with either of these lipids (diluted to a 10^{-4} (v/v) concentration in control media, and then added to an equal quantity of α-tocopherol media) did not support the optimum population growth observed in α-tocopherol media. These results indicate that neither CLO nor IO are able to maintain a continuous population. Furthermore, they suggest that both lipids, if diluted in control media (in the absence of α-tocopherol) inhibit growth and reproduction. When used together with α-tocopherol, the results are better than with no α-tocopherol, but worse than with α-tocopherol alone.

A more accurate evaluation of the effects of CLO on reproduction can be seen on Table 44. This table shows the reproductive capacity of paired cultures supplemented with CLO in their growth media. It should be noted that CLO appears to increase the

Table 44

The Effect of CLO as a Total or Partial Substitute for α-Tocopherol on Reproductive Capacity

Exp.	Origin of[1] Nematodes	Experimental Media (concentration)	Dauer-larvae (%)	Fertilized (%)	Mean Onset of Rep. Period±S.D. (day)	Mean Length of Rep. Period ± S.D. (days)	Mean # of offspring ± S.D. per pair
A	Control	Control	4	60	11.1±1.3	4.5±1.4	45±15
B	α-Toc.	Control	2	85	9.3±1.4	4.1±1.5	23±13
C	α-Toc.	Control + α-Toc. (10^{-4} M)	2	96	8.8±1.3	*7.0±0.1	*65±20
D	Control	Control + α-Toc. (10^{-4} M)	0	80	*9.6±1.3	*6.5±1.7	*66±26
E	Control	Control + CLO (10^{-4} M)	20	50	**11.5±1.5	**4.5±1.5	**28±16
F	α-Toc.	Control + CLO (10^{-4} M)	24	70	9.5±1.0	**5.5±1.0	**25±15
G	Control	Control + α-Toc. + CLO (10^{-4} M)	24	80	**11.5±1.5	7.0±2.3	55±10
H	α-Toc.	Control + α-Toc. + CLO (10^{-4} M)	32	80	9.0±0.5	6.5±2.0	**47±12

(n = 48)

[1]Transferred to experimental media before birth

* $\alpha < .05$ for Student t-test as compared to respective origin in control media (A, B)

** $\alpha < .05$ for Student t-test as compared to respective origin in α-tocopherol media (C, D)

number of non-maturing nematodes, as compared with any of the non-CLO-supplemented cultures. Student t-tests used to determine the significance of the differences between CLO-supplemented cultures and non-CLO-supplemented cultures, indicate that supplementation of control nematodes (B) with CLO, does not inhibit reproductive capacity of the nematodes that do mature to a significant extent, as compared with non-CLO-supplemented control nematodes (A). This group shows a significantly lower reproductive capacity ($\alpha<.05$) than control nematodes supplemented with α-tocopherol (D).

CLO-supplemented α-tocopherol media (G) appears to slow down the maturation of control nematodes (G) ($\alpha<.05$), but has little effect on the progeny produced, as compared with the non-CLO-supplemented group (C). On the other hand, α-tocopherol nematodes supplemented in this way (H) do not appear to be affected with respect to maturation. The only significant difference observed is in the number of progeny ($\alpha<.05$). These results show that CLO does not affect the reproductive capacity of Turbatrix aceti in the way α-tocopherol does, furthermore, CLO appears to have a deterimental effect on the maturation of this species.

DISCUSSION

In this discussion, the effects of α-tocopherol on the young nematode will be considered, followed by evaluation of the effects of dietary α-tocopherol on aspects of the aging nematode.

Effects of α-tocopherol on Growth and Development

In this section, there will be discussion of the effects of α-tocopherol on the reproduction processes of the nematode Turbatrix aceti, by examining the differences in growth and development between nematodes cultured in control media, and those cultured in α-tocopherol-supplemented media. The literature dealing with this and other nematode species will also be examined.

A great deal of work has been done on the embryology and development of the free-living, self-fertilizing, hermaphroditic nematode species C. elegans and C. briggsae. Many investigators have studied the development and maturation response of these two species to different chemically-defined media, and to an undefined "growth factor" (Dougherty et al., 1950; Dougherty, 1951; Dougherty et al., 1959; Sayre et al. 1963). Embryological analyses, have been carried out by many investigators, taking

advantage of the eutelic characteristics of these two species (Byerly et al., 1976; Klass et al., 1976; Klass, 1977; Sulston & Horwitts, 1977; Laufer et al., 1980). The only detailed descriptions of the development and anatomy of Turbatrix aceti are those of Pai (1928) and Peters (1927). These investigators were unable to define exact larval stages.

In the present study, it has been assumed that there are similarities between Turbatrix aceti and the Caenorhabditis species, with respect to their post-embryonic developmental stages. This view is supported by the facts that they belong to closely related families, that they are free-living, and that most nematodes go through four molts before reaching maturity (Lee, 1965). Based upon these assumptions, upon observations of molted cuticles, functional gonads, and upon measurements of body length, eight post-embryonic stages, from birth to adulthood, were defined (Table 1). Body length was used as the main criterion for growth. Some investigators feel that a more accurate measure is obtained by measuring the volume of the nematode (Kisiel et al., 1972). Measurement methods used here would not have produced sufficiently accurate estimates of width, however, and hence volume could not have been adequately measured.

The developmental stages thus defined were used as a base for the comparison of control and α-tocopherol-cultured nematodes. These comparisons show that α-tocopherol produced a faster rate of growth and maturation in both male and female Turbatrix aceti. The differences observed were more apparent in females than in males, because females reach a larger size earlier than males (Figures 2a and b). An interesting observation is the large standard deviation from the mean length in nematodes cultured in control media, particularly during the later stages of development (Figures 3a and b). This suggests that a significant number of larvae are slower than others in reaching maturity. This was observed in both male and female larvae and may be indicative of a nutritional deficiency in the control media.

Closer observation suggests that the growth and maturation of α-tocopherol-cultures may not have been accelerated, but rather, that it uniformly occurred at an early stage. It may be that supplementation of α-tocopherol does not induce a faster daily growth rate, but that it ensures that the bulk of the population reaches maturity at an optimum age. This is supported by the fact that the α-tocopherol group showed considerably less variation in the rate of growth

and maturation, resulting in a faster mean growth and maturation than that observed in control nematodes.

Having found that Turbatrix aceti appeared to mature faster when supplemented with α-tocopherol, attempts were made to discover the stage at which the presence of α-tocopherol is required in order to produce this effect. In particular, testing was done to determine wheter this effect if dependant upon the origin of the parent, or whether α-tocopherol is required during somatic cell division (embryogenesis) or whether it is required during formation of the germ cells (gonadogenesis). I examined the maturation of two generations following the supplementation with, or depletion of α-tocopherol, to determine how long it took for the presence or absence of α-tocopherol to have its effect.

In the first set of these experiments, gravid females (F_0) were transferred to α-tocopherol media just before giving birth. The daily growth rates of the F_0 nematodes, their progeny (F_1) and the F_1's progeny (F_2) were observed (Figure 5). I found that the F_1 nematodes grew significantly faster than the F_0 nematodes, and almost as fast as the F_2 nematodes. The F_2 nematodes matured at about the same rate as nematodes grown in α-tocopherol-supplemented media for generations. Assuming that the F_1 nematodes

had completed most of their embryogenesis when supplemented with α-tocopherol, one can conclude that α-tocopherol is not required during early embryogenesis to produce early maturation.

The depletion experiments, shown in Figures 7 and 8, support this conclusion. In these experiments, nematodes of α-tocopherol origin (F_0) were transferred to control media just before giving birth, and measurements similar to those mentioned above were made. These experiments showed that F_1 nematodes grew significantly more slowly than F_0 nematodes (or at least, that they went through the most marked growth phase a day later than the F_0 nematodes). This suggests that α-tocopherol is required in the growth media after embryogenesis in order to ensure early maturation. These results also suggest that the effects of α-tocopherol are not transferred from one generation to another.

The next set of experiments (Table 2) were designed to provide more detail, and to test the preceding conclusions. Again, as in the previous experiment, control nematodes were transferred to α-tocopherol media, and vice versa, just before they gave birth. After birth, each litter was isolated, allowing observation of litters that had been

transferred to α-tocopherol or control media at different stages of embryogenesis. Early litters were, therefore, transferred during very late stages of, or after, embryogenesis. Late litters were transferred before embryogenesis started, or during its early stages. Maturation rates were measured by counting the number of days it took each litter to reach each of two defined stages of development. The results obtained from the supplementation experiment cast considerable light on the results of the preceding experiments. It was observed that the early litters matured significantly more slowly than the later litters. Furthermore, the early litters showed maturation rates similar to those observed in control nematodes. Late litters matured significantly faster than control groups, but somewhat more slowly than α-tocopherol-cultured nematodes.

These results suggest that α-tocopherol is required during certain stages of embryogenesis, as well as after embryogenesis, in order to produce significantly earlier maturation in this species. These results also explain why the opposite conclusion was reached in the preceding experiment. In this case, the combination of early and late litters produced a mean maturation rate significantly faster than that of

control nematodes.

In considering these results, we ought also to consider that problems with the absorption of α-tocopherol may be affecting our results. It may be that well-developed embryos may be unable to absorb this molecule in quantities sufficient to affect growth and maturation. It has been shown in some ovoviviparous nematode species that embryos in the late stages of development have thick impermeable egg walls, which would prevent such absorption (Chitwood & Chitwood, 1974; Anya, 1976). This would also explain why the early litters did not show the effects of α-tocopherol.

At first glance it would appear that the depletion results for this experiment do not support the conclusion that α-tocopherol is required during some stage of embryogenesis. The early F_1 litters, whose embryogenesis took place in α-tocopherol-supplemented media, showed a significantly slower maturation rate than that of α-tocopherol-cultured nematodes. The litters, in fact showed a maturation rate similar to that of control nematodes. However, it should be noted that the later F_1 nematodes, which spent less of their embryogenesis in α-tocopherol-supplemented media, had a maturation rate significantly slower than that observed in early litters. Furthermore, their

maturation rate was significantly slower than that observed in control nematodes. These results suggest that the presence of α-tocopherol during embryogenesis is not sufficient to produce early maturation. The fact that the maturation of late litters was slower than that of control groups suggests that depletion has a stressful effect on these nematodes, and this is a complicating factor which must be considered when analyzing these results.

The results discussed thus far suggest that while α-tocopherol may be required during part of embryogenesis, its presence is also required beyond this stage. It is also known that the presence of α-tocopherol in this nematode's growth media during parental gonadogenesis does not result in early maturation. The results may or may not be in accordance with those of Gilbert and his co-workers (1968), who have reported on the effects of α-tocopherol on the morphology of another lower animal. They found that α-tocopherol induces body wall outgrowth , a phenotypic characteristic in a number of rotifer species. This change, however, is a result of cytoplasmic enlargement, rather than an increase in mitotic activity (Gilbert & Thompson, 1968). Their finding that this induction can be achieved by α-tocopherol

supplementation of new-born larvae of this eutelic organism further supports this belief. In comparing these studies with those on rotifers, it should be kept in mind that rotifers are unusually sensitive to α-tocopherol, and that this is not the case with Turbatrix aceti.

There was another experiment which resulted in valuable information about maturation. Table 2 shows the results of observations made on the variation in the maturation rate among litters of control origin. Later litters appeared to mature significantly earlier than early litters. Included in the term "late litters", are all litters after the forth one, because of the short reproductive period (four to six days) observed in control nematodes. The slower maturation rate in the very late litters is difficult to explain. This may be the result of a genetic or environmental variable. It is interesting to note that this kind of variation among litters is not observed in α-tocopherol-cultured nematodes.

In a study done on C. elegans, Beguet and Brun (1972) found that larvae from elderly parents hatched more rapidly than those bred from young parents. This phenomenon was attributed to a possible modification in the oocytic cytoplasm of the mother, due to a

disturbance in vitellogenesis. These investigators postulated that this disturbance leads to an intensification of metabolism in the embryo, shown by an acceleration in cellular multiplication, possibly caused by an increase in mitotic activity extending to gametogenesis. Direct evidence for this postulate was not shown, however.

The exact mechanism of this disturbance in vitellogenesis is not clear, but it was postulated by Beguet and Brun (1972) that it could be attributed to an increase in the DNA of cellular organelles, or to cytoplasmic transformations that arise from changes in enzyme activity in the maternal oocyte, a phenomenon frequently found in aging nematodes (Erlanger & Gershon, 1970).

From the results in this study it can be postulated that α-tocopherol inhibits the variation in maturation among litters by affecting one of the above-mentioned mechanisms. It could also be that the induction of early maturation is the result of the effect of α-tocopherol on a similar mechanism.

Although these results show that the maturation of gonads is ensured by α-tocopherol, there is no direct evidence about the effect of α-tocopherol on the development of the embryo. These results do indicate

that the presence of α-tocopherol is not required before embryogenesis to induce early maturation. This indicates that the pathway affected by α-tocopherol which governs the rate of maturation is determined after the maternal egg has been fertilized. Although this rules out a genetic effect (via the maternal oocyte), it does not exclude the possibility of a cytoplasmic effect, either during vitellogenesis, or post-mitotic cell growth.

The main conclusion that can be drawn from the experiments in this section is that α-tocopherol's presence in the growth media during embryogenesis is not sufficient to produce a significant effect on the maturation of Turbatrix aceti. It is not clear whether its presence after embryogenesis only would have this effect. It was also shown that the effects of α-tocopherol do not appear to be transferred from one generation to the next. Although α-tocopherol appears to accelerate growth and maturation in Turbatrix aceti, the difficulty in distinguishing between gonad differentiation, and cell growth does not allow us to be conclusive about whether α-tocopherol affects cell division, or whether it merely ensures rapid cell growth. The difficulty in obtaining large numbers of syncronized nematodes made isotopic studies on DNA turnover unsuccessful.

The possible variation in absorption at different stages makes it difficult to be precise about the effects of this vitamin on a particular stage of development. This complicating factor must be considered when evaluating the effects of α-tocopherol in animals to which it is administered via the growth media.

The Effects of α-Tocopherol on Reproduction

The first recognized function of vitamin E was its role as a fertility factor in the laboratory rat (Evans & Bishop, 1922). Since then, it has been demonstrated that vitamin E is needed for the maintenance of numerous other function, and the study of these functions has tended to over shadow the earlier studies on its role in animal reproduction. Many investigators believe that in most animals, E-avitaminosis has detrimental effects on other systems, which appear before there are noticeable effects on the reproductive organs (Mason, 1954). This has contributed to the relative neglect in the study of the effects of vitamin E on the reproductive system.

Intrauterine death and resorbtion of the fetus in well nourished rats was the phenomenon responsible for the discovery of vitamin E (Mason, 1933).
Several lower animals have also been shown to have

requirements for vitamin E. The house cricket Cacheta domestica requires vitamin E for spermatogenic activity and egg production, when raised on purified diets (Meikle & McFarlane, 1965). In a study of the nutritional requirements of the snail Biomphalaria glabrata raised under axenic conditions, Vieira (1967) observed that vitamin E was required for normal egg production.

Most of the studies that have attempted to evaluate the role of vitamin E on reproduction have been based on observations of animals grown with vitamin E-deficient diets, with a view to determining the direct effect of α-tocopherol. However, it is difficult to deplete a diet of vitamin E without depleting it of other essential nutrients. Supplementation of α-tocopherol was chosen as the testing method for this study. This factor should be considered in evaluating these findings.

It was decided to study the effects of dietary α-tocopherol on the reproductive capacity of Turbatrix aceti in axenic media, because of the very noticeable effect of this vitamin on this basic physiological function. It was hoped that these studies would complement the results obtained in the studies on aging.

The effects of α-tocopherol on reproduction were studied on young, senescing and nutrient-depleted worms. Several approaches were used in evaluating the role of α-tocopherol on reproduction. First, the effects of various reproductive parameters were accurately measured to determine which ones were affected by dietary α-tocopherol. The second approach was to measure the effects of α-tocopherol supplementation and depletion at different stages of development on reproduction. In this way an attempt was made to narrow the effect down to a specific stage of development, and to distinguish between a maternal and a direct effect (Riggs et al.,1972). The third approach was to measure the effects of α-tocopherol on nematodes mated in alternate ways, to allow evaluation of reproductive performance of both male and female nematodes.

A review of some of the characteristics of the reproductive cycle or this species may at this point be valuable. Several studies have been published on the fecundity, and the duration of the reproductive period of Turbatrix aceti under varying experimental conditions. Axenic culture conditions for this and other species were developed recently. Rothstein and Cook (1966) were able to produce favourable growth conditions for the reproduction of mass axenic cultures

of Turbatrix aceti at 30°C. In a similar medium, and at 27°C., Kisiel and Zuckerman (1974) were able to show that Turbatrix aceti mated on day seven after birth, started to reproduce on day eight, and that reproduction lasted eleven days. Females gave birth to an average of 106 offspring.

In this set of observations, nematodes cultured in control medium for generations were compared with those cultured in α-tocopherol media for more than two generation. The following reproductive parameters were followed: (1) the percentage of nematodes fertilized; (2) the time of mating and of onset of reproduction; (3) the length of the reproductive period; and (4) the number of live offspring. I will also discuss, where relevant, differences between the different culture methods used (single, mass, individually mated pairs).

The percentage of nematodes fertilized was defined by the number of nematodes producing live progeny. The fact that a significantly higher percentage of α-tocopherol nematodes was fertilized (Table 5), as compared to control nematodes, may be caused by an effect of α-tocopherol on the maturation of germ cells. Since the control nematodes showed no gross morphological abnormalities (as observed under the light microscope), and had a low number of dauerlarvae, the problem of

infertility probably does not lie in the maturation processes of the embryos, but rather in those of the germ cells (spermatogenesis and oogenesis). Observation of occasional unfertilized but mature eggs in paired cultures, and of non-fertile females in single cultures, confirms that both spermatogenesis and oogenesis were sometimes impaired in control cultured nematodes.

Further histological analysis of ovarian and spermatogenic tissue of these infertile nematodes would be required to determine the stage at which impairment occurs. Specific biochemical analysis of these cells would be required to discover any variation in DNA and RNA turnover, impaired protein synthesis or diminished mitotic or meiotic activity.

Nematodes cultured in α-tocopherol mate approximately one and a half days earlier than control cultured nematodes (Figure 9). This finding is in accordance with the maturation results shown in previous sections. Mating was measured in single cultures, and hence it is not surprising that the onset of the reproductive period was one and a half days earlier in single α-tocopherol cultures than in single control cultures. Again, this supports the theory that α-tocopherol ensures early

maturation.

One of the most interesting results found in this experiment was that while there was a significant difference between mean time of onset of reproduction in control paired groups and control single groups, there was no such difference between α-tocopherol groups. The later mean onset in control paired groups suggests that there is a great variability in the age at which these nematodes reach sexual maturation. Single cultures allow a measure of female maturation, since the large number of males present in the mass cultures prior to isolation of the female greater increases the probability of having a matured male. In paired cultures, both male and female must be mature before reproduction can occur. Thus, the fact that α-tocopherol single cultures had an earlier onset than control single cultures demonstrates that α-tocopherol has an effect on female maturation. The fact that paired cultures have a similar time of onset to α-tocopherol single cultures demonstrates that α-tocopherol also assures early male maturation.

Paired α-tocopherol cultures had a longer mean reproductive period than did paired control cultures. However, the mean reproductive period was similar for α-tocopherol and control single cultures. Control

single cultures had a significantly longer mean reproductive period than did control paired cultures. Taken together, these results give rise to several interesting conclusions. The first conclusion is that the maturation time of the male may be the key limiting factor in determining the length of the reproductive period. This explains the fact that control paired cultures, which had a later onset than did control single cultures, also had a shorter reproductive period. This is supported by the observation that the length of the reproductive period was similar in control and α-tocopherol single cultures, where variability of male maturity would not be a significant factor. This observation also suggests that α-tocopherol does not affect the length of the reproductive period per se. Rather, it affects male maturation, which may affect the length of reproduction.

I postulate that for this nematode, there is an optimum reproductive period, which starts as soon as the female is able to start reproduction, and which is not affected by the time of actual fertilization. Thus, the longer after the beginning of this period that fertilization occurs (depending upon male maturity), the shorter the observable reproductive period will be. The length of the optimum reproductive period does not

appear to be affected by the time the female takes to mature, since α-tocopherol single cultures, which matured earlier than did control single cultures, still had a reproductive period of approximately the same length. Thus, α-tocopherol does not appear to affect the length of the reproductive period directly. These results support the conclusions in the preceding section, which suggest that α-tocopherol ensures early growth and maturation.

The conclusions I have reached about both the time of onset and the length of the reproductive period suggests a further interpretation. This is, that α-tocopherol does not actually accelerate cell division in these nematodes but rather it ensures prompt maturation.

The mean onset of reproduction is earlier in single α-tocopherol groups, but the mean reproductive length is about the same. In most animals acceleration of the onset of reproduction leads to a shorter reproductive period (Snell & King, 1977). I suggest that the earlier mean onset observed in the results is not caused by acceleration, but by less variation, or greater consistency, in the time of onset. The control values for measurements of growth and maturation had a much greater range than did the α-tocopherol values,

suggesting that α-tocopherol is possibly assuring prompt maturation, rather than accelerated cell division. Instead of advancing the onset of reproduction, it prevents delays in the onset of reproduction.

The advantages and disadvantages of using the mean number of progeny as a parameter to evaluate reproductive capacity has been discussed in previous sections. If one asumes that the genetic variability causing the variation among nematodes of the same group is similar for control and α-tocopherol cultures, comparisons between the number of offspring produced by these groups are probably valid. A measure of the number of offspring is valuable because it allows estimation of the number of viable germ cells produced.

It is tempting to relate the number of live offspring to the length of the reproductive period. The α-tocopherol pair cultures and single cultures, which had similar reproductive periods, also had similar numbers of live progeny. Control single cultures had a longer reproductive period than control pair cultures, and also had more offspring than control pair cultures. The most interesting result is that α-tocopherol single cultures had significantly more offspring than did control single cultures, although the reproductive periods were not significantly

different.

In most free-living nematodes sperm (mature or immature) are stored in the female after mating, until fertilization occurs (Duggal, 1978). Females of closely related species are believed to produce oocytes throughout the reproductive period. Duggal (1978), has reported that a constant number of oocytes are produced daily. Detailed reproductive patterns of Turbatrix aceti have not been reported, however. In any event, studies made on closely related species and other animals make it reasonable to assume that the limiting factor in the production of offspring is the number of oocytes, rather than the number of sperm. The results suggest that this is true, since the numbers of offspring in α-tocopherol single and pair groups are approximately the same. If the number of sperm were a limiting factor, We would expect fewer offspring in pair groups despite equally long reproductive periods.

The higher number of live offspring produced in α-tocopherol single cultures than in control single cultures, despite similar reproductive periods, suggests that α-tocopherol affects the production of oocytes. It should be noted from Figure 14 that the difference in the number of offspring produced in these groups lies in the number of progeny they produced on

the first day. The rate of offspring production during the rest of the reproductive period was similar for both groups. It is possible that α-tocopherol females store more mature oocytes before being fertilized with mature sperm. This suggests that α-tocopherol ensures early maturation of viable oocytes. Females have been shown to mature approximately one day earlier than males in this species (Zuckerman, 1974). This explains why in fact both groups produce significantly more offspring on the first day than on any other day.

In summary, the observable effects of α-tocopherol on reproduction of Turbatrix aceti are probably the result of an α-tocopherol affect on maturation.

Having established that α-tocopherol has an effect on the reproduction of Turbatrix aceti, studies were done whereby α-tocopherol was added to or removed from the media at different stages of development. By ascertaining the earliest stage at which supplementation with α-tocopherol affected various reproductive parameters, and the latest stage at which depletion affected them, I hoped to determine at just what developmental stage the presence of α-tocopherol was required in order to produce a significant effect on reproduction.

A significantly earlier onset of reproduction

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was achieved by supplementing progeny before birth. Supplementation after birth, up until 6 hours of age, also produced an earlier onset of reproduction, although this value was of borderline significance. Depletion experiments showed that the onset of reproduction was significantly delayed if α-tocopherol nematodes were transferred to control media as late as 0 to 6 hours after birth. Depletion after this time had no significant effect. This suggests that α-tocopherol is required as late as six hours after birth, in order to ensure early onset of reproduction (assuming minimum stress due to handling at this early age). The supplementation results suggest that α-tocopherol is required during embryogenesis (before birth), as well, in order to ensure an early onset of reproduction in this nematode species. The maturation studies also included supplementation and depletion experiments (Table 3). The depletion results in these experiments are in agreement with the above. Depletion in late embryogenesis (just before birth) caused a later onset of reproduction. Although these results suggest that α-tocopherol is required after embryogenesis, it does not rule out its requirement during embryogenesis.

A number of complicating factors must be considered in evaluating these results. It is

possible that embryos in very late stages of development are unable to absorb this lipid molecule. Viviparous nematode embryos have been shown to have egg walls impermeable to a large variety of molecules (Anya, 1976). I mentioned above that Riggs and his co-workers (1972) reported that the rotifer is the only lower animal in which α-tocopherol effects have been induced by direct supplementation of α-tocopherol to the larvae rather than via the mother. On the other hand, stress due to handling of young larvae could be masking some of the beneficial effects of α-tocopherol supplementation. In summary, then, the results suggest that α-tocopherol is required before the onset of gonadogenesis (but after birth) to ensure a significantly earlier onset of reproduction. Whether it is required during embryogenesis is not clear.

α-Tocopherol appears to be required during, but not earlier than, the late stages of gonad development in order to produce a significantly longer reproductive period. This suggests that the pathway that determines the length of the reproductive period is determined during late gonadogenesis (oogenesis or spermatogenesis). Generally, the results show that the longer the nematodes are kept in α-tocopherol, the longer the reproductive period will be.

Depletion experiments showed that α-tocopherol depletion before 12 hours of age significantly affected fecundity (% fertilized) in this nematode. Supplementation experiments on the other hand are not clear, and are probably affected by an absorbtion factor. In any event, it appears that for α-tocopherol to have an effect on this parameter, its presence is required in the growth media during the later stages on gonadogenesis.

Observations of the mean number of live offspring of paired cultures showed a significant decrease when α-tocopherol was removed from the nematodes growth media prior to the later stages of gonadogenesis. α-Tocopherol increased the number of live progeny even when added 120 hours after birth. These results again suggest that the number of offspring produced in paired cultures varies with the length of the reproductive period. It may be that both of these parameters (length of reproductive period and number of offspring) are governed by the effect of α-tocopherol in ensuring the viability of early oocytes, or possible spermatocytes. Once again these effects are most likely a result of early maturation.

It was demonstrated that α-tocopherol causes an increase in the reproductive capacity of this nematode.

Another interesting effect of α-tocopherol is the formation of dauerlarvae. Oddly enough, supplementation or depletion of α-tocopherol at specific stages of development results in the formation of more dauerlarvae than the usual number present in either control or α-tocopherol cultures. Gilbert et al. (1968) induced mictic females in rotifers supplemented with α-tocopherol in their growth media. These females are formed to overcome poor environmental conditions. Daurlarvae were induced with supplementation and depletion of α-tocopherol. It may be that the sudden addition of α-tocopherol to the nematodes' growth media at a specific stage of development induces poor conditions. Since α-tocopherol has not been shown to induce adverse conditions with respect to reproduction, it is possible that the interaction is between this lipid and the molting hormones, which likely have an essential role in this developmental process. The presence of large numbers of dauerlarvae as a result of depletion is not so surprising, although their absence in nematodes depleted before twenty-four hours of age (Table 12) is. Even more puzzling is the presence of these poorly developed larvae in the next two generations, in particular because these progeny were not the result of dauerlarvae parents.

I also observed the reproductive parameters of the progeny of the nematodes transferred to or from α-tocopherol before birth and before the onset of reproduction. I found that after depletion there were no significant effects of α-tocopherol transferred from one generation to the next. What these results did show is that the poor reproductive capacity that results from α-tocopherol depletion during development is passed on to these parents' progeny.

Although these results do not allow us to identify with precision the mechanism affected by α-tocopherol, they do suggest that more than one mechanism related to reproduction is affected in this nematodes species. I have also shown that most, if not all, of the effects of α-tocopherol on the reproduction of Turbatrix aceti are related to an effect of this molecule in ensuring maturation or viability of their germ cells. There is no evedence however, to distinguish between a direct effect on cell division and an effect merely on nutrient availability. The observed effects on the actual molting process, and the fact that α-tocopherol is required before, rather than during, gonadal cell division for an early onset or reproduction, and after gonad formation for an increased production of offspring, might suggest

that the effect of α-tocopherol under our culture conditions is that of ensuring the availability of essential nutrients.

There are several theories on the mode of action of α-tocopherol which would explain this availability. They are, however, beyond the scope of this study.

Effects of α-Tocopherol on Parental Senescence

So far, I have shown that progeny from aged parents have an increased mean maturation rate, as compared with progeny from young parents. I have also shown that α-tocopherol supplementation to the nematodes' growth media ensures early maturation in a larger number of nematodes.

To further evaluate the effects of parental senescence on the maturation of progeny, I examined the reproductive capacity of aged virgin nematodes mated to each other and to younger nematodes. We also examined the reproductive capacity of progeny from young and old parents.

A number of investigators have shown that parental senescence in higher organisms has an effect on the F_1 generation especially with regard to reproductive function (Lansing, 1947; Comfort, 1957; Klass, 1977). The variability observed in the reproductive behavior

among F_1 generations has caused some controversy, about this postulate. It was suggested by a number of investigators that it may be explained by the genotypic heterogeneity in some species (Beguet & Brun, 1972). Using the hermaphroditic nematode C. elegans, these investigators studied the effect of parental senescence on the sexual maturation of the F_1 generation.

Beguet and his co-workers were able to show that larvae produced by aged parents matured more rapidly than those bred from young parents. These investigators believe that this is caused by an intensified metabolism in the embryo due to an acceleration in cellular multiplacation or in mitotic activity. These investigators also found a reduction in the number of spermatozoa in progeny of aged parents, and attributed this observation to the duration of the male phase (spermatogenesis) of this hemaphroditic species. Furthermore, the small numbers of F_2 progeny produced by the F_1 generation were attributed to a large incidence of mortality in the eggs, rather than reduced sperm numbers in the F_1 generation.

In this experiment I found that the reproductive life of virgin females is longer in control-cultured

nematodes that in α-tocopherol supplemented females (Table 15), suggesting that early maturation leads to an earlier end of reproductive life in virgin nematodes. The significant decrease in the length of the reproductive period, and number of live offspring produced by these pairs as compared with young pairs, is not surprising since these nematodes mate late in their reproductive lives. Similar observations have been reported by Zuckerman (1974) in the same nematode species, although his study showed a much longer reproductive life for both male and females. These findings are in accordance with those discussed in previous sections. An earlier maturation in α-tocopherol nematodes would be expected to lead to an early end of the reproductive period.

In addition to these findings, we showed that senescing control parents produced a large number of larvae that died before maturation. Dead F_1 larvae were not observed in senescing α-tocopherol cultures.

If the presence of dead larvae in aged control cultures can be attributed to a disorder in the senescent germ cell of the parents, as has been suggested by Beguet and Brun (1972), it is possible that this disorder is related to misfunctions in the protein synthesis mechanism since this is an

essential function for the production of viable offspring (Lee, 1965; Anya, 1976). If this is the case, it may be postulated that α-tocopherol is able to prevent, or at least, mask this disorder. Supplementation of α-tocopherol to the growth media of Turbatrix aceti has been shown to have an effect on the patterns of enzyme activities in senescing nematodes (Bolla & Brot, 1975).

Comparisons between the reproductive capacity of early litters and the reproductive capacity of late litters, obtained from the same parents (Table 17), in both control and α-tocopherol cultures, showed that late control litters that managed to survive to reproductive age, had a significantly lower reproductive capacity than the comparable litters cultured in α-tocopherol medium. Late litters of control parents also showed a significantly lower reproductive capacity than early litters. Very late litters (rarely produced in control cultures) were unable to reproduce, although no gross abnormalities were observed with respect to maturation (as seen under the light microscope).

It should be noted that litters (IV, V, VI) which in previous experiments showed an accelerated maturation did not show an early onset of reproduction in this experiment. This might suggest that although embryogenesis and early gonadogenesis appeared to be

accelerated, possibly by the influence of the parental oocytes (Beguet & Brun, 1972), oogenesis and spermatogenesis might not have been accelerated. Bugnet and Brun (1972) found that spermatogenesis was reduced in the F_1 generation.

In examining the reproductive capacity of the α-tocopherol litters, I found that although very late litters in this group (XII, XIII) are able to reproduce, they show a somewhat lower fecundity (% fertilization) than the early litters in the same cultures. It should be noted that the other parameters of reproduction are also somewhat affected in the later litters (Table 17). The exact effect of α-tocopherol on late litters is difficult to interpret, due to the interaction between acceleration due to α-tocopherol supplementation, and acceleration due to senescent germ cells. The fact that these very late litters show a normal reproductive capacity, as compared with that observed in early control nematodes, does indicate that α-tocopherol has a significant effect on the mechanism that results in progeny with faulty reproductive capacity.

Although accurate measurements of the reproductive capacity of the progeny of these late litters (F_2) were not done due to low numbers, general observations indicate that their growth and maturation were normal

in both control and α-tocopherol culture groups.

The following can then be concluded from this particular set of experiments: 1) α-tocopherol nematodes have a mean reproductive life that begins and ends earlier as a result of an earlier mean maturation; 2) if mating is allowed as soon as sexual maturation is reached, α-tocopherol ensures normal reproductive capacity in all litters.

If the effect of parental senescence is to disturb the regulation of the gametogenic cellular division in the F_1 progeny (Beguet & Brun, 1972), and if we accept the premis that this is a result of a disturbance in maternal oocytes (Beguet & Brun, 1972; Clark & Rockstein, 1964), it could be postulated that under our experimental conditions α-tocopherol ensures normal vitellogenisis in the maternal oocyte. This postulate is further supported by the fact that changes in enzymatic activity and an increase in aberrant proteins with age has been reported for this and other nematode species (Rothstein et al., 1974; Erlanger & Gershon, 1970; Reib et al., 1975; Gershon, 1979; Prasanna, 1979). Furthermore, changes in enzyme activities have been reported following supplementation with α-tocopherol in Turbatrix aceti (Bolla & Brot, 1975). I postulate then that even if α-tocopherol

accelerated, possibly by the influence of the parental oocytes (Beguet & Brun, 1972), oogenesis and spermatogenesis might not have been accelerated. Buguet and Brun (1972) found that spermatogenesis was reduced in the F_1 generation.

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Although accurate measurements of the reproductive capacity of the progeny of these late litters (F_2) were not done due to low numbers, general observations indicate that their growth and maturation were normal

69 ± 4 days) lead these investigators to postulate a direct effect of antioxidants on the aging process.

Several reports have been published on the longevity of Turbatrix aceti under varying experimental conditions. Published results on maximum longevity range from 48 days (Pai, 1928) to ten months (Hennenberg, 1900). I found that under these experimental conditions Turbatrix aceti had a mean life span of 45 ± 29 days, a life expectancy of 58 days, and a maximum life span between 96 and 104 days. The weighted mean life span was 37 days. The α-tocopherol-supplemented nematodes had a mean life span of 64.4 ± 28, a life expectancy of 68 days, a weighted mean life span of 41 days, and a maximum life span of 112-120 days. It should be noted that the values that represent the overall survivorship are not in agreement with each other.

The survivorship curves obtained from three replicate experiments (Figure 24) indicate that the difference between the survivorship of control-cultured nematodes and that of α-tocopherol-cultured nematodes is most pronounced during the early (0 to 30 days) and late (72 to 104 days) stages of the nematodes' life cycle. Life tables obtained from these data (Figures 27 a and b) support these observations. These figures

indicate that control nematodes showed a close to normal distribution in the number of deaths per day (d_x); a distribution that would be expected in a senescing population (Lamb, 1977). α-Tocopherol-supplemented nematodes, on the other hand, showed a more spread-out distribution.

Maximum life span (the longest-living nematode in an experimental group) always appears to be lengthened in α-tocopherol-treated cultures. Gershon and his co-workers (1972) have postulated than an extension of the maximum life span is indicative of a direct effect of α-tocopherol on senescence. Due to the low number of nematodes involved in this measurement, I felt that for my purposes, the other parameters chosen to evaluate the effects of α-tocopherol on longevity would be more conclusive.

For the purposes of this discussion, the effects of α-tocopherol on longevity will be examined first in terms of effects that prevent or delay early mortality (death before 24 days), and then in terms of effects that delay senescence or late mortality. I will discuss these separately because of the different causal relations involved. Parameters used to evaluate the overall mortality (weighted mean life span, life expectancy) are used to evaluate the

effects of α-tocopherol on the overall life span.

The results show that α-tocopherol decreases the incidence of early mortality. Two explanations for early mortality can be advanced. One is that the high incidence of death is attributable to a stress factor, either induced by handling, or due to changes in environmental conditions. The other possible explanation is that early mortality is a result of congenital abnormalities, or abnormalities brought on by a defect present at birth.

In examining early mortality in detail (Figures 28 a and b) we found a significantly higher incidence of early mortality in control nematodes than in α-tocopherol nematodes. I also found a larger variance among the four control curves (representing experimental groups isolated on four different days during development) than among the four α-tocopherol curves. The control curves showed a lower mortality rate for the late isolation dates. This may be due to a difference in susceptibility to damage due to handling, but it is more likely the result of not being able to count the number of nematodes which had died before isolating the later groups. The incidence of early mortality in α-tocopherol groups was so low that there was no significant difference between the four isolation

days.

α-Tocopherol may act by diminishing the incidence of early mortality either by reducing the incidence of abnormal newborn, or by protecting young larvae against stress (or both). Normal vitellogenesis is required to produce normal larvae (Anya, 1976). Therefore, it could be that a higher incidence of abnormal vitellogenesis occurs in control cultures, and that α-tocopherol ensures that vitellogenesis will be normal. This effect could be correlated with the effect of α-tocopherol in preventing production of dead larvae by progeny of old parents. As has been mentioned above, the production of abnormal or dead larvae by senescing parents has been attributed to abnormalities in the cytoplasm of the maternal oocyte (Beguet & Brun, 1972). I have no evidence to rule out the possibility of an effect of α-tocopherol on a nuclear mechanism. The possibility of α-tocopherol playing a role in the induction of a repair mechanism has been suggested from its radioprotective effect (Dr. R. M. Roy, Personal communication).

The evidence presented on the effects on early mortality of the supplementation and depletion of α-tocopherol at various stages of the nematode's life cycle does not allow us to draw firm conclusions in

this respect. These results suggest that the presence of α-tocopherol in the growth media is required after birth (post-mitosis) and possibly before birth, in order to reduce the incidence of early mortality in Turbatrix aceti. Supplementation with α-tocopherol immediately after birth did not significantly reduce early mortality (Figure 29b). On the other hand, removal of nematodes from α-tocopherol media after birth did significantly increase the incidence of early mortality (Figure 30b). The removal of nematodes from α-tocopherol media after ninety-six hours of age did not result in increased early mortality. Furthermore, α-tocopherol supplementation after ninety-six hours of age did not reduce the incidence of early mortality. These results indicate that there is a period between twenty-four and ninety-six hours of age during which α-tocopherol is required to inhibit early mortality. It should be noted that this is the period when most active cell growth and membrane synthesis take place in these nematodes (Gershon et al., 1972). The effects of vitamin E deficiency on protein synthesis have been well-documented (Dinning & Day, 1957) and have recently been reviewed by Catignani (1980).

In suggesting that this time period is the crucial one, we are assuming that absorption occurs

at the time of supplementation. It is possible that supplementation between birth and twenty-four hours of age might have had a significant effect had these young larvae been able to absorb this lipid fast enough. Therefore, the fact that this group had a high incidence of early mortality does not necessarily mean that α-tocopherol is required before birth to have a significant influence in this regard.

I found that there was no significant difference between the mortality rates of nematodes supplemented with α-tocopherol at twenty-four and ninety-six hours. I also found that control nematodes transferred to control media at twenty-four hours of age, showed roughly the same incidence of early mortality as control nematodes so transferred at ninety-six hours. These results indicate that the depletion results, which indicate a significantly lower rate of early mortality for nematodes transferred at ninety-six hours than for those transferred at twenty-four hours, are due to the removal from α-tocopherol, and not due to the difference in susceptibility to damage due to transfer at these two ages.

I found that dietary α-tocopherol, supplemented in the nematodes' growth media for generations, delayed mortality in senescent nematodes. Studies

of α-tocopherol supplementation and depletion during various stages of the life cycle have shown that α-tocopherol supplementation as late as thirty days after birth had an effect in delaying late mortality. The removal of α-tocopherol after ninety-six hours caused earlier senescence and death. Thus, supplementation of α-tocopherol at any stage would have some effect on delaying the cumulative effects that lead to senescence. The finding that supplementation at such a late stage has such an effect rules out the theory that the action of α-tocopherol is limited to its influence on development and cell division, particularly synthesis of membranes (Gershon, 1972), that take place between twenty-four and ninety-six hours. Gershon et al. (1972) found that irreversible age-related damage was initiated at a very early age in C. elegans, and that treatment with α-tocopherol Q. delayed the effects that lead to mortality. These investigators suggested that this age-related damage could be attributed to damage of cellular components with very slow turnover, such as membranes, mitochondria and the like, and suggested that α-tocopherol Q. may play a role in the prevention of this damage.

In summary, then, the presence of α-tocopherol

in the nematodes' growth media between twenty-four and ninety-six hours reduces the incidence of early mortality. Whether its presence before birth is essential for this effect is not clear. α-Tocopherol appears to delay the processes that lead to senescence and mortality even if added to the media as late as thirty days after birth. These results suggest that the effects of α-tocopherol, on both early mortality, and mortality due to senescence, occur after cell division has terminated.

These suggestions are consistent with a number of aging theories presently available. Orgel (1963) attributes the phenomena of aging to an increased frequency of errors at either the transcriptional or translational level resulting in the synthesis of aberrant protein molecules. It was also postulated by this investigator that the frequency of these errors are very low in young animals, and that it increases exponentially due to the increased rate of accumulation of faults in the enzymes involved in protein synthesis. Orgel (1970) further hypothesized that once a critical level of faulty proteins is reached, death occurs. Implicit in this theory is the assumption that miscoding is the main mechanism for causing random amino acid substitutions, that leads

to catastrophic levels of errors in the last period of life of the cells. The exact threshold of the altered enzyme activity which becomes catastrophic to cellular functions was not clearly specified.

Large numbers of investigations have led to a great deal of controversy about Orgel's hypothesis (Holliday, 1979; Baird et al., 1975; Gershon, 1979). Although complete evidence for or against this theory is not yet available, this hypothesis has been useful in stimulating a great deal of work in this field.

Another theory, the postranslational error theory has been developed by Gershon and his co-workers (1979) over the past decade. These investigators have recently postulated that the errors that lead to aging and cell death are most likely due to post-translational deterioration of proteins rather than errors at the translational or transcriptional level. The exact reason for the accumulation of abberant proteins, however, is not yet clear. It has been postulated that it could be attributed to a defect in the degradation system of these abberant proteins in old animals either due to the inability of proteases to recognize altered proteins or a deterioration in the degradation system with age (Comolli et al., 1972).

In agreement with these postulates are studies

by a number of investigators which showed an increase in the half life of a number of proteins in aged animals as compared to young animals (Prassana & Lane, 1979). Prassana et al. (1979) attempted to assess the effect of aging on the stability of the protein degradation machinery in Turbatrix aceti. In spite of their utilization of DNA synthesis inhibitors as a tool for synchronization, these investigators postulated that the protein degradation system becomes dysfunctional with age in this species. These investigators attributed the progressive accumulation of defective nematode enzymes with age to a fault in this mechanism.

Additional evidence relating to protein or membrane instability in aging should also be considered in attempting to understand the role of α-tocopherol in retarding the mortality of Turbatrix aceti. Lysosomes may participate in the aging process in a number of ways. Under normal conditions lysosomal enzymes play a role in the brakedown of intracellular proteins and other material (Huang & Tappel, 1971).

We have mentioned a deficiency in the degradation machinery that can possibly be attributed to inadequate lytic activity within cells, cytoplasm and nucleus and into extracellular spaces in animals of increased

age (Tappel, 1965, 1968; Comfort, 1966; Packer, 1967).

Unfortunately studies like ours do not allow distinction between these theories without biochemical evidence. In any event any of these explanations are consistent with a number of presently known theories that explain how α-tocopherol might delay aging.

Effects of α-Tocopherol on the Life-Shortening Effects of Gamma Irradiation

The life-shortening effect of irradiation has led to a great deal of investigation by researchers attempting to correlate aging processes with radiation damage. In the early 1960's, these findings led to the somatic mutation theory. According to this hypothesis, natural aging and radiation-induced life-shortening are both due to damage in fixed postmitotic cell (Curtis, 1963). More recently, however, a large body of evidence, based mainly on studies on nonrenewing cell systems such as Drosophila and other insects (Lamb, 1964, 1965), has cast doubt on this theory.

Lamb (1964) suggested, that the long-term life-shortening effect of ionizing radiation occurs because radiation either accelerates the natural

aging process or causes precocious aging. Accelerated aging occurred if radiation caused an increased in the rate of the aging processes throughout life; precocious aging would occur if the animal aged rapidly in the period immediately after radiation.

Maynard Smith (1966) and Lamb (1974) favoured the accelerated aging hypothesis, basing their conclusions on Drosophila studies. Blair (1950) suggested that radiation injury causing life-shortening in mammals is partly reparable, and partly irreparable. Lamb (1964), proposed a model based on reparable (acute) and irreparable (chronic) effects to explain the radiation life-shortening effect in Drosophila.

In a discussion about the life-shortening effects of irradiation on animals without cell renewal systems, investigators contributing to a 1966 conference on radiation-induced life-shortening agreed that somatic mutations play no role in irradiation-induced aging in insects (Lindop & Sacher, 1966). These investigators also agreed that this conclusion does not necessarily have any implications for mammals. The dosages used to induce a life-shortening effect in insects are usually enormous, and probably affect enzymes and proteins directly, bypassing chromosomal damage. In mammals, on the other hand, the effects

seem to be chiefly at the DNA or chromosomal level. Furthermore, there is evidence that in insects, dose-effect curves show no shoulder, which indicates that radiation damage is strictly cumulative, and that there is no repair (Metalli & Ballardin 1966)

Another example of evidence against the somatic mutation theory in insects is the similar sensitivity to irradiation observed in males and ovaryless females, and in diploid and triploid Drosophila females (Lamb, 1965; Lamb & Maynard Smith, 1964). These findings and the fact that there is no direct evidence to date as to why adult non-cell-renewing insects die, have led a number of investigators to suggest that enzyme destruction is the cause of radiation-induced aging. (Lindop & Sacher, 1966).

In the survivorship studies, it was shown that α-tocopherol delays the processes that lead to natural senescence in Turbatrix aceti. I continued the investigation of this phenomenon by testing the effects of α-tocopherol on the life-shortening effects caused by gamma irradiation. Since Turbatrix aceti is eutelic, this organism provides a simple system in which some of the effects of α-tocopherol can be tested.

The results show that the nematode Turbatrix aceti is resistant to radiation induced aging. It was observed that

that more than 75 Kr. are required before a significant reduction in life span is observed (Figure 31).

If we agree with the explanations of radiation effects described above, it could be suggested that exposing Turbatrix aceti to low doses of gamma radiation has an immediate or reparable effect (DNA level), and that high doses of gamma radiation produce irreparable effects which are more obvious because they affect proteins, enzymes and possibly membranes.

In these studies, α-tocopherol was added both before and after irradiation (Malick & Roy, 1978), we observed that α-tocopherol after irradiation extended the survivorship in nematodes irradiated with low doses. Supplementation after irradiation appears to more effective in extending the survivorship of nematodes irradiated with high doses. The results also showed that at very low radiation doses, nematodes treated with α-tocopherol after irradiation, but not before, had a higher survivorship than non-irradiated nematodes in the same group. A similar phenomenon has been reported in unicellular animals (Horseley & Laszlo, 1971), and is thought to be attributed to induction in the repair mechanism or "over repair" (Howard & Cowie, 1976). The exact

mechanism responsible for this induction is not clear.

It was shown that α-tocopherol appears to make essential nutrients available, it could be postulated that low doses of radiation stimulate the synthesis of these essential nutrients even further. It is curious however, that this phenomenon was not as clear in nematodes which were supplemented with α-tocopherol before irradiation. This could be explained by the possibility that the beneficial effects induced by radiation are counteracted by detrimental effects of irradiated α-tocopherol molecules, present in the body prior to irradiation.

If I correlate these results with those obtained in Drosophila studies (Lamb, 1964, 1965), and speculate that damage induced by high gamma ^{60}Co radiation goes past the chromosomal level to proteins, enzymes and membranes in this nematode, then it could be postulated from the results that the presence of α-tocopherol in the nematodes' growth media before, rather than after, irradiation protects these proteins from damage. It is difficult to explain, however, why the irradiated α-tocopherol molecules would not have a detrimental effect at high dosages if they appeared detrimental at low dosages.

The fact that α-tocopherol added immediately

after irradiation shows a protective effect in mice suggests that α-tocopherol plays a role immediately after irradiation if the damage is chromosomal, and before irradiation if the damage is at the protein of membrane level.

The results suggest that further studies, with exposure to doses higher than 100 kR, would be required to be more conclusive about the effects of α-tocopherol supplementation before and after irradiation. The most conclusive evidence presented in this section is that nematodes of control origin have a higher mortality rate at high gamma ^{60}Co radiation doses than nematodes of α-tocopherol origin treated with α-tocopherol after irradiation. If irradiation-induced aging is indeed induced by damage to enzymes and proteins, we would postulate that α-tocopherol slows down the aging process by protecting these molecules from radiation damage.

Interaction of α-Tocopherol with Essential Nutrients Present in the Growth Media

The experiments discussed so far have suggested that α-tocopherol plays a role in assuring optimum maturation in most of the population. In the next experiment, I attempted to determine whether

α-tocopherol could be involved in synthesizing or ensuring the availability of essential nutrients for growth and maturation in this nematode's growth media.

The question of the specific metabolic role of vitamin E remains the subject of study and of speculation. One current theory postulates a role for vitamin E in heme synthesis. Dinning and Day (1957) found that nutritional anemia occurred in vitamin E-deficient monkeys. This evidence, and evidence about other anemias found in vitamin E-deficiency in several species (Majaj et al., 1963; Nafstad, 1965; Porter et al., 1966; Murty et al., 1970) have led a number of investigators to suggest that vitamin E may play a role in the heme biosynthetic pathway.

In a study to elucidate the origin of an α-tocopherol-deficiency anemia in monkeys, it was found that this species produced large numbers of immature red blood cells (Porter et al., 1962). Later investigations of the role of vitamin E in the regulation of heme synthesis have found that this lipid-soluble vitamin is capable of preventing, or at least reversing a number of anemia-related symptoms (Majaj et al., 1963). Nair (1972) has shown that vitamin E counteracts induction of hepatic ALA synthetase and ALA dehydrogenase in experimental porphyra. In a

more specific study, these investigators showed that vitamin E specifically blocks induction of hepatic ALA synthetase (Caasi et al., 1972).

The large body of evidence presently available has led a group of investigators to believe that vitamin E plays a role in the regulation of heme biosynthesis. Nair and his co-workers (1972) believe that α-tocopherol may control the mechanism of induction and repression of the enzymatic steps involved in the synthesis of ALA and PBG. These are synthesized by intramitochondrial, and soluble cytosolic enzymes respectively (Lehninger, (1975).

A number of theories that describe α-tocopherol as a regulatory factor in biochemical pathways involve functional components associated with a number of heme proteins, such as cytochromes (mitochondrial), CO-binding cytochrome (liver microsomal P-450), and catalase (peroxide scavenger) (Caasi et al., 1972). Some investigators have also shown that both vitamin E-deficiency and aging result in a significant decrease in four heme proteins (Caasi et al., 1972).

Contrary to these findings, other investigators have associated anemia due to α-tocopherol deficiency with non-maturing red blood cells (Dinning, 1963), shortened red blood cell life span (Porter, 1962) and increased

in vitro hemolysis (Rose & Gyorgy, 1952). Dinning found that bone marrow RNA and DNA increase in vitamin E-deficient monkeys; other investigators found that the increase in RNA was attributed to decreased degradation, and that the increase in DNA in bone marrow was due to an increased rate of nucleotide synthesis de novo (Pollard 1960). Contrary to these findings, further research on the syntheses of DNA in vitamin E-deficient monkeys indicated that the increased DNA in the bone marrow was apparently the result of erythropoieses to compensate for the short lived red blood cells that result from the deficiency of this vitamin (Porter , 1962). How α-tocopherol protects these erythrocyte precursors is not clear. There is evidence however against a direct effect of vitamin E on the enzymes involved in the heme pathway (Bartlett et al., 1974).

The requirement of a heme protein for optimum growth and reproduction by some types of free-living nematodes has been well documented (Vanfleteren, 1974). It was the maintenance of Turbatrix aceti, together with the observation of optimized growth and reproduction with the supplementation of α-tocopherol, that led to the next question in this study: does α-tocopherol ensure the availability of an essential nutrient, or can it substitution for this essential factor ? These results show that total replacement of

HLE with α-tocopherol in the growth medium does not allow maintenance of optimum growth and development of Turbatrix aceti. On the other hand, α-tocopherol appears to ensure the availability of the essential growth factor with the addition of minimal amounts of HLE. These concentrations of HLE in non-α-tocopherol supplemented growth media do not maintain growth and reproduction.

The results also show that there is a crucial age before which the presence of sufficient HLE or minimal HLE and α-tocopherol is required for the maintenance of a continuous culture. Furthermore, it should be noted that the discrepancy in these crucial ages between control and α-tocopherol-cultured nematodes, (96 - 72 hours) clearly indicates that this factor is essential up to a certain stage of sexual maturation.

Although α-tocopherol appears to ensure the availability of the essential growth factor, whether by promoting its synthesis or merely by ensuring its availability, the mechanism by which this nutrient is made available cannot be determined from experiments of this type. From evidence presently available on the mode of action of α-tocopherol, a number of possible explanations could be made in light of these

findings. One explanation is that while α-tocopherol cannot induce the synthesis of the essential nutrients de-novo with out chemically-defined media (soy peptone, yeast extract), it may promote the synthesis of essential nutrients (proteins) in the presence of small amounts of HLE. The possible effect of vitamin E in protein synthesis (other than heme synthesis) has been well-documented (Catignani, 1980).

A second explanation is that the role of α-tocopherol under these experimental conditions is merely to ensure the availability of the essential nutrients. A number of possible explanations of how this might be achieved can be postulated: 1) α-tocopherol may be affecting the permeability of the membranes, thus increasing, or perhaps decreasing, the flow of certain nutrients across the membranes, and therefore increasing the availability of some of the more favored nutrients (Lucy, 1972). 2) Alternatively, α-tocopherol may act as a carrier, or vector, of essential molecules required for the maintenance of Turbatrix aceti cultures. One of the theories about the molecular make-up of the growth factor postulates that there are two essential parts necessary for optimum maintenance of a number of free-living nematodes in axenic medium: i)

a heme protein, and ii) a lipid molecule (Hieb & Stokstad, 1970; Vanfleteren, 1974). It is thought that the latter, or, a third component of the growth factor may act as a vector in the transport of a protein molecule to the cells (Vanfleteren 1975). 3) α-Tocopherol may be making small amounts of proteins available by reducing the competition of other proteins for specific sites, by altering their composition (possible antioxidant effect; Corwin & Schwarz, 1963). These postulates may or may not relate to the effects of α-tocopherol on heme synthesis.

The data obtained from experiments in which we measured the reproductive capacity of nematodes cultured in stale media (Table 42) may have some implications for the findings we discussed above. The results showed that nematodes cultured in stale α-tocopherol media were able to maintain a continuous population, whereas in the absence of α-tocopherol, nematodes failed to reproduce. It can be postulated from these results that α-tocopherol ensures the availability of nutrients from denatured proteins, by inducing a protein synthesis mechanism, or by preventing the proteins from losing their nutrient value. In any event, it appears that α-tocopherol has an effect on the growth of these nematodes. It

is not clear, however, whether it affects the cells directly, or whether this effect is mediated via a nutrient in the medium.

A number of additional points may be of interest to the discussion of these results. Recent studies have shown that heme may play a major role in the regulation of globin protein synthesis in nematodes (Adamson, 1971; Datta, 1977). The regulation of protein synthesis by hemin is thought to be of great importance to nematodes during early embryogenesis and molting (Smith & Lee, 1963). The hemin moiety is also thought to be used for the synthesis of cytochromes needed in large amounts by the developing embryo and larvae (Cain, 1976). The role of this molecule in the male, however, is not clear.

Since previous experiments have indicated that α-tocopherol has an effect on embryogenesis and molting with respect to maturation, it might be interesting to speculate that there is a connection between the effect α-tocopherol has on the availability of the heme molecule, and the effects α-tocopherol has on maturation.

The purpose of this study was to test the effects of α-tocopherol on a simple *in vivo* system. It was hoped to avoid the problems associated with more

complex *in vivo* systems, and *in vitro* systems. A great deal of the present controversy about vitamin E is the result of early studies on its effects on major physiological mechanisms in complex *in vivo* systems. The complexity of the animals used has caused much confusion, and has prevented determination of the exact mode of action of vitamin E. I chose a simple animal, and used the approaches of early investigators, along with the wealth of knowledge now known about this vitamin to attempt to clarify some of this confusion. the results only allow us to understand how some of the early studies came to their conclusions, but they do not allow us to conclude whether α-tocopherol has a direct effect on these physiological functions or whether the effects observed are mediated by the effects of α-tocopherol on another molecule or structure.

I conclude that this free-living nematode is a good *in vivo* system for morphological aging studies, but unless the problem of synchronization is overcome it does not appear to be ideal for aging studies that involve functional problems.

SUMMARY

I investigated the effects of α-tocopherol on some of the major physiological functions of the free-living nematode Turbatrix aceti. It was shown that α-tocopherol supplementation in this nematodes growth medium ensured optimum growth and maturation; which in turn led to an increase in reproductive capacity. It was also shown that when the nematodes' growth medium was supplemented with this lipid, it resulted in an increased life span in both irradiated and non-irradiated nematodes.

The results present ample opportunity for generalization about the effects of this vitamin on fertility, life span and aging. The stage of development at which α-tocopherol influences the major physiological functions in this nematode remain to be determined.

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Appendix I

Test of DL,α-tocopherol-solubility in basal media.

Table 45

Test of DL α-Tocopherol-Solubility in Basal Growth Media

Solvent	Concentration of α-Tocopherol	Concentration[1] of Solvent	General Appearance of Media	Generation Time (days)	Population Increase on day 21
Tween 80	1.0×10^{-2}	1.0×10^{-2}	cloudy	16	+
Tween 80	1.0×10^{-2}	9.0×10^{-3}	very cloudy†	N.T.	-
Tween 80	1.0×10^{-3}	9.0×10^{-3}	cloudy†	N.T.	-
Tween 80	1.0×10^{-4}	9.0×10^{-4}	cloudy	12	++
Tween 80	1.0×10^{-4}	9.9×10^{-3}	very cloudy	18	+
Tween 80	1.0×10^{-5}	9.9×10^{-4}	slightly cloudy	14	++
Tween 80	-	9.0×10^{-4}	clear	-	+
G. acetic acid	1.0×10^{-2}	9.0×10^{-2}	cloudy†	N.T.	-
G. acetic acid	1.0×10^{-3}	9.9×10^{-2}	slight cloudy	10	+++
G. acetic acid	1.0×10^{-3}	9.0×10^{-3}	cloudy†	8	++++
G. acetic acid	*1.0×10^{-4}	9.9×10^{-3}	slightly cloudy	7	+++++
G. acetic acid	1.0×10^{-5}	9.9×10^{-4}	slightly cloudy	8	+++
G. acetic acid	-	9.0×10^{-4}	clear	8	+++

[1] Concentration in basal media

† Large lipid droplets

* Concentration used in this study

Appendix II

Statistics

Table 46

Test Statistic for Paired Comparisons Between Survivorship Curves of Nematodes Irradiated with Various Dosages of ^{60}Co

Treatment Groups			Dosage (Kr) 0	10	50	75	100
C / C	vs	α-Toc./α-Toc.	$t_{(12)}=2.18^*$	$t_{(12)}=1.4$	$t_{(12)}=7.4^*$	$t_{(33)}=3.3^*$	$t_{(12)}=3.6^*$
C / C	vs	C /α-Toc.	N.S	$t_{(12)}=2.8^*$	$t_{(12)}=6.6^*$	N.S	$t_{(12)}=1.9$
α-Toc./α-Toc.	vs	α-Toc./ C	$t_{(12)}=2.59^*$	$t_{(12)}=2.3$	N.S	N.S	N.S

* $\alpha<.05$

Table 47

Test Statistics[1] for Comparison of Slopes and LD_{50} Between Treatment Groups

Day of Life Span	Treatment Groups			Test Statistic: Slopes $t_{(10)}$	Test Statistic: LD_{50} $t_{(10)}$
20	C / C	vs	α-Toc./α-Toc.	1.75	*2.87
	C / C	vs	C /α-Toc.	1.09	1.61
	α-Toc./α-Toc.	vs	α-Toc./ C	*2.80	*1.90
	C / C	vs	α-Toc./ C	0.20	0.40
40	C / C	vs	α-Toc./α-Toc.	1.22	*3.00
	C / C	vs	C /α-Toc.	0.00	1.26
	α-Toc./α-Toc.	vs	α-Toc./ C	0.75	1.70
	C / C	vs	α-Toc./ C	0.44	0.75
60	C / C	vs	α-Toc./α-Toc.	*2.92	*1.85
	C / C	vs	C /α-Toc.	*2.60	*4.50
	α-Toc./α-Toc.	vs	α-Toc./ C	0.27	0.24
	C / C	vs	α-Toc./ C	*3.48	1.00

[1]Stanley (1963)

*$\alpha < .05$

Table 48

Significance Test Between Slopes Used in Analysis of Radiation Data (Stanely, 1963)

$$T = \frac{byx^1 - byx^2}{SE_{diff}}$$

$$SE_{diff} = \sqrt{N_1\ SDy\ (1-r^2_{yx_1})\quad N_2\ SDy_2\ (1-r^2_{yx_2})\ \frac{1}{N_1\ SD^2_{x_1}} + \frac{1}{N\ SD^2_{x_1}}}$$

$$SD_x = \sqrt{E(x - \bar{x})^2}$$

$$SD_y = \sqrt{E(y - \bar{y})^2}$$

Significance Test Between LD_{50} Values Used in Analysis of Radiation Data

$$T = \frac{diff}{SE_{diff}}$$

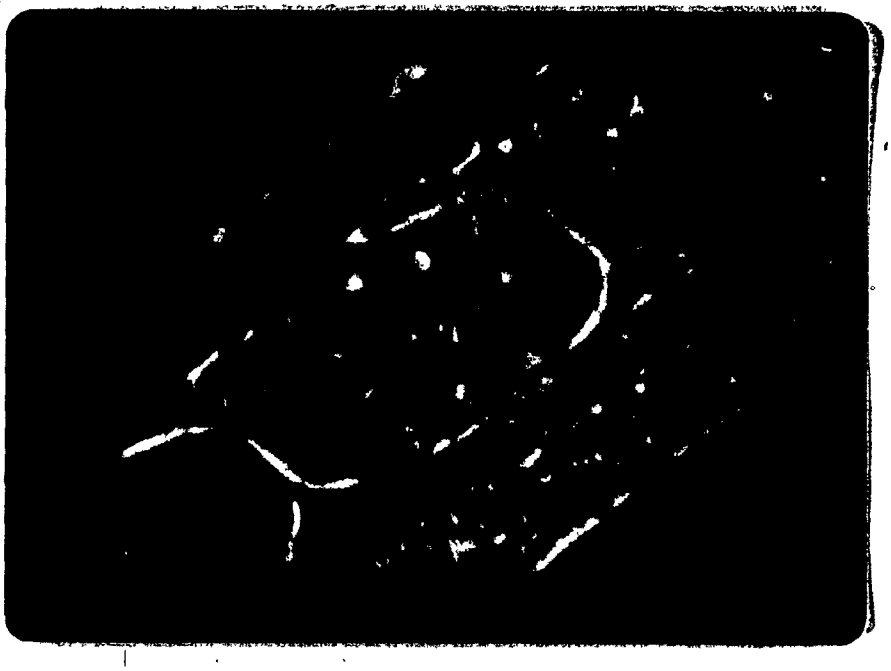
$$SE_{diff} = \sqrt{SE_1^2 + SE_1^2}$$

Appendix III

Photomicrography

PLATES 1,2. Photomicrographs showing embryos at various stages of development, in an α-tocopherol-cultured mother. The numbers 1-7 indicate embryos at increasingly later stages of development. (i), intestine; (u), uterus.

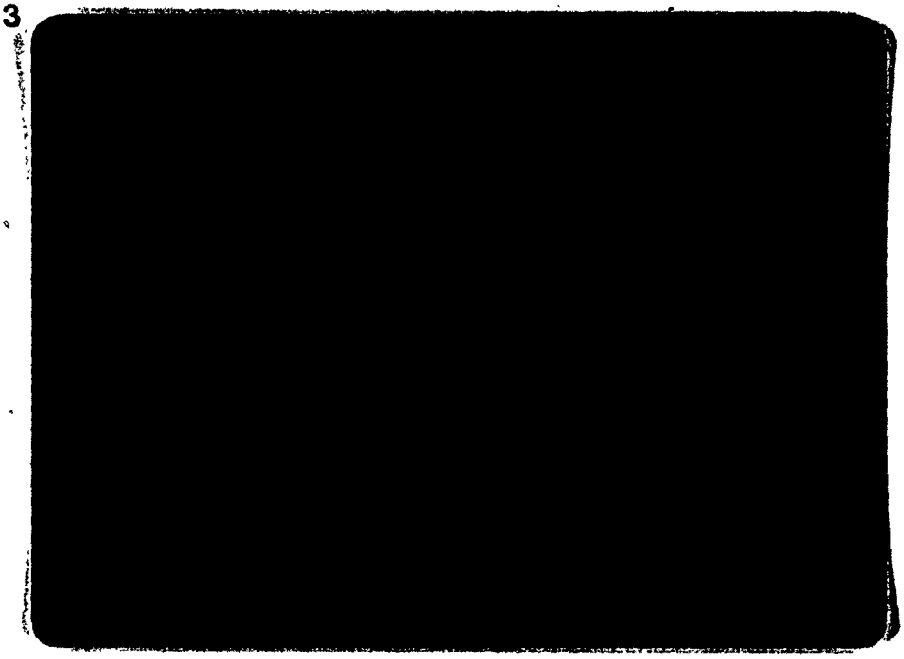
1



2

PLATES 3,4. Photomicrographs showing larvae (control) which have been removed prematurely from their mothers. (3), normal; (4), dead.

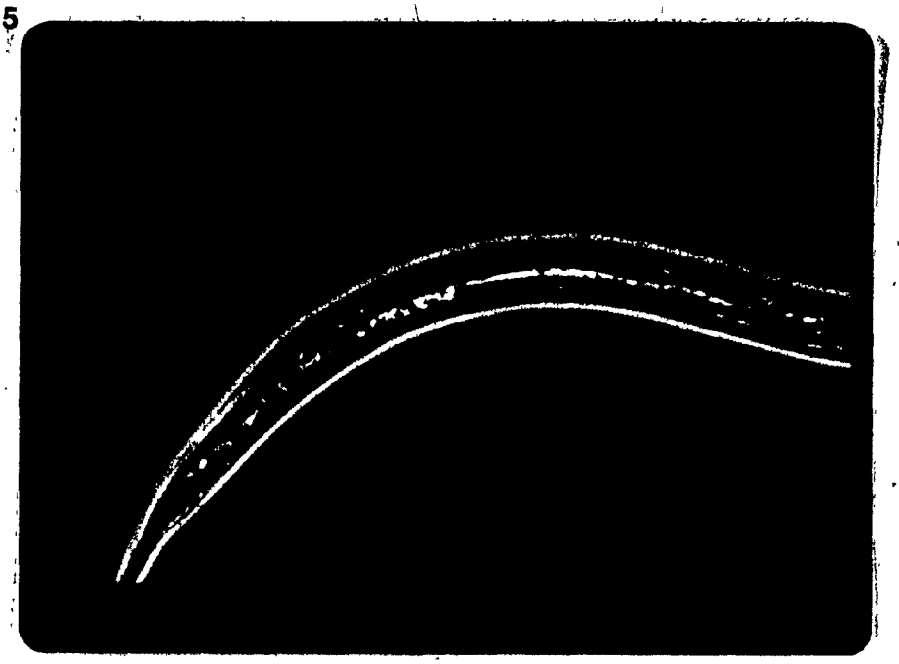
3



4

PLATES 5,6. Photomicrographs of three day old larvae. Note that at this stage and magnification, the only distinguishing characteristic is the shape. (5), control (female); (6), α-tocopherol (male). Note the droplet like particles in the area where the spicule will develop in the α-tocopherol worm (arrow).

5

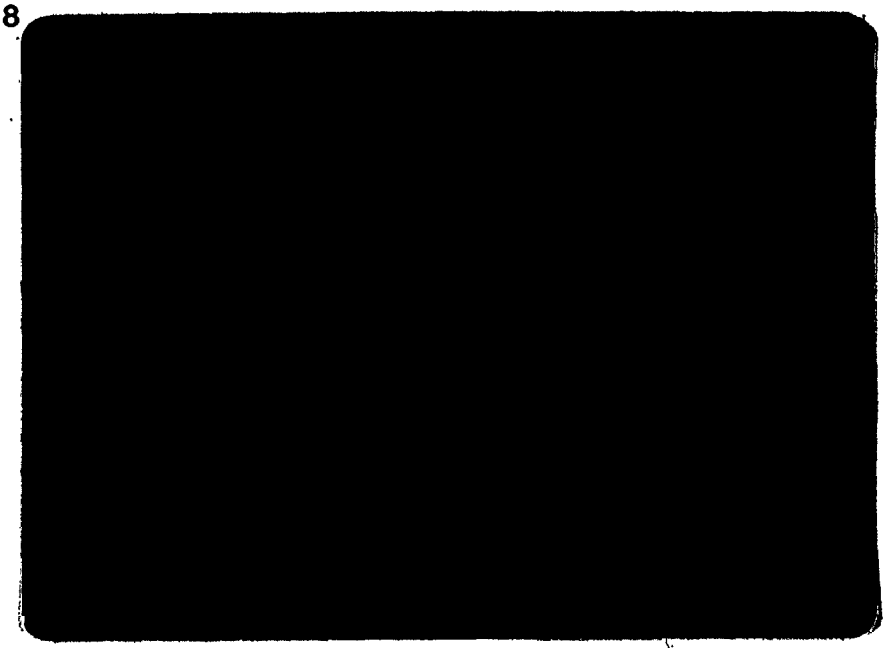


6

PLATES 7,8. Photomicrographs of 5 day old larvae. Note the difference in gonad development, between the control larvae (7) and the α-tocopherol larvae (8). (ov), ovary; (pc), gonad precursor cell.

7

8



PLATES 9,10. Photomicrographs of 6 day old larvae. Note the difference in gonad development between the control larvae (9) and the α-tocopherol larvae (10). (t), testis; (pc), gonad precursor cell.

9

10

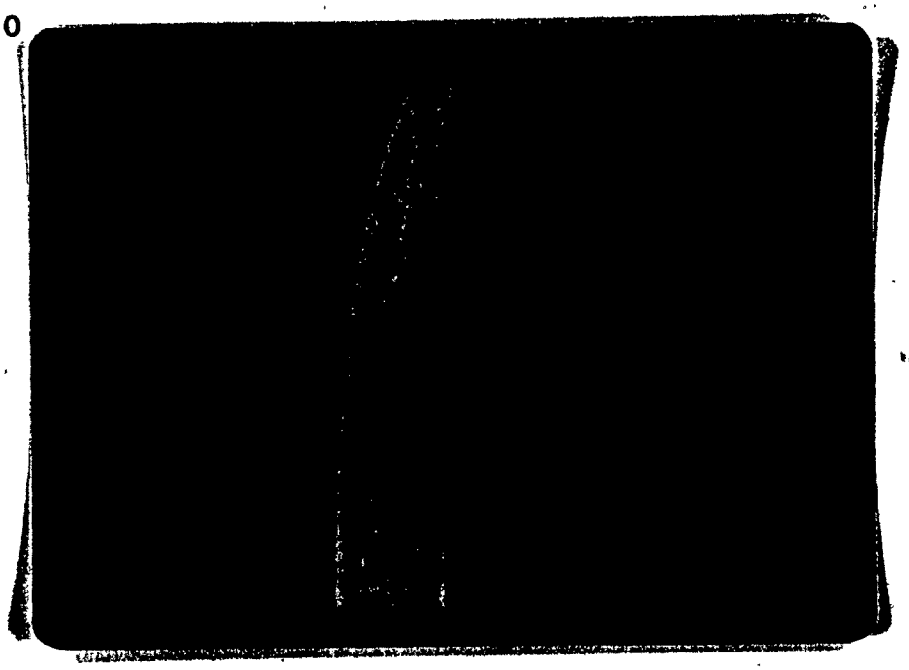


PLATE 11. Photomicrograph of an α-tocopherol-cultured male after the fourth molt. Note that the external sex organs are easily distinguised at this stage. (s), spicule; (cu), cuticle.

11

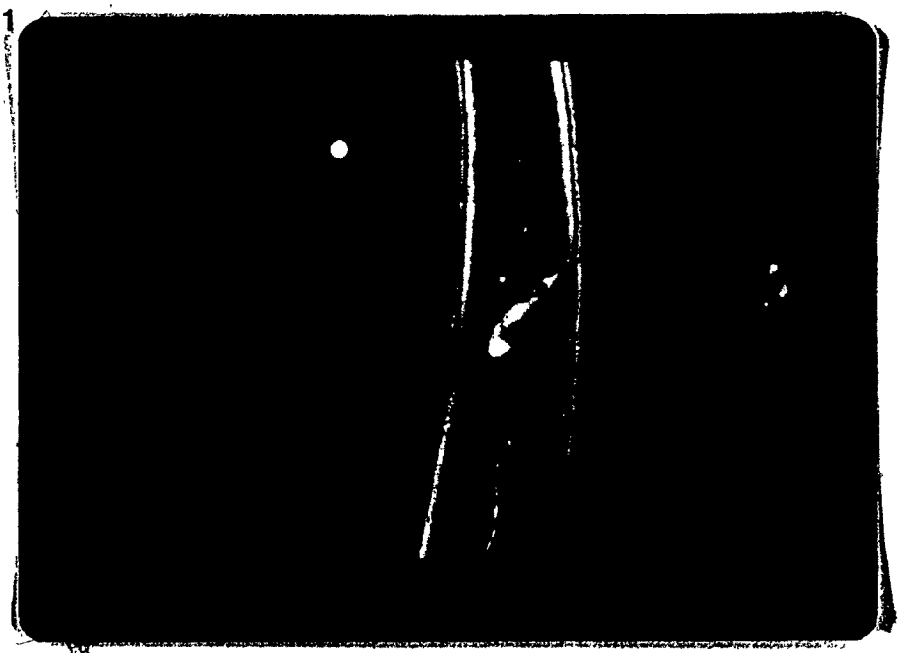
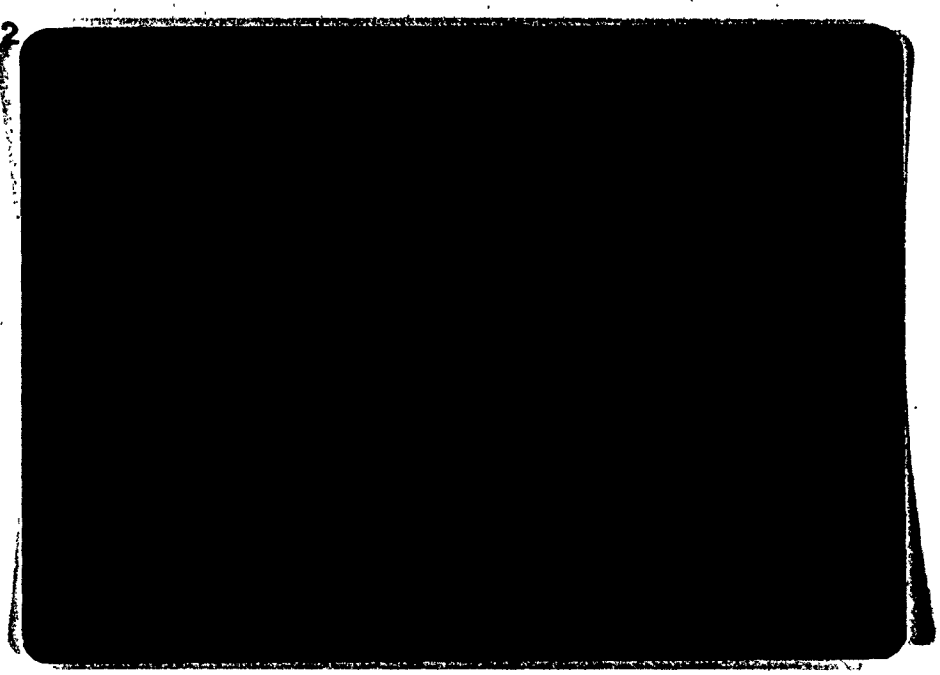


PLATE 12. Photomicrograph showing various zones of the ovary of a young female. Note the well defined gonadal cell. (ge),germinal zone; (gr),growth zone.

PLATE 13. Photomicrograph showing various zones of the testis of a young male. (ge),germinal zone; (gr), growth zone; (s),spermatid. Stain: methylene blue; no fixative.

12



13

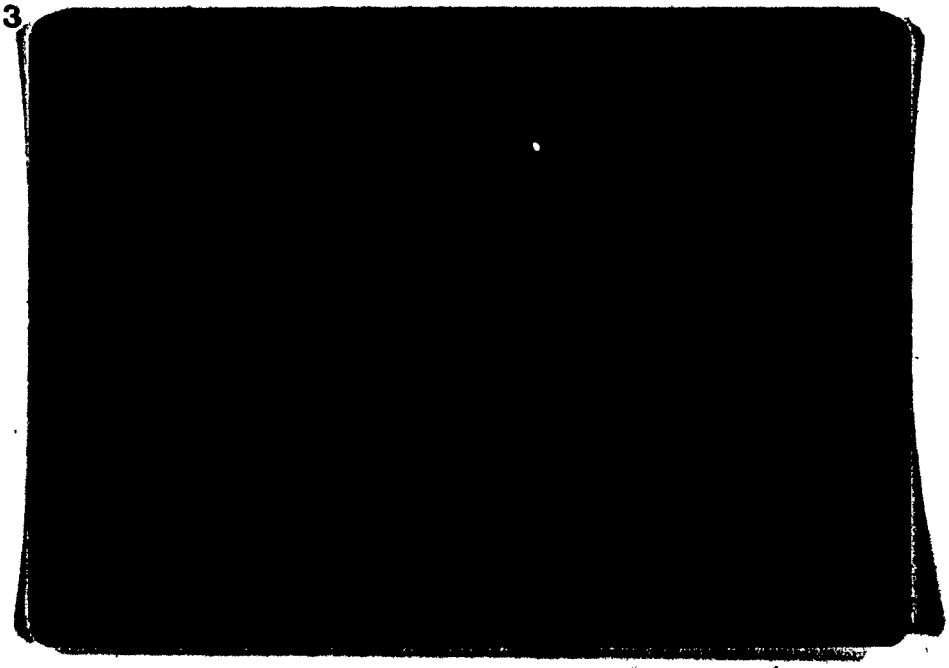
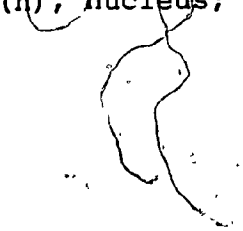


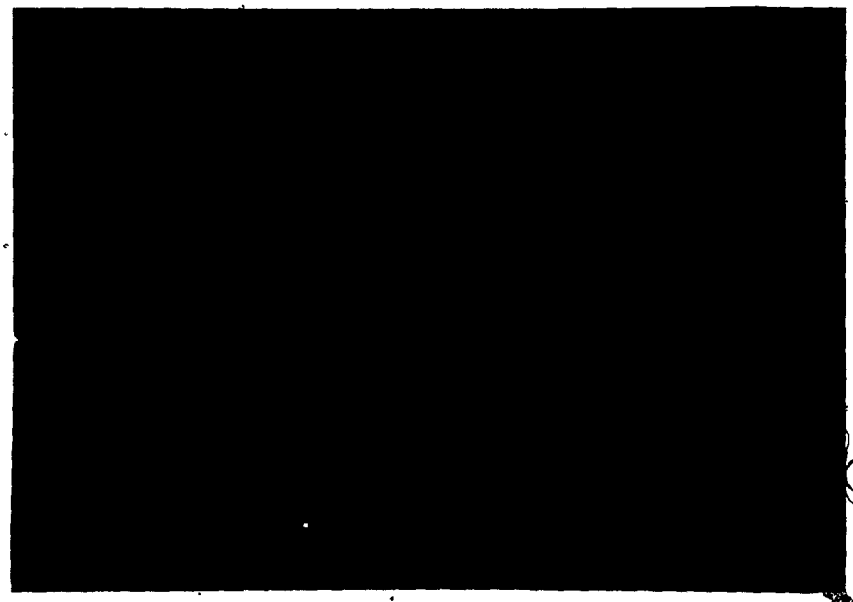
PLATE 14. Photomicrograph showing oocytes produced by a young control female before the second meiotic division. (c), cytoplasm; (n), nucleus; (nu), nucleolus.



14

PLATES 15,16. Photomicrographs showing oocytes produced by an α-tocopherol-cultured female. (15), early stage and (16), late stage of vittelogenesis.

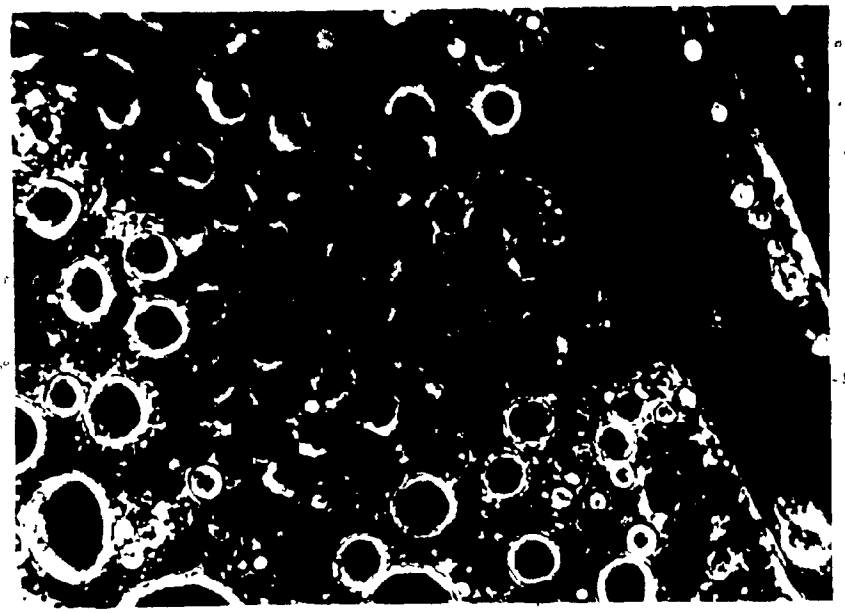
15



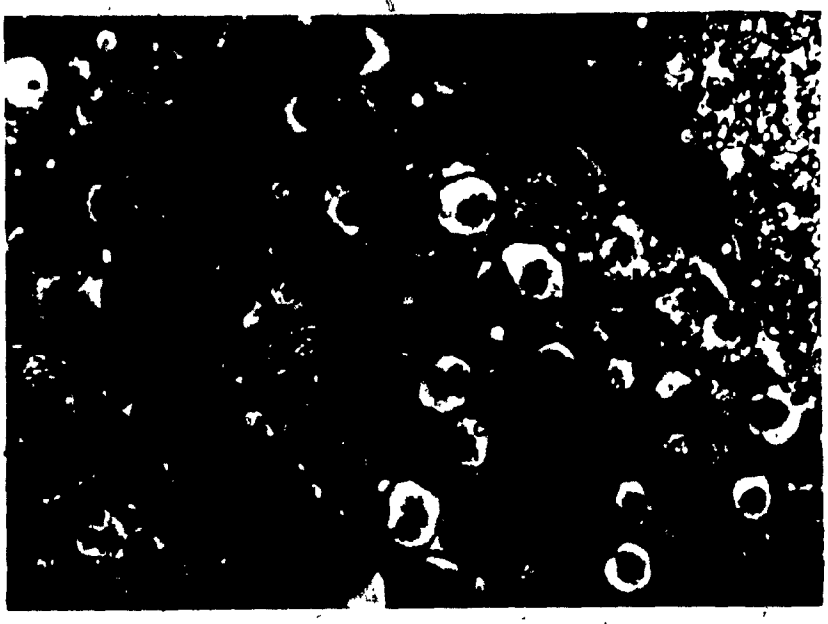
16

PLATES 17,18. Photomicrographs showing the difference between oocytes extracted from a ten day old control female (17), and a ten day old α-tocopherol-treated female (18). Note that the cytoplasm (c), nucleus (n) and nucleolus (nu) are not as well-defined in the control cells as in the α-tocopherol oocytes.

17

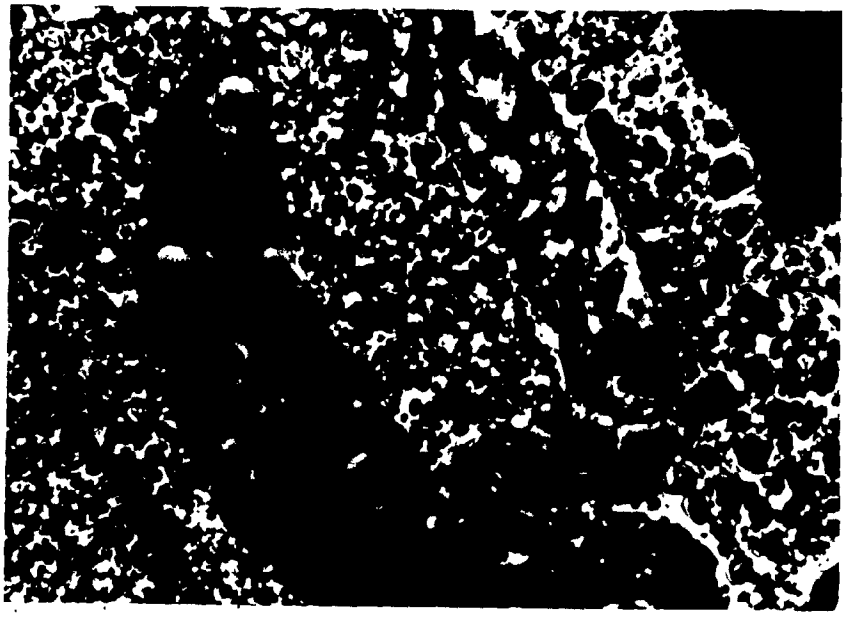


18



PLATES 19,20. Photomicrographs showing the difference in the number of oocytes present in the zones of vitellogenesis of the ovaries of a control female (19) and an α-tocopherol-treated female (20) of approximately equal ages.

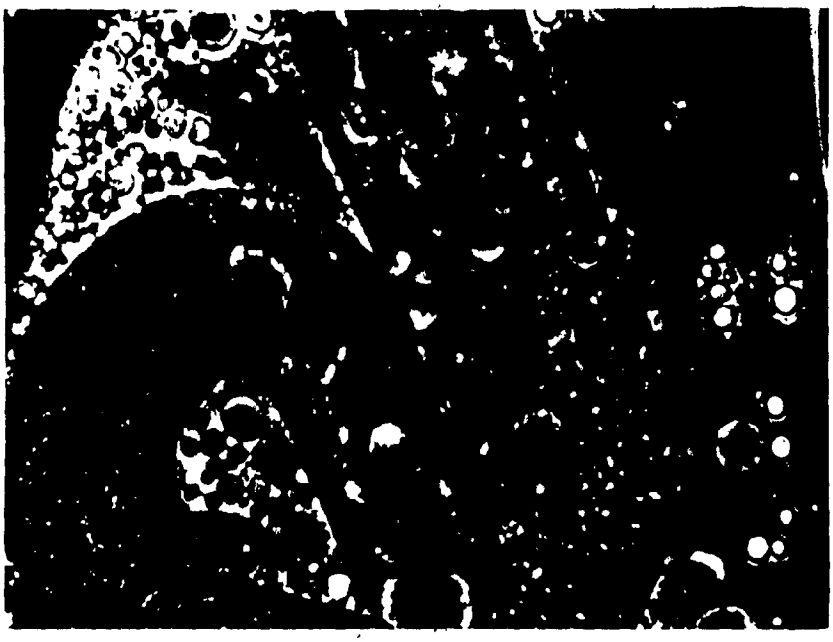
19



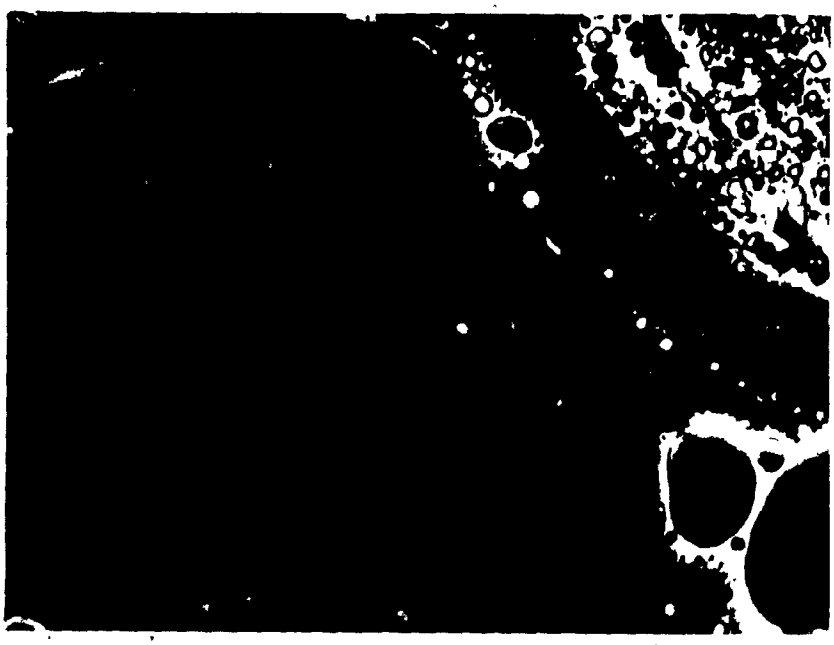
20

PLATES 21,22. Photomicrographs showing the difference between the vitellogenic zones of the ovaries of a control female (21) and an α-tocopherol female (22) at the end of their reproductive periods. Note the difference in the cytoplasmic material between the control oocytes (c) and the α-tocopherol (c).

21

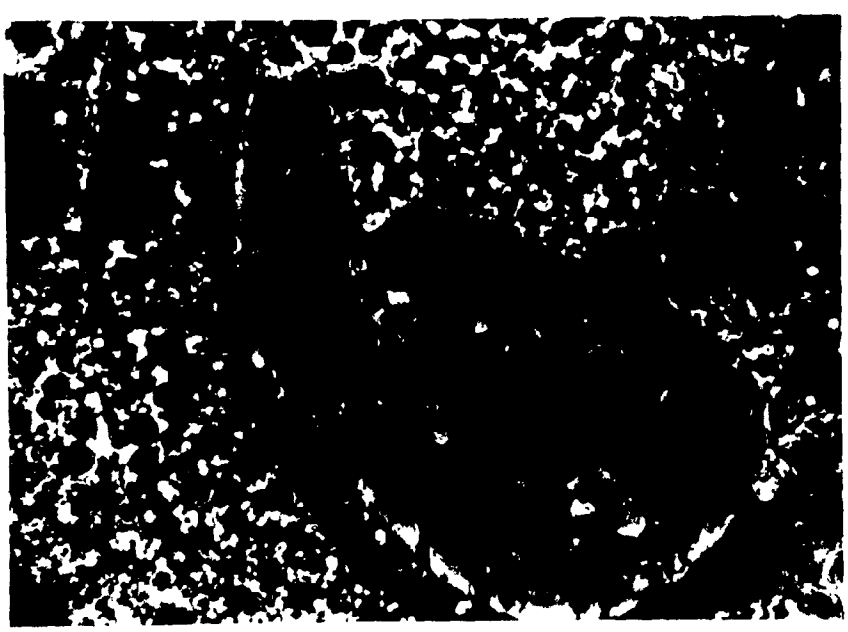


22



PLATES 23,24. Photomicrographs of the germinal zones of an infertile ovary of a control female (23), and of a fertile ovary of an α-tocopherol-treated female (24). Stain: neutral red, no fixative.

23



24

PLATES 25,26. Photomicrographs showing the difference between the ovarian walls of a senescing control female (25) and a senescing α-tocopherol female (26). Note the large number of lipid-like particles in the α-tocopherol ovary (arrow).

25

26

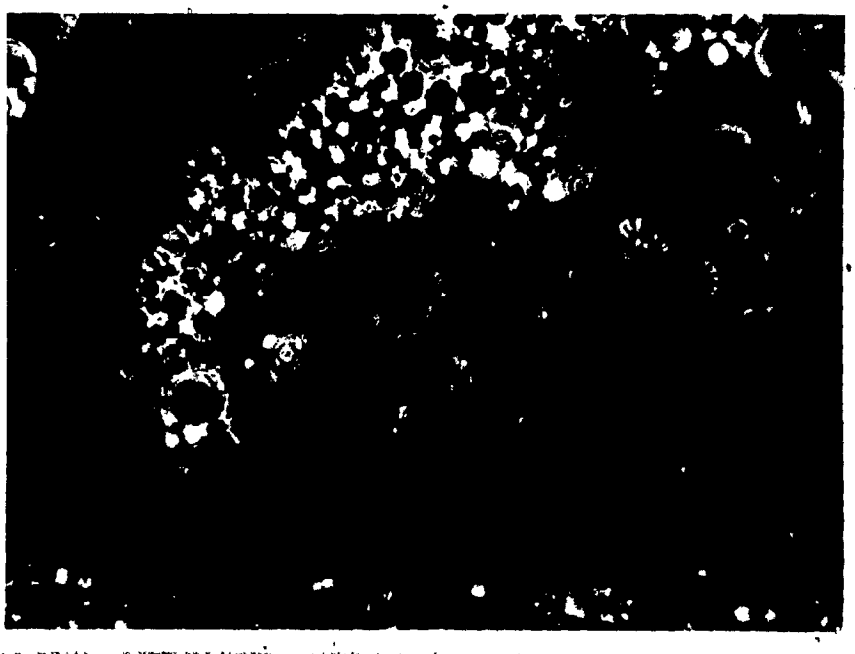


PLATE 27. Photomicrograph of the growth zone of the ovary of a senescing control-cultured female. (c), cytoplam; (n), nucleus.

27

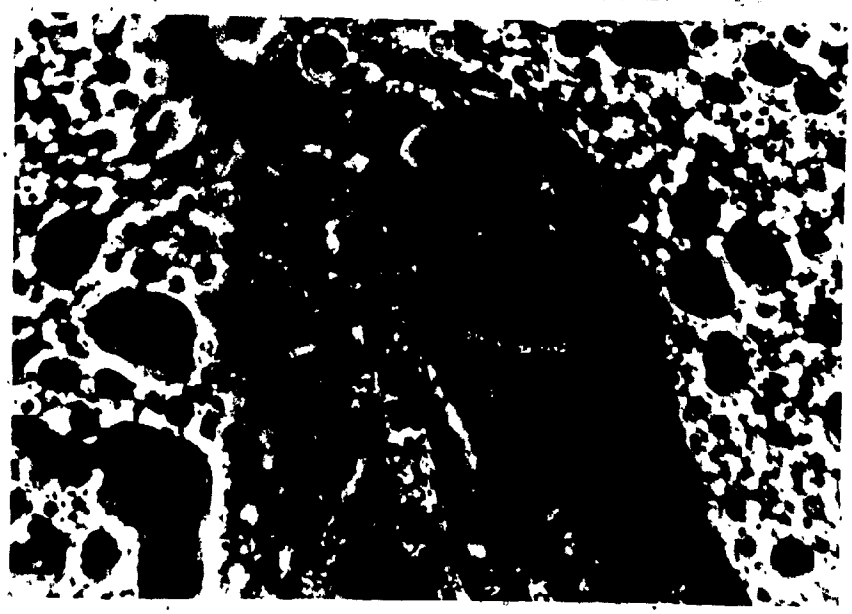
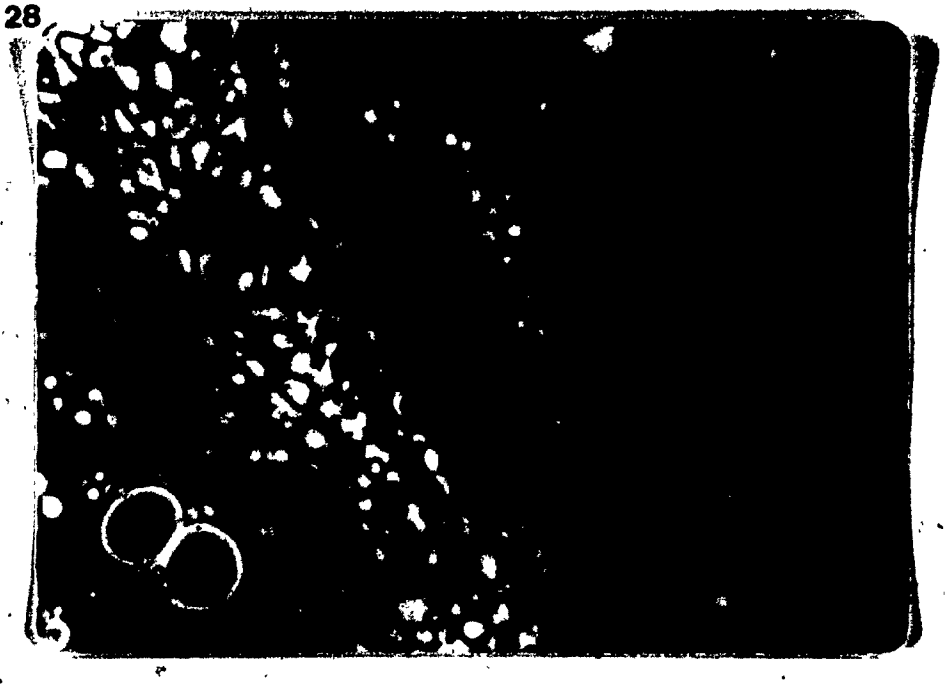


PLATE 28

Photomicrograph of oocytes produced by an α-tocopherol-treated female late in the reproductive period. Note the lipid-like particles conglomerated around the nucleus (arrow). Stain: methylene blue; no fixative.

28



PLATES 29,30 — Photomicrographs of the testes of a young control male (29) and an aged male (30). Stain: neutral red; no fixative.

29

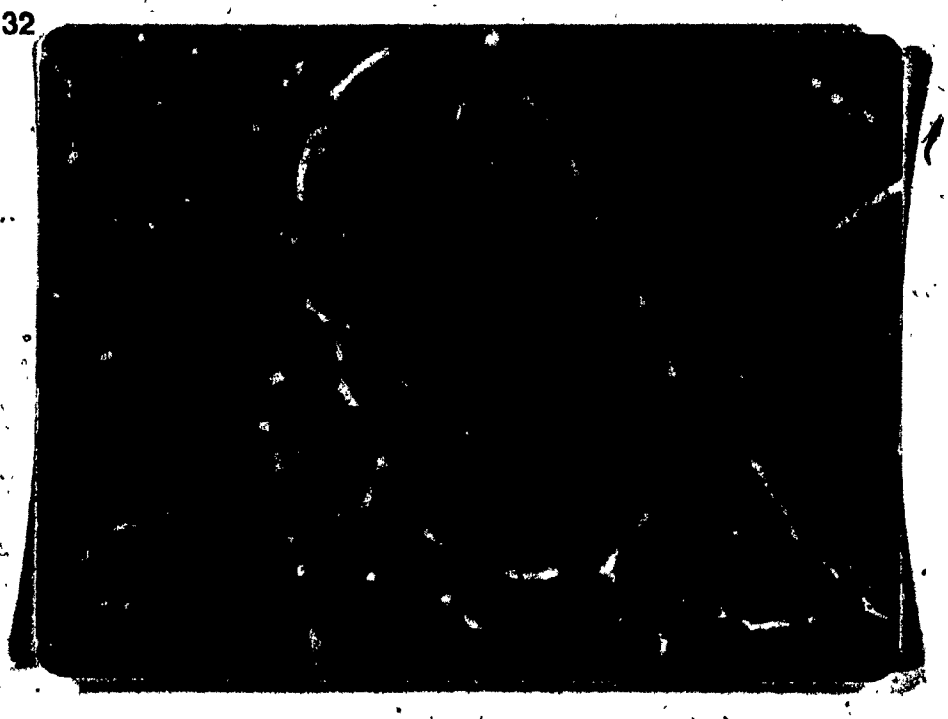
30

PLATE 31. Photomicrograph of an egg produced by a control female showing the sperm penetrating the egg membrane. Note the granulated cytoplasm. (n), nucleus; (s), sperm.

PLATE 32. Photomicrograph of a fertilized egg produced by an α-tocopherol treated female after having gone through a number of cell divisions. (c), cytoplasm; (n), nucleus; (cr), chromatin; (sq), somatic cell (s), sperm. Stain: methylene blue; no fixative.

31

32



PLATES 33, 34. Photomicrographs showing the difference between fertilized eggs produced by an aged control female (33) and an aged α-tocopherol-treated female (34).

33

34