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Post-Irradiation Modification of Survival and Hematopoiesis by Tocopherol

Tewfik J. E. Bichay

A Thesis

in

The Department

of

Biology

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ABSTRACT

POST-IRRADIATION MODIFICATION OF SURVIVAL AND HEMATOPOIESIS BY TOCOPHEROL

Tewfik J. E. Bichay

An increase from 6.6 Gy to 7.0 Gy was observed in the $LD_{50/30}$ of CD-1 female mice receiving 2.5 mg of dl-α-tocopherol immediately post-irradiation. The mechanism of protection is uncertain but appears to involve hematopoietic colony forming units (CFU). The antioxidant effect of tocopherol does not readily explain the observed effects. Endogenous spleen colonies were found in greater numbers in the tocopherol-treated mice after radiation damage. The vitamin, however, must be present within five hours after irradiation to have this effect. The increase in endogenous CFU caused by tocopherol may be due to a stimulation of recovery or repair of the cell. The split-dose assay suggests that maximal repair of hematopoietic stem cells takes place at 7-9 hours after irradiation. Tocopherol appears to

increase the 7-9 hour recovery peak of the split-dose assay. There is also evidence that tocopherol caused an earlier onset of CFU division after radiation stress. This, however, requires further experimentation. The observed tocopherol-induced increases in the number of mouse spleen colonies in these experiments are probably not due to an altered CFU seeding efficiency resulting from an improved spleen microenvironment. Tocopherol did not alter the shoulder in the exogenous spleen colony assay (SCA) of bone marrow derived or spleen derived hematopoietic stem cells. There is a probable slope change in the higher dose region (4.3 Gy) of the bone marrow exogenous SCA curves for the vehicle-injected, and the non-injected groups. The tocopherol-injected group, however, showed no evidence of slope change up to the highest dose used (5.0 Gy). The results suggest that tocopherol stimulates the recovery of hematopoietic stem cell damage in the higher dose range of bone marrow syndrome. This in turn improves the chance of animal survival.

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INTRODUCTION

Evans and Bishop (1922) observed a reduction in the rate of fetal resorption of animals fed various lard diets if supplemented with a fat soluble substance. Sure (1924) classified this substance as a vitamin. At this time vitamins A,B,C and D had been discovered. The fat soluble substance was designated Vitamin E. The name tocopherol was suggested by Evans. It is a composite of the Greek words tocos, meaning childbirth, phero to bring forth and the ol for alcohol (Horwitt 1980).

Since the time of its discovery, it has been exceedingly difficult to ascribe a specific unique biochemical or physiological function to vitamin E. Attempts at reducing tocopherol stores of the body have largely been unsuccessful. This is largely due to the ability of the body for extensive storage of tocopherol (Bieri 1975). However, diseases of malabsorption and premature infant subjects have provided data on the effect of low tocopherol concentration in the body. Normal levels of tocopherol in human plasma average 10.5 ug/ml. However, in infants, the concentration tends to be lower. At ages of 4 months to 6 years, the level is approximately 6.4 ug/ml (Bieri and Farrell 1976). Adults whose plasma

vitamin E concentration drops to, or below, 5 ug/ml are generally considered deficient in tocopherol (Farrell 1980). However, because of the association of tocopherol and polyunsaturated fatty acids (PUFA), a meaningful tocopherol concentration must always include PUFA levels (Horwitt 1962).

A 6 year study on low tocopherol intake in humans consisting of 3 mg/day as opposed to the average daily intake of 15 mg/day, was carried out by Horwitt (1960, 1962). The peroxide hemolysis test indicated a marked enhancement in erythrocyte peroxidation sensitivity in the tocopherol deficient group. There was also a shortened mean circulating erythrocyte survival time as measured by ^{51}Cr release. The plasma tocopherol levels of the deficient groups reached a minimum plateau at 2 ug/ml.

Besides studies using "artificial deficiency" purposely brought about by diet, clinical cases of tocopherol deficiency may exist due to problems of malabsorption especially related to steatorrhea. Cystic fibrosis patients have a reduced capacity of intestinal lipid absorption, and a concomitant decrease in tocopherol absorption (Farrell 1980). Nitowsky et al. (1962) reported the existence of creatinuria suggesting myopathy in cystic fibrosis patients. Tocopherol administration reduced the high urinary creatinine excretion.

Vitamin E has also been shown to play a role in

blood and circulation related errors. Intermittent claudication (limping due to a recurring reduction of arterial blood flow in a leg) has in some cases been reversed by vitamin E supplementation. Haeger (1978, 1982) showed an increase in arterial blood flow of patients with intermittent claudication when they were supplemented with tocopherol. This suggests that circulation is improved upon the addition of tocopherol.

Premature infants have usually, at the time of their birth, not had a chance to store a reserve of tocopherol in their tissues. Furthermore, their gastrointestinal tracts are not yet sufficiently developed to allow adequate absorption of vitamin E from their diet (Bieri and Farrell 1976). Such children offer further clues as to the function of tocopherol by the clinical symptoms manifested during this apparent deficiency. Guggenheim *et al.* (1982) showed evidence of improvement in neurologic disease when tocopherol-deficient children were supplemented with the vitamin. Two other common premature infant syndromes, retrolental fibroplasia and hemolytic anemia respond to tocopherol treatment (Bieri and Farrell 1976).

Patients receiving total parenteral nutrition (TPN) may become vitamin E deficient due to the lack of variety of vitamin E containing foods normally consumed. Thurlow and Grant (1982) showed that 50% of TPN patients

were deficient in tocopherol and had an elevated sensitivity to erythrocyte peroxide hemolysis test. There was also marked platelet hyperaggregation. Tocopherol administration decreased peroxide hemolysis and platelet hyperaggregation.

Tocopherol inhibits the metabolism of arachidonic acid to prostaglandin by inhibiting the enzyme lipoxygenase (Panganamala and Cornwell 1982). This in turn may result in an effect on blood flow and immunity (Machlin 1978).

Animals supplemented with tocopherol have been shown, in some cases, to have an improved immune response. Tengerdy, et al. (1978) reported enhanced resistance of chicken and mice to bacterial challenge by increased antibody production and phagocytosis in mice fed on tocopherol supplemented diets. Mice immunized against sheep red blood cells or tetanus toxoid had increased numbers of plaque-forming cells and hemagglutinin titers when fed high tocopherol diets (Nockels 1979). Antibody production was reportedly increased in vitro from bone marrow lymphocytes even in the absence of macrophages with tocopherol stimulation (Campbell et al. 1974). T-cell activity is enhanced in mice fed tocopherol supplemented diets (Corwin and Gordon 1982, Tanaka et al. 1979).

There is evidence of increased cell proliferation with vitamin E supplementation. Cornwell et al. (1979),

Miller *et al* (1980) and Gavino *et al*. (1981) have shown a tocopherol-induced increase in smooth muscle proliferation, while Corwin *et al*. (1981) have shown increased T-cell mitogenisis with tocopherol supplementation.

While the effects of tocopherol appear to be widespread and its mode of action unclear, there is one effect which has become well established and has received almost universal acceptance. Tocopherol both *in vivo* and *in vitro* is a very effective antioxidant. Reduction of lipoxygenase-catalyzed end-products of arachidonic acid results in decreased lipid peroxidation of alveolar macrophages (Ingraham 1981). Dougherty *et al*. (1981) reported an eight-fold increase in lipid peroxidation after iron administration in vitamin E deficient rats. Gavino *et al*. (1981) reported a decrease in lipid peroxidation of cultured fibroblasts supplemented with tocopherol. The lipid peroxide by-product lipfuscin is found to accumulate to a lesser extent in the heart cells of mice after a single *i.p.* injection of tocopherol at two months of age and assayed at 3 or 5 months of age (Kruck and Enesco 1981). Carbon tetrachloride (CCl_4) increases lipid peroxidation and may result in hepatic toxcity, coma and death. Administration of tocopherol prior to the insult protected male rats dramatically. Interestingly, female rats were not protected. Two other antioxidants,

butylated hydroxytoluene (BHT) and ethoxyquin, protected both male and female rats provided they were administered 48 hours prior to the insult. Vitamin E at these time intervals did not protect female rats from CCl_4 death (Green 1970).

Tocopherol is thought to play a role in the stabilization of lipid membranes by associating through physio-chemical interaction between the phytyl side chains of tocopherol and the fatty acyl chains of polyunsaturated phospholipids (Diplock and Lucy 1973). This close proximity also provides close contact for efficient protection of lipid peroxidation.

Tocopherol is also protective during radiation stress (Sakamoto and Sakka 1973, Kónings and Drivjner 1979, Srinivasan and Weiss 1981, Sahu et al. 1981). Radical production and ionizations are produced at an accelerated pace by ionizing radiation (Coggle 1971, Copeland 1978, Gofman 1981). The protective effect is taken as further evidence of the antioxidant property of tocopherol. Irradiated synthetic diets had a lower yield of lipid peroxide when supplemented with tocopherol as compared to controls. This was also true of the antioxidants, butylated hydroxyanisole (BHA) and BHT (Wills 1980). Dawes and Wills (1972) reported a decrease in lipid peroxidation after treatment with tocopherol of irradiated mouse tissue homogenates.

In the last ten years, data has begun appearing on the antimutagenic effects of tocopherol. There is increasing evidence that tocopherol plays an important role in decreasing the mutation rate in a number of organisms. Kalinina et al. (1979) reported a reduction of N-nitroso-N-methylmonourea (NMU) induced mutations in Salmonella if tocopherol was administered to the cultures prior to the NMU. The reduction varied from 30% to 75% depending on the concentration of NMU. Alekperov and Akhundova (1974) reported a reduced number of chromosomal alternation in Allium fistulum and Vicia faua arising spontaneously and after X-irradiation if tocopherol was administered. Chromosomal alteration in rat bone marrow cells induced by dimethyl sulfoxide (DMSO) were reduced by tocopherol supplementation. The reduction lowered the mutation rate to that of untreated controls (Patterson et al. 1981). A reduction was also observed in bone marrow chromosomal aberrations due to potassium bichromate (Bigaliev and Elemesova 1978). Tocopherol also reduced the mutation rate in E coli after nitrosoguanidine treatment (Kalinina et al. 1981). The interpretation of this activity of tocopherol is mainly based on the supposition of its action as an antioxidant.

Recently, reports have appeared suggesting a possible cellular repair involvement of tocopherol. (Fonck and Konings 1978, Malick et al. 1978, Kalinina et al.

1981, Roy et al. 1982). A few experiments have been carried out investigating the effect of tocopherol supplementation following radiation exposure. Malick et al. (1978) and Roy et al. (1982) have shown decreased radiation effects in mice injected i.p. with tocopherol post-irradiation. The radicals induced by ionizing radiation are extremely short-lived 10^{-14} to 10^{-3} seconds (Coggle 1971). It is therefore justifiable to assume the classic theory of the antioxidant property of tocopherol is not implicated in this protection due to the addition of the vitamin following the degradation and primary damage induction by the radicals.

Studies of mouse survival after irradiation in the range of dosage causing bone marrow syndrome have generally demonstrated a protective effect of tocopherol (Sakamoto and Sakka 1973, Malick et al. 1978, Konings and Drijver 1979, Srinivasan and Weiss 1981). At the dose used, 2 Gy to 10 Gy (1 Gy = 100 rads), there is a breakdown in the hematopoietic system. The animal dies within 30 days due to multiple infections and anemia. The infections are due to neutropenia, and anemia is due to thrombopenia and hemorrhage. The effect of radiation in this dose range (2 to 10 Gy) has been termed "bone marrow syndrome" (Bond et al. 1965 , Coggle 1971). Virtually all deaths associated with bone marrow syndrome occur within 20-25 days.

During bone marrow syndrome, the renewal system of peripheral blood is damaged. As peripheral differentiated cells are more radioresistant than undifferentiated stem cells, their destruction is not immediate (Coggle 1971). Mature cells will die out eventually (erythrocyte life-span is approximately 60 days, thrombocytes 10 days and granulocytes 5-6 hours) and have to be replaced. Surviving stem cells proliferate to repopulate the hematopoietic system. Extensive cell division takes place in the bone marrow from a few hours to a few weeks following the irradiation in an attempt to repopulate the hematopoietic system.

Till and McCulloch (1961) demonstrated a method of measuring the proliferative capacity of hematopoietic stem cells. They showed that after a dose of radiation in the range causing bone marrow syndrome, surviving stem cells form colonies on the spleen (colony forming units). After suitable staining, these colonies can be counted macroscopically. The endogenous spleen colony assay (SCA) makes use of this phenomenon.

These spleen colonies contain precursor cells which differentiate and repopulate the hematopoietic system and increase the animal's chance of survival. The colonies are generally erythrocytic at the center of, and myelocytic at the periphery of, the spleen. An increase in the number of proliferating colony forming units (CFU)

increases the chance of animal survival by combatting infection and anemia (Bond et al. 1965, Coggle 1971).

Some compounds have been found to increase survival of mice in bone marrow syndrome with an associated increase in CFU in the spleen. Uray et al. (1980) reported an increase in endogenous spleen colonies and increased $LD_{50/30}$ after irradiation in mice treated with leukotrophin. Mori (1973), Mori and Katamura (1976) reported increased endogenous CFU and enhanced animal survival in mice receiving an injection of carbon particles prior to irradiation.

Tocopherol injection improves animal survival in bone marrow syndrome (Malick et al. 1978, Roy et al. 1982), this would suggest that some protection or stimulation of the hematopoietic system is implicated. Tests will be carried out to assess whether the increased animal survival is indeed associated with increased survival or proliferation of hematopoietic CFU. An endogenous SCA (Till and McCulloch 1963) will be carried out with and without tocopherol at a number of doses. The endogenous SCA is generally applied to assess CFU response at the higher dose range ( )5.5 Gy) of bone marrow syndrome (Till and McCulloch 1963, Smith et al. 1966, Mori 1973). Lower doses result in a larger number of endogenous hematopoietic stem cells forming colonies on the spleen which crowd and overlap one another making counting

difficult or impossible. To observe the response of hematopoietic stem cells at lower doses of X-irradiation, the exogenous spleen colony assay (Till and McCulloch 1961) will be carried out.

In the exogenous SCA, a known number of CFU are transplanted from the marrow of healthy donor mice to lethally irradiated recipients having no surviving endogenous CFU. These exogenous CFU seed the spleen and form colonies as in the endogenous system. The transplanted CFU are irradiated and assayed for survival *in vivo* in a model that mimics the *in situ* (endogenous SCA) system. The shape of the survival curves of the transplanted CFU can then be compared for the tocopherol treated and control groups. Two main parameters can be compared. The extrapolation number (n) is obtained by extrapolating the exponential survival curve back to 0 Gy dose. A change in n suggests an altered capacity of the cell to accept sublethal damage. The inverse of the slope of the exponential part of the survival curve, Do, may also change suggesting an altered radiosensitivity at the exponential region of the survival curve.

Hematopoietic stem cell repair kinetics commence immediately post-irradiation and are virtually complete by five hours (Till and McCulloch 1963). For this reason, tocopherol injections will be carried out in one group of mice immediately post-irradiation, and in another group at

five hours post-irradiation. In this manner, tocopherol will be present during the recovery period in one group but not in the other. If an increased number of endogenous CFU survive in one group compared to the other, some implications as to the tocopherol effect will be discussed.

It is equally possible that any observed increase in CFU on the spleen could be due to increased stem cell proliferation and not strictly enhanced recovery (repair). The split-dose assay (Till and McCulloch 1963) will be carried out on endogenous CFU to estimate the recovery time and early proliferation kinetics of the tocopherol treated and untreated mice.

Increased numbers of hematopoietic stem cells arising because of increased division or stimulation of repair or other means would be expected to increase the chance of animal survival in the dose range encompassing bone marrow syndrome. The stem cells would be capable of proliferating and repopulating the depleted hematopoietic system resulting in the reinstatement of the immune response.

The purpose of this study is to investigate the capacity of tocopherol to protect mice given doses of ionizing radiation encompassing bone marrow syndrome, and to investigate the possibility that any evidence of enhanced recovery is due to an effect at the level of the

hematopoietic stem cell. To ensure any observed tocopherol effects are not simply due to radical scavenging during the irradiation process, tocopherol will be administered immediately after mouse total-body-irradiation, at which time induced radicals and ionizations will have degenerated.

MATERIALS AND METHODS

Animals

All animals used in the following experiments were female CD-1 mice (Charles River of Canada, St. Constant, Québec). Mice were normally purchased one or two weeks before the commencement of the experiments, allowing adaptation to the animal room prior to experimental treatments.

Bedding (Beta Chip, Charles River) was replaced and water bottles replenished at 2 to 3-day intervals. Food (Charles River Mouse, Hamster and Rat Chow) and water were available ad libitum.

Mice were kept in either small (28 x 17 x 13 cm housing 3-6 mice), or large (27 x 20 x 15 cm housing 5-9 mice) plastic cages. Room temperature was maintained at 21 °C, and humidity at 50%. The animal room was on a regular 12 hour light: 12 hour dark cycle.

Irradiation Procedures

X-Radiation was generated from a Mueller MG-300 X-ray machine operated at 260 KVp and 8mA with a 1 mm aluminum filter added. Exposure dose rate was measured

using a victoreen condenser R-meter, model 570 and absorbed dose by ferrous ammonium sulphate chemical dosimetry. The target-to-object distance in all experiments was 80 cm. The dose rate was .31 Gy/min.

Groups of 15-30 mice were irradiated in large plastic cages. The top of the cage was covered with perforated paper. This allowed aeration without attenuating the X-ray beam. Food and water were not available during the irradiation.

Intraperitoneal Injections

Intraperitoneal injections (i.p.) were given on the right ventral side of the animal using a 25 or 26 gauge needle affixed to a 1-cc plastic disposable syringe. Animals that were i.p. injected were given either 0.10 ml aqueous micellar suspension of tocopherol as described by Newmark et al. (1975), containing 25 mg/ml dl-α- Tocopherol (U.S. Biochemical Corporation, Cleveland,Ohio) or 0.10 ml vehicle only, or no injection. The vehicle consisted of 10% (v/v) propylene glycol, 10% (v/v) ethanol, 10% (v/v) mulgophen EL 620 (GAF Corporation, Montreal), 1% (v/v) benzyl alcohol, 0.25% (w/v) glacial acetic acid, 0.9% (w/v) sodium chloride, 0.03% (w/v) sodium acetate and 0.01% (w/v) disodium edetate.

Tocopherol (1.1 IU/mg) was dissolved in the

vehicle. Normally 250 mg of tocopherol was added to 10 ml of the vehicle. This mixture was repeatedly shaken for 1-2 hours at room temperature to ensure complete suspension.

Intravenous Injections

Lateral tail veins of the mouse were used as the site of injections of the bone marrow or spleen cell suspensions. Due to the small size and fragility of the veins, absolutely no animal movement was tolerated. To achieve sufficient restraint, mice were placed in a perforated 50-cc polyethylene centrifuge tube fixed onto a solid platform.The tube was stoppered with a foam plug through which the tail was allowed to protrude. To facilitate venous location and puncture, mice were heat treated for ten minutes before i.v. injections so as to dilate the tail veins. This was achieved by placing animals in 10 x 20 cm glass containers with partially perforated covers. Two lamps, each with a 60 watt bulb, were placed at either side of the jars at a distance of 10 cm from the wall of the containers. The temperature rose quickly and was maintained at 30 °C. One-cc syringes with 27 gauge needles were used for the i.v. injections. The needle was inserted into the vein at a very shallow angle of approximately 5° . After the injection, the needle was carefully withdrawn and light pressure was placed on the puncture site until bleeding stopped.

Dose Survival Curves

Groups of 24 mice (48 in the case of 7.1 Gy dose), were given radiation doses ranging from 5.6 Gy to 7.1 Gy. In all, 648 animals were used. There were three experimental groups: vehicle-injected, tocopherol-injected and non-injected. Mortality was recorded daily up to 30 days post-irradiation.

Spleen Colony Counting Technique

Eight days post-irradiation, animals were sacrificed using carbon dioxide gas, spleens were excised and placed in Bouin's solution following the method of Till & McCulloch (1961). Before fixation in Bouin's, the colonies could not be easily distinguished from the rest of the tissue as both were dark red in colour. After fixation, the macroscopic colonies stood out as light spots, about 0.5-1.0 mm in diameter, on a darker background.

Endogenous Spleen Colony Assay (SCA)

The endogenous SCA (Till & McCulloch 1963) was performed according to standard procedures. Mice weighing 20-22 g were subjected to a range of X-ray doses. Five groups of mice were used per dose for a total of 1,299

mice. Two groups of mice were injected immediately (5-10 minutes) post irradiation. One of these (0E), received 2.5 mg of tocopherol suspended in a vehicle while the second (0V) group received the vehicle only. Two other groups were injected similarly but at 5.0 hours after the irradiation. One group (5E) received 2.5 mg of the tocopherol suspension and the other the vehicle only (5V). The last group (C) served as a non-injected control.

At day eight after the irradiation, the spleens were removed and colonies were counted.

Exogenous Spleen Colony Assay

To examine the response of spleen and bone marrow haematopoietic stem cells after radiation injury, the method of Till & McCulloch (1961), with some modification (Lord & Hendry 1973), was used.

i) Bone Marrow Graft

Recipient mice, (those receiving the marrow graft) weighing 18-22 g. were irradiated with a dose of 8.0 Gy X-rays 16-24 hours before the transplant. This served to suppress the endogenous colony forming units (CFU) to less than one colony per spleen. This dose was experimentally determined and was found to be the highest dose only causing mortality due to bone marrow syndrome (10-30 days post-irradiation) with few early deaths (4-6 days post

irradiation) in gastrointestinal syndrome range.

The donor bone marrow cells were prepared from non-irradiated mice (18-22 g) which were sacrificed by cervical dislocation. Both femurs were removed from each mouse and rinsed in Hank's balanced salt solution, (BSS, TC-199 + phenol red; Difco Laboratories, Detroit) + 0.35 g sodium bicarbonate per litre and CO_2 to pH=7.2. One epiphysis was removed from each femur and discarded. A 1-cc syringe, with a 25 gauge needle, containing 0.5 ml ice cold BSS was inserted into the hollow shaft. The marrow was then flushed out into a chilled test-tube.

The number of donor mice used per radiation dose varied from 5-20. A greater number of cells were required for the higher doses as CFU survival in the higher range was extremely low. Generally, 15 recipient mice were used per group per dose for a total of 466 recipient mice.

Bone marrow suspensions were pooled and cell concentration was measured using a hemocytometer. Briefly, 0.10 ml of cell suspension was added to 0.90 ml of a 1% acetic acid solution containing 0.05% methylene blue. This treatment destroys non-nucleated erythrocytes. Only nucleated cells remain intact. Once the concentration was determined, the stock cell suspension was brought to an appropriate concentration. The concentrations used varied from 3.3×10^5 cells/ml at the lower radiation doses of 0 Gy - 0.25 Gy, to 47×10^6 cells/ml at the highest

dose of 5.0 Gy. The cell suspension was then injected *in vivo* into the recipient animals. After 1-2 hours, the animals were subjected to the appropriate 'second dose' which damaged the newly injected cells. In this manner, the exogenous CFU obtained from the donor animals were irradiated *in vivo* in the recipient animals.

At the end of the second irradiation, mice were completely randomised. The animals were then injected with tocopherol, vehicle, or were not injected. Eight days later, spleens were excised and colonies counted.

The surviving fraction was calculated by dividing the number of colonies per 10^5 cells injected appearing in an irradiated group by the number appearing in the group not receiving the second dose (0 Gy).

$$\text{Surviving fraction} = \frac{\text{CFU}/10^5 \text{ at dose X}}{\text{CFU}/10^5 \text{ non-irradiated}}$$

Normally 5-6 hours pass from the time of the first injection to the last. To ensure optimal CFU survival during this time, the bone marrow cell suspension was always kept at 0 °C. A survival of CFU at 0 °C over a period of time was carried out. The criterion of survival was taken to be the ability of the CFU to form colonies on the spleen of recipient mice. The transplants were injected *in vivo* into irradiated recipient mice at 1, 3, 5, 7, 9, and 24 hours after cell collection.

ii) Spleen Cell Graft

Transplants of spleens cells followed a similar protocol to that of bone marrow. The differences are noted here:

Donor mice were sacrificed and spleens were removed and rinced in ice cold BSS. Spleens were then disrupted by pushing them through a 50 gauge wire mesh of a cellector-homogenizer. Spleen clumps were then broken up into a single cell suspension by flushing them twice through a 25 gauge needle.

Cell concentrations were then determined and dilutions were made ranging from 2×10^6 cells/ml for low doses (0 - 0.25 Gy) to 112×10^6 cells/ml for the highest dose (3.4 Gy). A total of 273 mice were used as recipients.

Split-Dose Assay

To estimate the time required for maximal recovery after X-irradiation, the split-dose assay was used (Till and McCulloch 1963). Briefly, groups of mice weighing 20-25 g were given 6.9 Gy total body irradiation in two equal dose fractions each of 3.44 Gy. The time interval between the two doses ranged from 0-24 hrs. A total of 11 intervals were used at approximately 30 mice per group for a total of 1,229 mice.

Three groups of mice were used per time interval. One group of mice received an injection of 0.05 ml of the vehicle after each dose fraction. A second group received an injection of 0.05 ml of the tocopherol suspension after each dose fraction. The third group was not injected. This was repeated with a fresh batch of mice at each time interval. In this manner the two injected groups received a total of 0.1 ml of either vehicle only, or tocopherol suspended in vehicle. This was done to keep the total volume of injected material in this experiment the same as that of the other experiments.

RESULTS

Optimal Tocopherol Dose

To ascertain the optimal dose of tocopherol required to increase animal survival after radiation stress, groups of mice were given 6.5 Gy total-body-irradiation. The animals were randomized and injected with variable doses of tocopherol in 0.1 ml vehicle, 0.1 ml vehicle only, or were not injected. Six groups of mice were used at 24 mice per group, for a total of 144 mice. The optimal tocopherol dose was found to be 2.5 mg per mouse (Figure 1).

Survival Study

The effect of tocopherol, post-irradiation, on mouse lethality was studied in the dose-range causing bone marrow syndrome. Dose-mortality curves of total-body-X-irradiated mice from 5.6 to 7.1 Gy were transformed to straight lines for the three groups of data (Table 1, Figure 2).

There is a significant difference ($P<.01$) between the $LD_{50/30}$ of the control groups (vehicle and non-injected) and the tocopherol-injected group. The $LD_{50/30}$

of the vehicle-injected group and the non-injected group is not significantly different (Table 2).

The slopes of the three lines suggest that the tocopherol-injected group, having a lesser slope of 1.1 Gy^{-1}, compared to that of the vehicle-injected group of 1.8 Gy^{-1}, and the non-injected group 1.9 Gy^{-1}, has an improved rate of survival (Table 2). This is especially true at the higher doses where the absolute difference between control and tocopherol groups is larger (Figure 2). The differences between slopes however is not significant (X^2 test for differences between slopes, Stanley 1963).

Endogenous Spleen Colony Assay

To determine the effect of tocopherol on hematopoietic stem cell survival, after total-body-irradiation, the endogenous spleen colony assay (SCA) of Till and McCulloch (1963) was carried out. Five groups of mice were irradiated over a dose range of 5.8 to 6.9 Gy. There were two groups of animals receiving tocopherol injections, one group immediately post-irradiation (0E) and the other five hours after irradiation (5E). Two groups of mice receiving vehicle only were injected at the same times as the tocopherol groups (0V, 5V). The fifth group (C) was the non-injected control.

Figure 1. Survival curves of mice receiving variable doses of tocopherol immediately after 6.5 Gy total-body irradiation.

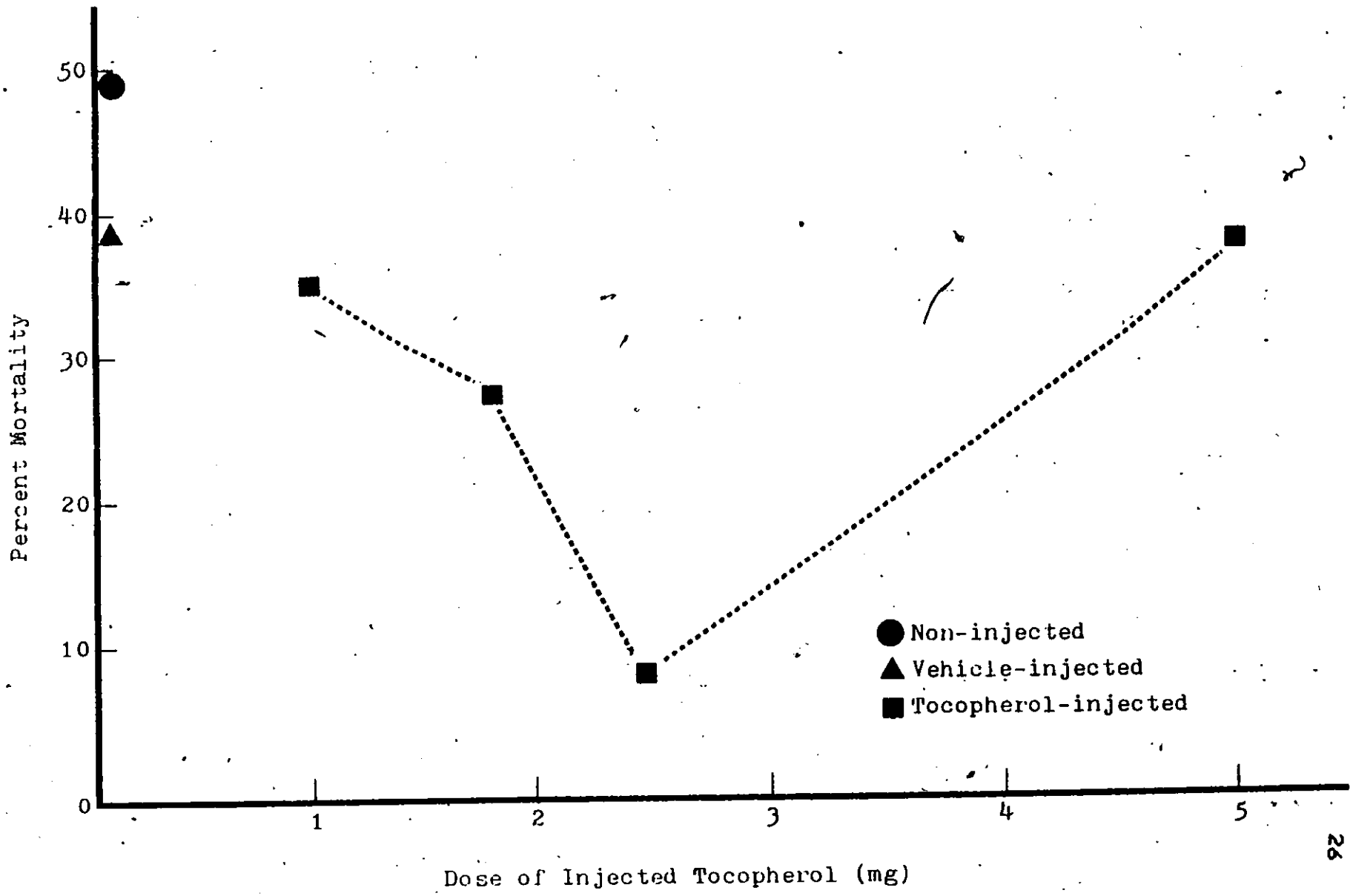

Percent Mortality
50
40
30
20
10
0
1
2
3
4
5
Dose of Injected Tocopherol (mg)
Non-injected
Vehicle-injected
Tocopherol-injected

Figure 2: Probit lines of dose-mortality data for tocopherol-injected, vehicle-injected or non-injected mice.

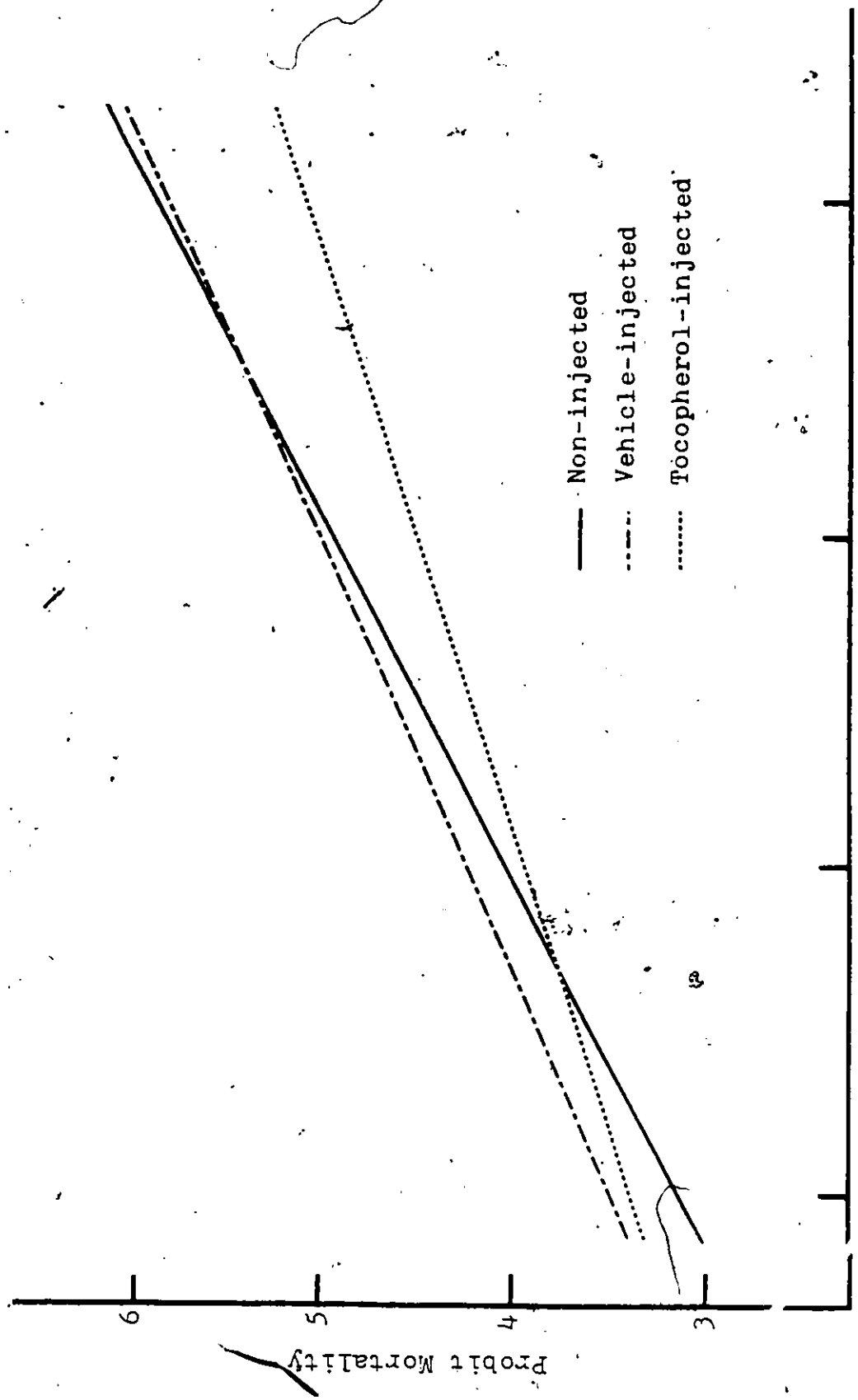

Probit Mortality
6
5
4
3
5.5
6.0
6.5
7.0
Dose (Grays)
Non-injected
Vehicle-injected
Tocopherol-injected

TABLE 1

THIRTY-DAY DOSE-MORTALITY STUDIES OF

TOTAL-BODY X-IRRADIATED ANIMALS

Dose (Gy)	% Mortality C	V	E	Number of Animals Per Treatment Group	Probit Conversion C	V	E
5.6	4.2	0	4.2	24	3.09	3.45	3.36
5.8	0	4.2	12.5	24	3.49	3.78	3.61
6.0	25.0	29.0	4.2	24	3.90	4.12	3.86
6.2	29.0	41.7	33.3	24	4.30	4.46	4.10
6.4	38.0	45.8	20.8	24	4.70	4.80	4.35
6.7	29.0	50.0	20.8	24	5.11	5.14	4.59
6.9	71.0	66.7	45.8	24	5.51	5.47	4.84
7.1	88.0	77.1	56.3	48	5.91	5.81	5.09

C Non-injected control group. (Stanley 1963)

V Vehicle-injected group.

E Tocopherol-injected group.

TABLE 2

SLOPES OF PROBIT LINES OF THIRTY-DAY SURVIVAL STUDIES OF ANIMALS RECEIVING TOTAL-BODY IRRADIATION FROM 5.6 - 7.1 Gy.

Group	Slope (Gy^{-1})	Deviation from Linearity	$LD_{50/30}$ ± SE (Gy)
C	1.88	> 0.05	6.60 ± 0.05
V	1.77	> 0.3	6.58 ± 0.06
E	1.14	> 0.2	7.02 ± 0.11 *

Curves are accepted as linear if $p > 0.05$ using X^2 for linearity.

C Non-injected control group.

V Vehicle-injected group.

E Tocopherol-injected group.

* Significantly different ($p > 0.01$ t-test).

Values obtained from Probit Analysis Test (Statpack from Concordia University computer center and Stanley 1963.).

The 5-hour interval was selected as the hematopoietic stem cell recovery is normally complete by five hours after radiation injury (Till and McCulloch 1963). For this reason, introduction of tocopherol at 5 hours post-irradiation assured that the vitamin would not be present during the major recovery period and could not take part in associated repair.

Due to the skewed nature of the endogenous colony counts, (large number of spleens with no colonies at high doses), individual spleen colony counts were transformed to log (see below). Combined group data at a particular dose was then detransformed to mean colony count as recommended by Smith et al. (1966). Statistical analysis were carried out on the detransformed mean colony counts. Briefly, individual colony counts (x) were transformed using the formula; $X_t = \log (x+1)$, where X_t is the transformed spleen colony count. In this manner, the problem of dealing with the log of zero was circumvented. The mean for the group was then obtained and detransformed by the formula $\hat{\bar{X}}_d = (\text{antilog } \bar{X}_t) - 1$, where $\bar{X}_t$ is the transformed mean response of a group at a particular dose and $\bar{X}_d$ is the detransformed mean colony count. Spleen colony dose response curves were fitted by weighted regression analysis (Figure 3,4).

The endogenous SCA data suggest that the number of CFU surviving (ie. capable of proliferating into a spleen

colony) after doses of 5.8 Gy to 6.9 Gy, can be increased by an injection of 2.5 mg. of dl-α-tocopherol immediately post-irradiation (Figure 3, Table 3). This effect is not apparent if tocopherol administration is delayed by five hours (Figure 4, Table 3). The vehicle also showed some protective effects if administered immediately after the irradiation. The effect declined with increasing dose and reached the control level at the higher doses (Figure 3, Table 3). In the case of tocopherol, injection immediately post-irradiation increased the spleen colony count compared to both vehicle-injected and non-injected controls. This is especially true at the higher doses. The 5-hour post-irradiation tocopherol-injected group had a similar response to that of the non-injected control group.

In this study, there was no apparent effect of tocopherol when injected at five hours post-irradiation. To alter the endogenous spleen colony counts, tocopherol had to be present within five hours of irradiation.

Exogenous Spleen Colony Assay

To investigate further the tocopherol effect on CFU within the recovery period of the endogenous system, a dose-response curve of exogenous bone marrow and spleen CFU was carried out. An increase in the 'shoulder' of the dose-response curve would indicate that there is an

Figure 3. Endogenous spleen colony assay of mice receiving tocopherol, vehicle or no treatment immediately after 5.8 to 6.9 Gy of total-body X-irradiation.

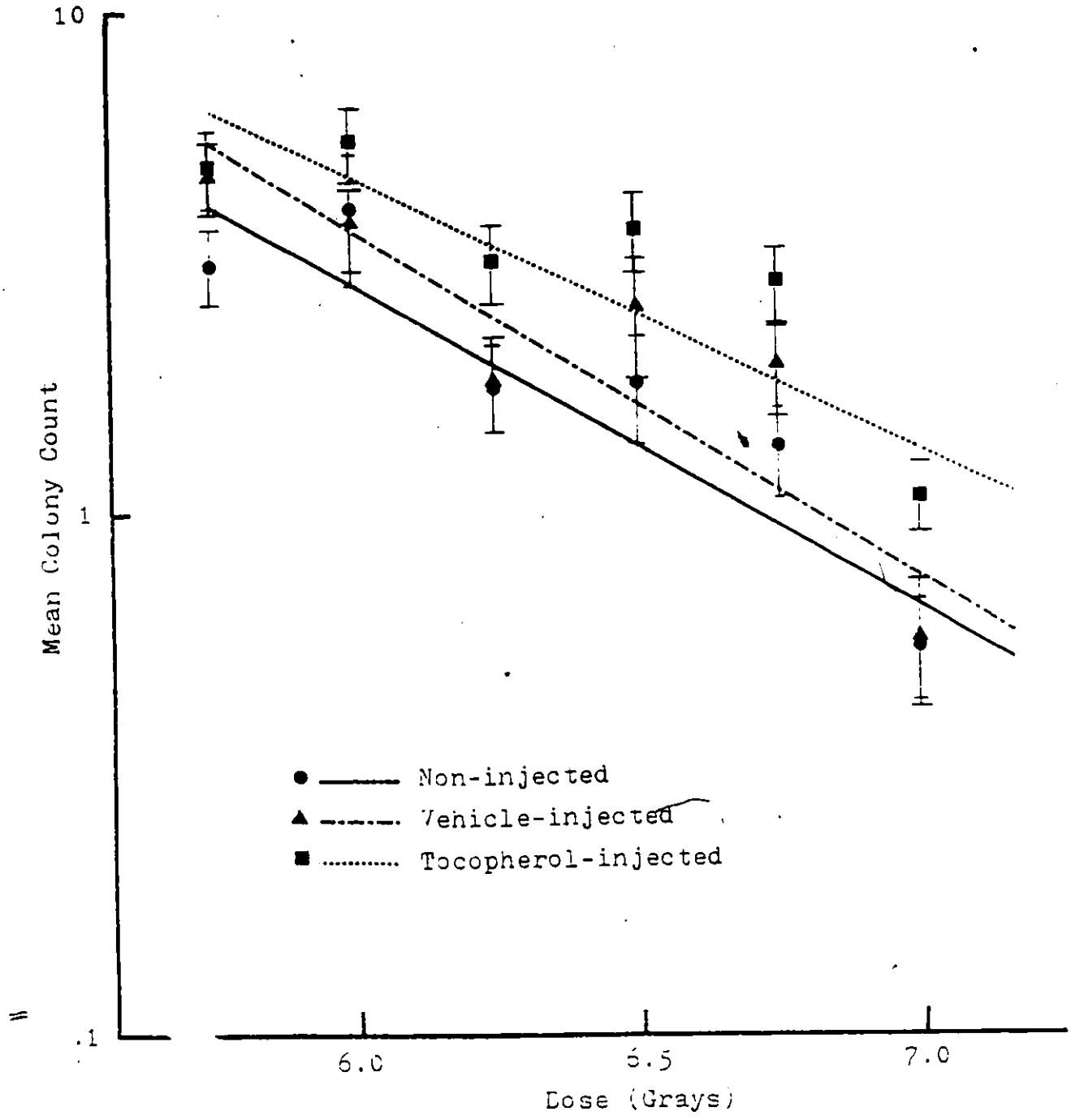

10
1
.1
Mean Colony Count
6.0
6.5
7.0
Dose (Grays)
Non-injected
Vehicle-injected
Tocopherol-injected

Figure 4. Endogenous spleen colony assay of mice receiving tocopherol, vehicle or no treatment five hours after 5.8 to 6.9 Gy total-body X-irradiation.

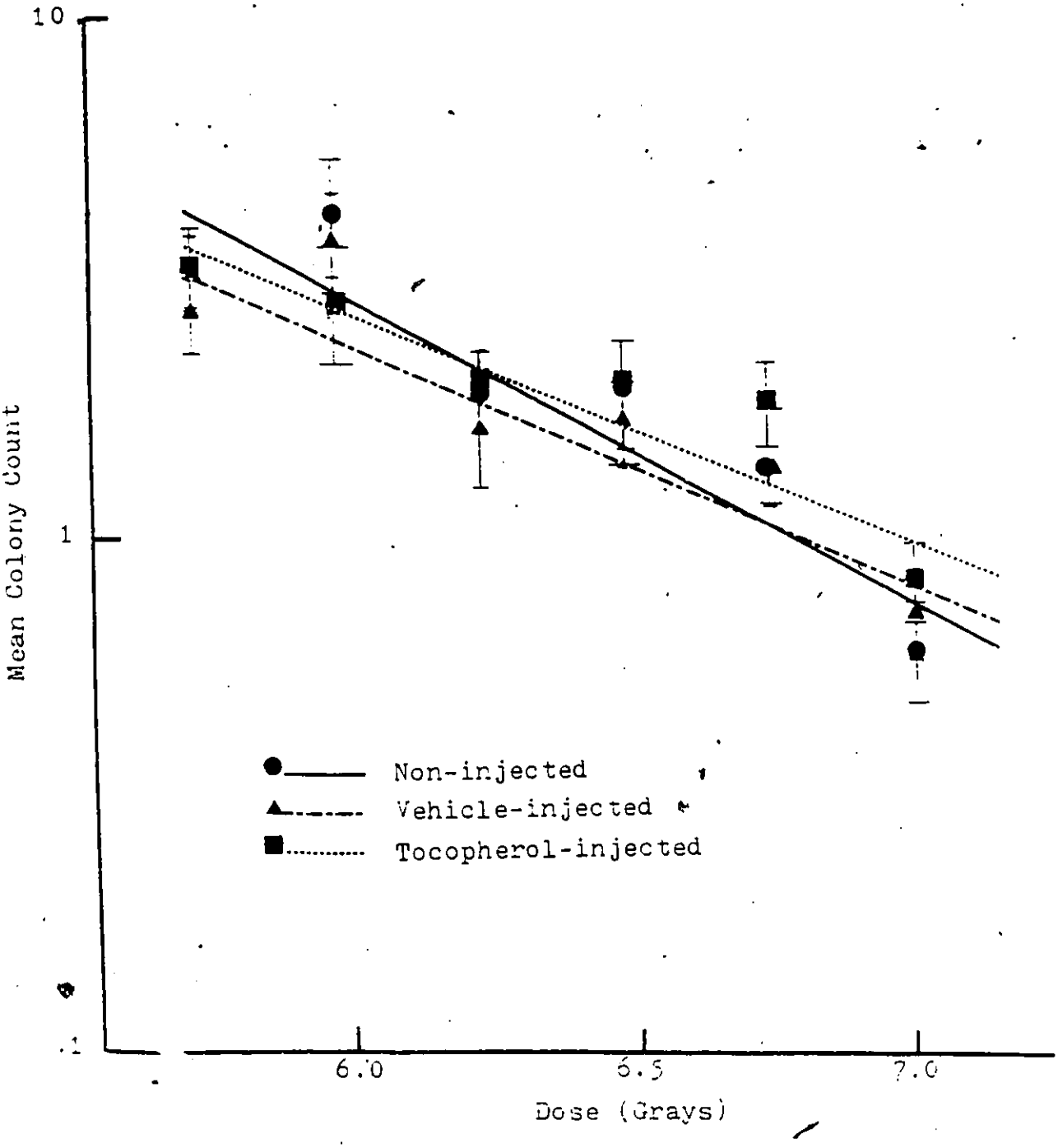

10
1
.1
Mean Colony Count
6.0
6.5
7.0
Dose (Grays)
Non-injected
Vehicle-injected
Tocopherol-injected

TABLE 3. ENDOGENOUS SPLEEN COLONY ASSAY OF MICE RECEIVING VARIABLE DOSES OF TOTAL-BODY X-RADIATION.

Dose Gy	Mean colony counts ± SE				
	C	OV	OE	5V	5E
5.8	3.20±0.57 39[a]	4.78±0.79 36	4.96±0.89 37	2.66±0.44 40	3.27±0.67 36
6.0	4.07±1.18 20	3.80+0.70 25	5.55±0.93 25	3.67+0.96 19	2.83±0.71 20
6.2	1.86±0.35 38	1.88±0.40 32	3.24±0.54* 35	1.57±0.37 35	1.96±0.38 32
6.4	1.84±0.43 38	2.63±0.41 38	3.70±0.67* 39	1.64±0.34 39	1.67±0.35 40
6.7	1.39±0.27 40	2.04±0.42 39	2.90±0.50* 35	1.38±0.30 40	1.74±0.31 39
6.9	0.57±0.12 84	0.60±0.11 93	1.12±0.18** 88	0.67±0.13 86	0.78±0.14 92

a Sample size.

C Non-injected group
Do= 0.60 Gy.

OV Vehicle-injected group immediately post-irradiation
Do= 0.54 Gy.

OE Tocopherol-injected group immediately post-irradiation
Do= 0.71 Gy.

5V Vehicle-injected group 5 hours post-irradiation
Do= 0.76 Gy.

5E Tocopherol-injected group 5 hours post-irradiation
Do= 0.77 Gy.

* Significantly different from non-injected group ($P<0.05$).

** Significantly different from vehicle-injected group ($P<0.05$)
(t-test, Sokal and Rohlf 1969).

increase in the capacity of the cell to accept accummulating damage (Elkind and Sutton 1960, Till and McCulloch 1961, Alper 1977, 1979). Alternatively, this may in turn be interpreted as an increase in repair capacity (Elkind and Sutton 1960 , Fonck and Konings 1978).

For logistic reasons, the suspension of hematopoietic cells had to be kept in saline for a period of 1 to 7 hours during an experiment. Typically, the first animal would receive the intravenous (iv) transplant 6 hours before the last animal would. Hence, it was necessary to ascertain the survival of CFU in balanced salt solution (BSS) at 0 °C for a period overlapping the maximum length of time for which the cells would be stored. The results for bone marrow CFU are summarized in Table 4. At 7 hours, the maximum time of storage, cell viability is approximately 96%. This error was judged too low to necessitate correction for animals injected at the later time. To minimize error, animals receiving a particular dose were first iv injected, irradiated, and then randomized before receiving ip injections of tocopherol, vehicle, or being assigned to non-injected groups.

At 6.9 Gy, the number of spleen colonies arising from endogenous CFU was approximately 0.7 per spleen in the control groups, and 1.4 in the tocopherol-injected group (Figure 3). To ensure that an insignificant number

of endogenous CFU would survive and colonize the spleen during the cell transplant experiments, a dose greater than 6.9 Gy was given to recipient mice. Too high a dose however, would shift mortality from bone marrow syndrome (11-21 days) to gastrointestinal syndrome (4-8 days). This would result in many animals dying before the spleen could be excised on day eight. To determine the appropriate dose of X-rays, a range of doses from 7.7 to 9.9 Gy were given to groups of 16 mice (Figure 5). A dose of 8.0 Gy was chosen for the recipient mice. This dose yielded 0.3 colonies per spleen for endogenous colony forming units.

The response of exogenous CFU from the spleens of donor mice was not modified by the post-irradiation injection of tocopherol, under the present experimental conditions (Figure 6). The Do, Dq and extrapolation number (n) for the three groups are approximately equal (Table 6). The lack of a shoulder suggests that spleen CFU have little or no capacity to accept sublethal damage. This response does not appear to be modified by tocopherol in the present case.

The response of bone marrow hematopoietic stem cells differed from those arising from the spleen. Although the Do were similar, Dq and n were larger. As in the case of spleen CFU, no tocopherol effect was observed up to 3.4 Gy; however, there appears to be a change in slope for the vehicle-injected and non-injected groups at

TABLE 4

CAPACITY OF BONE MARROW CFU TO FORM SPLEEN COLONIES

AFTER SUSPENSION IN BSS FOR VARIOUS TIME INTERVALS

Time in Solution (Hours)	Colony Count[a] ± SD	Number of Animals Injected	Survival[b] Ratio
1	14.0 ± 2.0	5	1.0
3	14.0 ± 1.7	5	1.0
5	13.8 ± 2.4	5	0.99
7	13.4 ± 1.8	5	0.96
9	12.8 ± 3.0	5	0.91
24	8.5 ± 3.4	4	0.61

a 2×10^5 cells were injected per mouse.

b Relative to colony forming ability at 1 hour.

TABLE 5

DATA FOR DOSE-RESPONSE CURVES OF EXOGENOUS BONE MARROW AND SPLEEN HEMATOPOIETIC COLONY FORMING UNITS IRRADIATED IN VIVO IN MICE.

Group	Correlation coefficient(r)[b]	Sample size	Slope ± SD (Gy^{-1})	Do ± SD (Gy)	Extrapolation number (n)	D ± SD
Bone marrow						
C	0.99	56	-0.44±0.03	1.00±.06	1.5	0.42±0.02
V	0.99	75	-0.50±0.02	0.84±0.04	2.2	0.67±0.03
E	0.99	82	-0.44±0.02	0.98±0.04	1.8	0.58±0.02
Spleen						
C	0.99	82	-0.42±0.01	1.03±0.03	1.2	0.14±0.01
V	0.99	67	-0.43±0.03	1.01±0.09	1.0	0.02±0.00
E	0.99	63	-0.44±0.01	0.98±0.02	1.1	0.02±0.00

C Non-injected

V Vehicle-injected.

E Tocopherol-injected.

b Correlation coefficients were calculated on the linear (exponential) portion of the survival curves (1.5 - 5.0 Gy for bone marrow and 0 - 3.4 Gy for spleen).

Figure 5. Time-mortality study of mice receiving doses of 7.7 Gy to 9.9 Gy total-body X-irradiation.

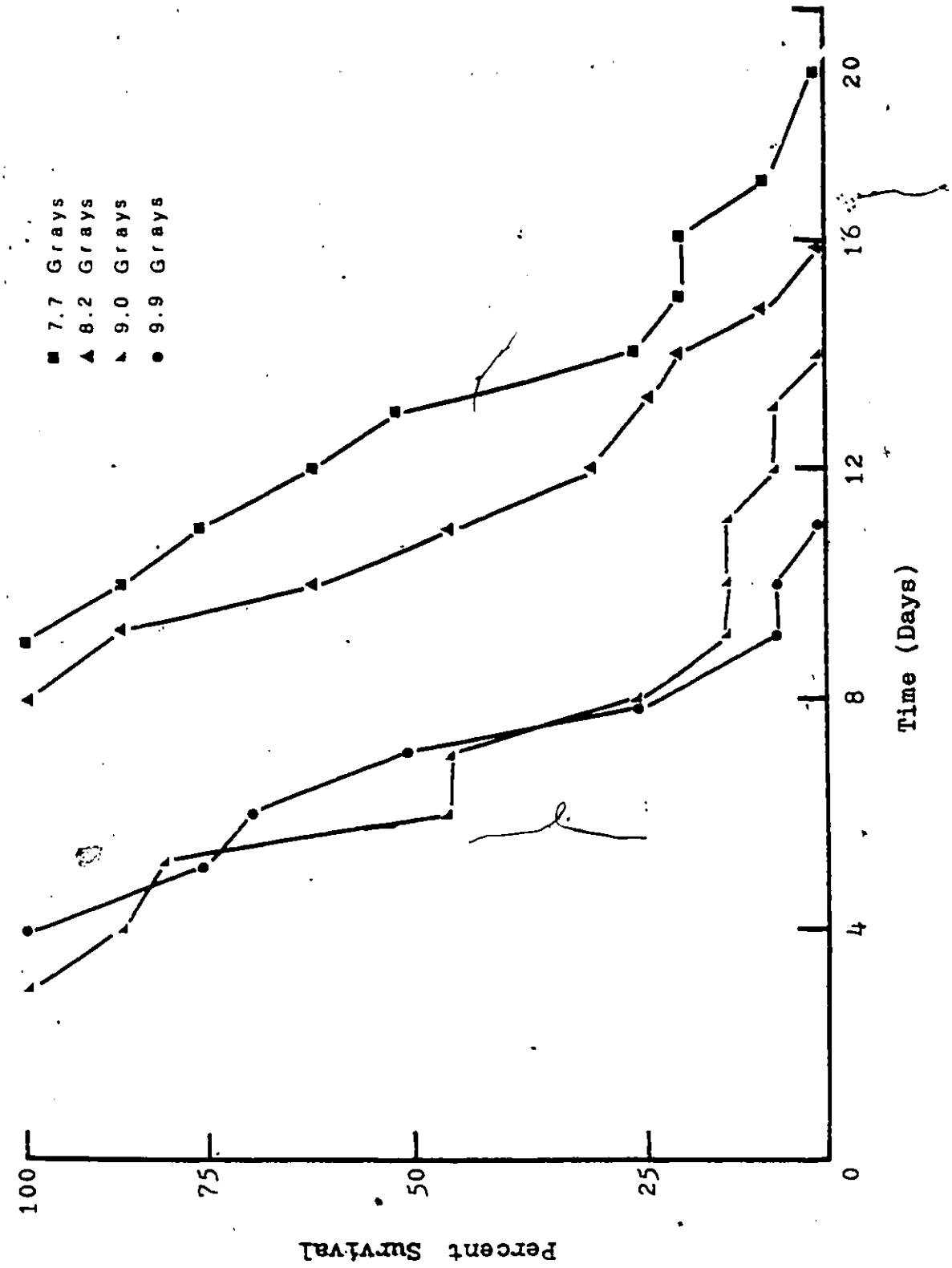
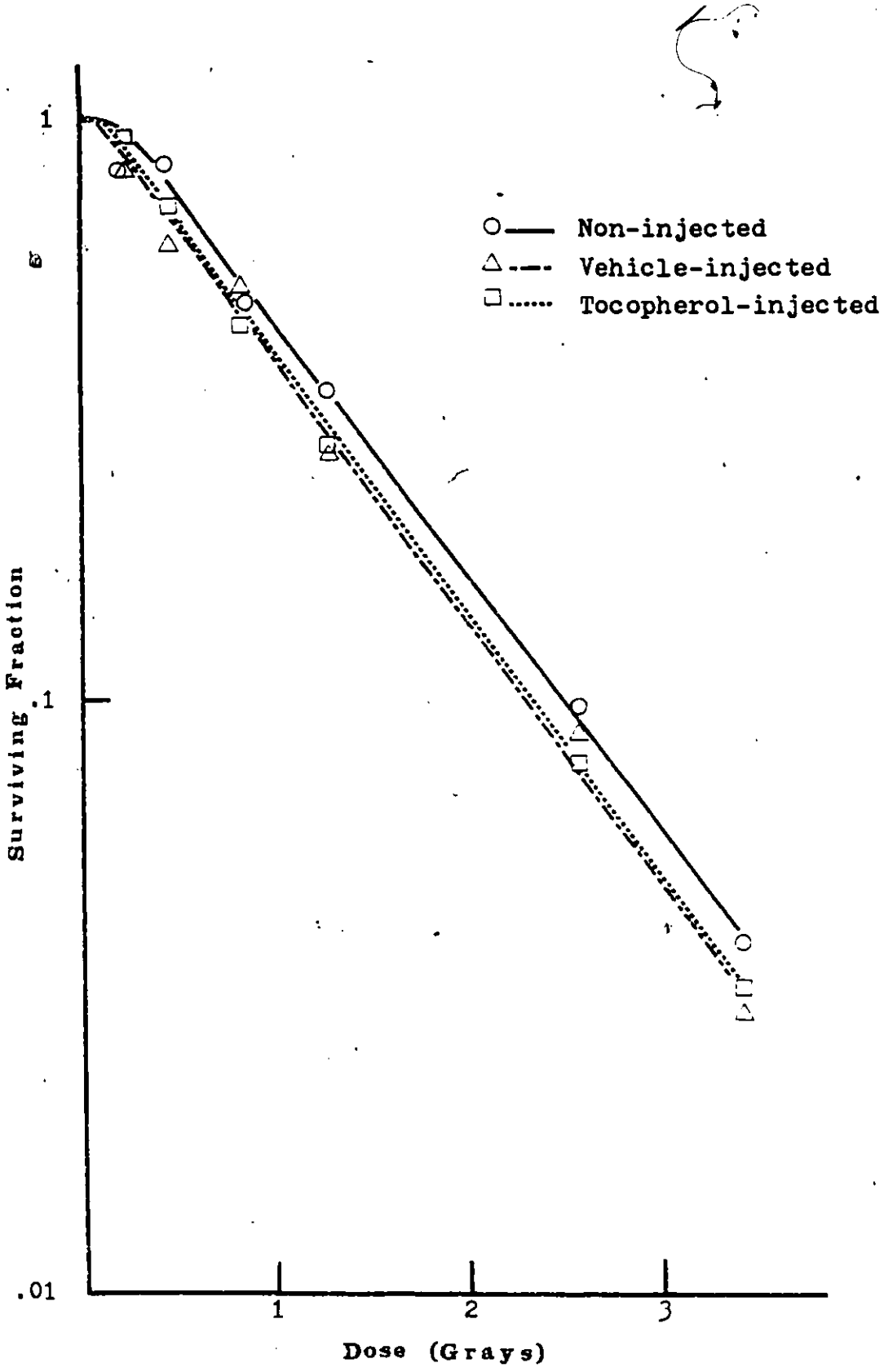

7.7 Grays
8.2 Grays
9.0 Grays
9.9 Grays
Percent Survival
100
75
50
25
0
4
8
12
16
20
Time (Days)

Figure 6. Dose response—curves of exogenous spleen colony forming units irradiated *in vivo* in recipient mice injected with tocopherol, vehicle, or non-injected immediately post-irradiation.


1
.1
.01
Surviving Fraction
1
2
3
Dose (Grays)
Non-injected
Vehicle-injected
Tocopherol-injected

the higher doses (Figure 7). There is a significant difference between the mean colony counts at the higher doses of tocopherol-treated groups and control groups (Table 6). The tocopherol group does not appear to undergo a slope change at doses less than 5.0 Gy. This is the highest dose that could be achieved within the present experimental design. Technical difficulties prevented proceeding to higher doses.

An attempt to reach the same dose range with CFU arising from the spleen was not successful. At 3.4 Gy, 57-112 x 10^5 spleen cells had to be transplanted (i.v.) to yield 6-7 colonies per spleen (Appendix, Table 9-11). To give a dose of 4.3 Gy, approximately 17 x 10^6 spleen cells per mouse would be required for the same number of colonies. However, when the i.v. transplants were made, death of the recipients resulted in 1 to 3 minutes.

Spleen Microenvironment

Treatment of lethally irradiated recipient mice with tocopherol immediately after bone marrow or spleen derived hematopoietic stem cell transplant did not increase the number of CFU seeding the spleen (Table 7). These results suggest that the increased number of surviving endogenous CFU appearing in the tocopherol-treated mice were not more likely to form colonies on the spleen simply due to an improved microenvironment.

TABLE 6

SUMMARY OF EXOGENOUS HEMATOPOIETIC CFU RESPONSE TO DOSES OF X-RADIATION.

Surviving fraction (x 10^{-2})

Dose (Gy)	0	0.22	0.43	0.86	1.30	2.60	3.40	3.90	4.30	4.70	5.0
Group											
Bone marrow											
C	100	96.6	85.7	60.4	45.7	13.3	4.2	---	2.3	0.71	0.37
V	100	94.8	80.6	70.4	50.8	13.9	3.8	2.9	1.3	0.95	0.29
E	100	86.1	88.8	65.2	52.3	14.5	4.8	---	2.6**	1.2*	1.1**
Spleen											
C	100	82.5	84.8	49.3	35.8	9.9	4.1	---	---	---	---
V	100	82.6	57.3	50.0	26.9	9.0	3.8	---	---	---	---
E	100	92.7	70.8	43.8	27.8	8.2	3.3	---	---	---	---

C Non-injected mice

V Vehicle-injected mice

E Tocopherol-injected mice

* Significantly different from non-injected group ($P<0.05$)

** Significantly different from vehicle-injected group ($P<0.05$)

(t-test, Sokal and Rohlf 1969)

TABLE 7

COLONY FORMING ABILITY OF BONE MARROW AND SPLEEN HEMATOPOIETIC STEM CELLS INJECTED INTO IRRADIATED RECIPIENT MICE RECEIVING TOCOPHEROL INJECTION, VEHICLE INJECTION OR NO FURTHER TREATMENT.

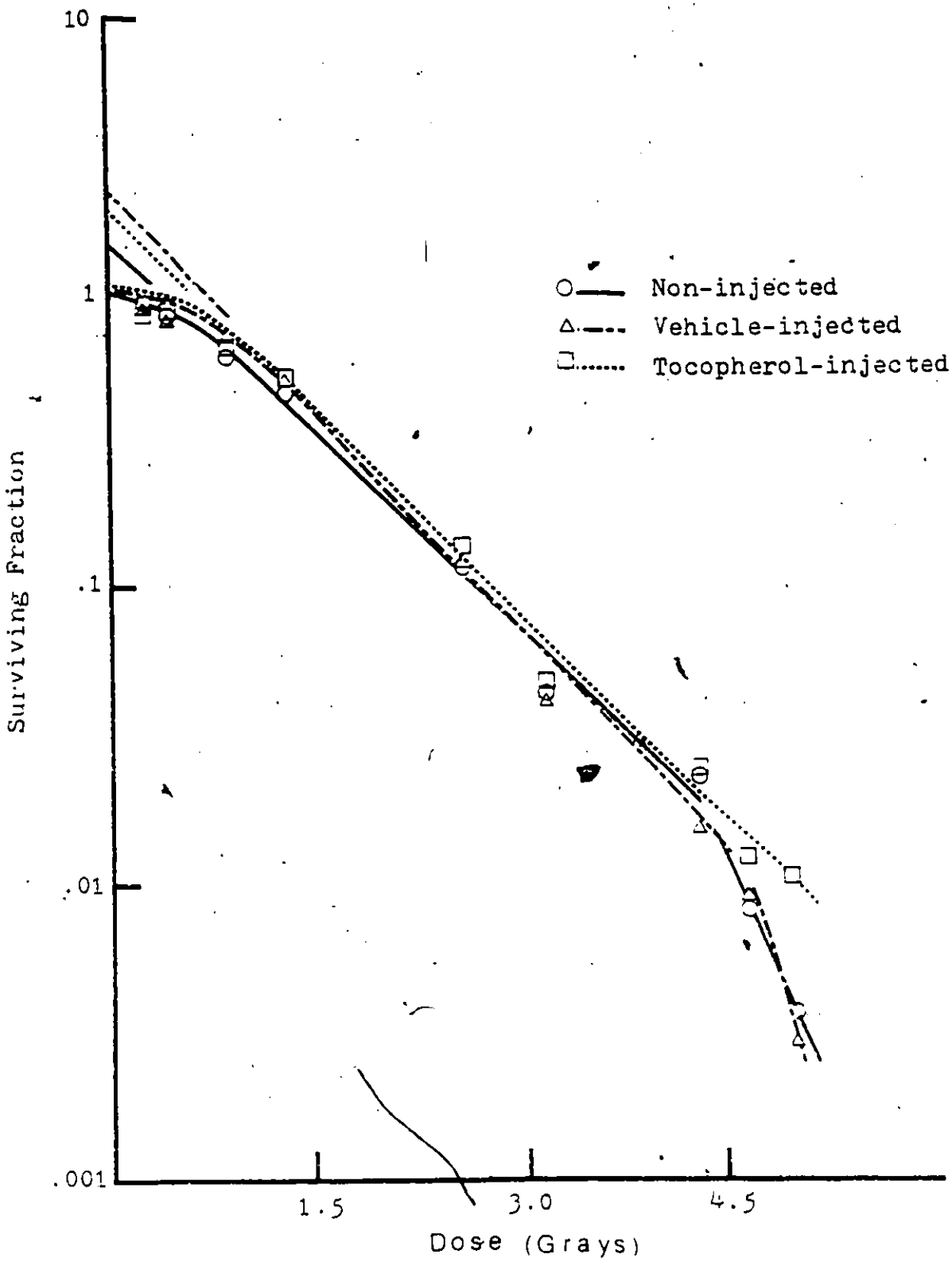
	Bone Marrow			Spleen		
	C	V	E	C	V	E
Cells inj x 10^5	2.0	2.0	2.0	4.4	4.4	4.4
Mean colony count ± SD	17.7 ± 5.1	19.2 ± 8.1	18.0 ± 6.3	9.2 ± 2.7	8.5 ± 4.0	8.2 ± 4.6
Sample size	11	8	9	10	10	5
CFU/10^5 cells	8.9	9.6	9.8	2.1	1.9	1.9

C Non-injected recipient mice

V Recipients receiving vehicle injection post-stem cell transplant

E Recipients receiving tocopherol injection post-stem cell transplant

Figure 7. Dose-response curves of exogenous bone marrow colony forming units irradiated *in vivo* in recipient mice injected with tocopherol, vehicle, or non-injected immediately post-irradiation.


10
1
.1
.01
.001
Surviving Fraction
Non-injected
Vehicle-injected
Tocopherol-injected
1.5
3.0
4.5
Dose (Grays)

Split-Dose Assay

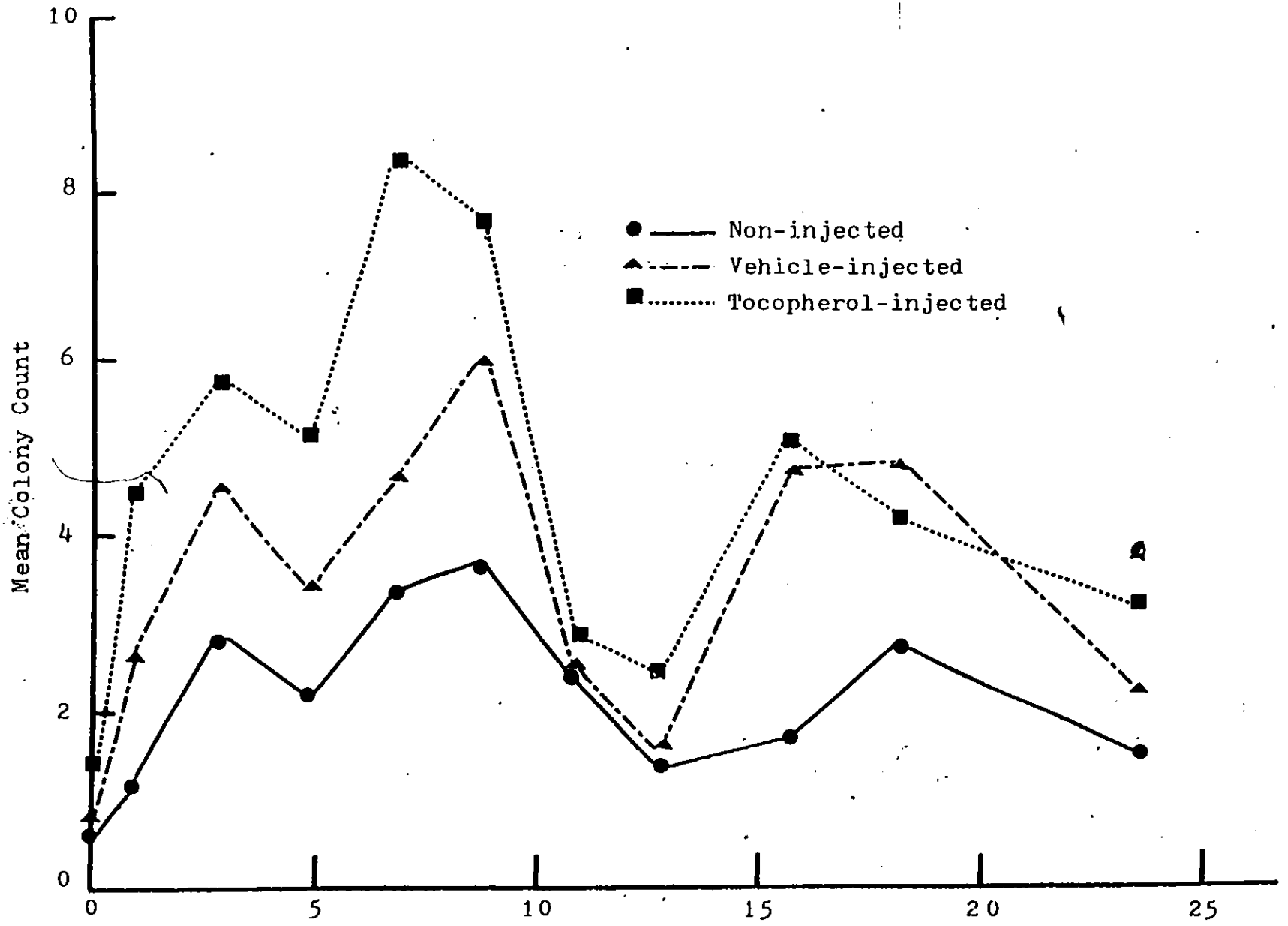
After receiving a dose of X-irradiation, hematopoietic stem cells commence cellular recovery. This recovery is thought to be mediated by repair of the induced damage and is often referred to as "Elkind Recovery" (Elkind and Sutton 1960, Till and McCulloch 1963, Alper 1977). The peak of such recovery in endogenous or exogenous hematopoietic CFU of mice is at approximately 5 hours (Till and McCulloch 1963).

If the post-irradiation tocopherol effect involves stimulation of Elkind recovery, one may expect to observe two possible modifications of the split-dose response. The time-course of recovery may be altered or the magnitude of recovery observed over the same recovery period may be changed.

To evaluate the effect of tocopherol on 'Elkind' recovery, a fractionated dose-response study was carried out. Two doses, each of 3.4 Gy total-body-irradiation, were given to three groups of animals with a variable recovery time between the doses of 0 to 24 hours (Figure 8, Table 8).

The results of the fractionated-dose experiments suggest that a maximal recovery from irradiation occurred at approximately 9 hours for the vehicle-injected and non-injected groups. A somewhat earlier maximal recovery at

Figure 8. The effect of dose fractionation on the recovery of endogenous hematopoietic stem cells of mice receiving tocopherol injection, vehicle injection or no injection immediately post-irradiation.


Mean Colony Count
10
8
6
4
2
0
0
5
10
15
20
25
Non-injected
Vehicle-injected
Tocopherol-injected

approximately 7 hours was observed in the tocopherol-injected group. There is a modification of split-dose response for both the vehicle-injected and tocopherol-injected groups as compared to the non-injected controls (Table 8). The difference between the tocopherol-injected and vehicle-injected groups is significant at the intervals of 0 hours and 7 hours (Table 8).

TABLE 8

ENDOGENOUS SPLEEN COLONY ASSAY OF SPLIT-DOSE STUDY. MICE WERE GIVEN TWO DOSES OF X-RADIATION SEPARATED BY A VARIABLE TIME INTERVAL

Interval (hours)	C		V		E	
	n	$\bar{X}$	n	$\bar{X}$	n	$\bar{X}$
0	84	0.57 ± 0.12	93	0.60 ± 0.11	88	1.12 ± 0.18***
1	27	1.26 ± 0.28	29	2.63 ± 0.56*	31	4.58 ± 0.83*
3	30	2.87 ± 0.68	29	4.65 ± 0.92	29	5.85 ± 0.96*
5	59	2.24 ± 0.39	56	3.47 ± 0.55	59	4.99 ± 0.76*
7	30	3.45 ± 0.83	30	4.70 ± 1.04	29	8.42 ± 1.33***
9	30	3.74 ± 0.82	30	6.12 ± 0.81	30	7.63 ± 1.43*
11	30	2.37 ± 0.56	30	2.50 ± 0.63	29	2.82 ± 0.62
13	29	1.34 ± 0.30	29	1.61 ± 0.36	30	2.40 ± 0.54
16	30	1.74 ± 0.46	33	4.74 ± 0.86*	27	5.15 ± 1.17*
19	30	2.79 ± 0.54	29	4.85 ± 0.78*	30	4.17 ± 0.82
24	26	1.47 ± 0.44	25	2.15 ± 0.47	29	3.19 ± 0.68*

C Non-injected.

V Vehicle-injected.

E Tocopherol-injected.

X Mean colony count ±SE.

n Sample size.

* Significantly different from non-injected group.

** Significantly different from vehicle-injected group. (t-test, Sokal and Rohlf 1969).

DISCUSSION AND CONCLUSION

This discussion will focus on the experimental results related to the modification of animal and CFU survival when tocopherol treatment is administered after radiation injury.

The results of this series of experiments suggest that the dl-α-tocopherol form of vitamin E, has a beneficial effect on animal survival when administered to mice at the doses of radiation causing bone marrow syndrome (6.1-7.1 Gy). The results also suggest that the reduced lethality may be due to the increased survival of the irradiated hematopoietic stem cell population (CFU) as shown in the increased colony counts of endogenous spleen colony assay. The higher doses required to demonstrate a tocopherol effect in the endogenous spleen colony assay (SCA) parallel the findings in the whole-animal survival experiments. In both series of experiments the tocopherol effect was pronounced at the upper radiation dose range and insignificant at the lower dose range.

Survival Study

Mice pre-treated with tocopherol have been shown to have an increased survival after total-body-irradiation

as compared to controls (Sakamoto and Sakka 1973, Srinivasan and Weiss 1981). Mice fed diets deficient in vitamin E have a greater sensitivity to radiation as measured by survival when compared to animals fed supplemented diets (Konings and Drijver 1979). These experiments have been interpreted, at least partially, on the assumption that the observed tocopherol effect is due to its antioxidant properties. Malick et al. (1978) showed that mice receiving various diets, some restricted and some supplemented in tocopherol, all responded with 100% mortality in thirty days after 8.0 Gy total-body-irradiation. However, regardless of diet, an injection of tocopherol immediately post-irradiation improved the thirty-day survival. The dose-response curves of animal lethality in the present experiments are consistent with these results and have gone further to show that a post-irradiation injection of tocopherol can improve the thirty-day survival over the dose range causing bone marrow syndrome (Figure 2). The $LD_{50/30}$ was increased significantly ($P<.01$ using t-test, Stanley 1963) from 6.6 Gy in the vehicle-injected group and non-injected group, to 7.0 Gy in the tocopherol-injected group (Table 2).

The increase in $LD_{50/30}$ obtained in this experiment with tocopherol treatment, is similar to that of Sakamoto and Sakka (1973) who injected mice with tocopherol thirty minutes prior to irradiation. It is

doubtful that this short time interval prior to irradiation was sufficient to allow and *i.p.* injection of tocopherol to be fully incorporated into the target tissues. Roy *et al.* (1982) have shown that after an *i.p.* injection of tocopherol (^{3}H labelled), up to 3 to 6 hours may be required for tocopherol uptake in the spleen, bone marrow and serum. The amount and rapidity of uptake was greatly enhanced after irradiation. Other information on tocopherol uptake and distribution after parenteral administration is scant (Machlin 1980). However, data is available on orally administered tocopherol. A peak of absorption from the gut in rats is observed at 2-4 hours followed by a second peak at 8-10 hours (Gallo-Torres 1980). Machlin and Gabriel (1982) have shown that in human plasma, the early peak appears at 5-8 hours after the ingestion of α-tocopherol. In rats, *i.v.* injection of the vitamin reaches peak uptake in the spleen at 6 hours (Gallo-Torres 1971).

These absorption data suggest that it is unlikely in the experiment of Sakamoto and Sakka (1973) that tocopherol was able to accumulate in large enough quantities in critical tissues upon being injected thirty minutes prior to the insult. The results suggest that the tocopherol effect may be effectively post-irradiation in this case.

The point of interest in previous experiments of

pre-administration of tocopherol and those of post-administration presented here in this study is the reduced animal lethality in both cases.

Endogenous Spleen Colony Assay

The endogenous spleen colony assay (SCA) results suggest that at least part of the increased $LD_{50/30}$ in the tocopherol-treated group may be due to an increase in the survival of endogenous colony forming units (CFU) lodging in the spleen. An alternative explanation which will be discussed later considers the possibility that vitamin E improves the microenvironment of the irradiated spleen thereby increasing its efficiency in enabling the seeding of CFU within its tissue.

The major cause of death at these doses (5.8-6.9 Gy), as described in the introduction, is a failure of the hematopoietic system. The animal dies of severe multiple infections and anemia. Not only are mature blood cells damaged, but so are the non-committed progenitor cells (ie. CFU) which would normally give rise to a new population of hematopoietic cells. An increase in colony number would suggest that a greater fraction of CFU are surviving the radiation insult and are capable of forming erythrocytic and granulocytic colonies on the spleen. In the endogenous SCA, such an increase was found in the tocopherol-injected group receiving the vitamin

immediately following irradiation (Figure 3). Due to the time of injection, tocopherol would have been present during the recovery period of the irradiated CFU population (see discussion of split-dose assay) estimated for the CD-1 female mouse at 7-9 hours (Figure 8). This is probably still true when one considers the time loss for uptake of tocopherol. The mechanism involved in the increased CFU survival is unknown. Either by direct, or indirect means tocopherol has increased the number of CFU yielding spleen colonies (Figure 3). When the injection of the vitamin is delayed to five hours post-irradiation, after the recovery peak (considering time for uptake), no effect is observed on endogenous colony counts (Figure 4). These results suggest that there is some repair, or recovery modification by tocopherol.

Two established criteria often interpreted as establishing the degree of recovery from radiation damage are the Dq of the exogenous SCA and the split-dose effect. These two tests and the significance of their results will be discussed later.

The slope of the survival curves of CFU in the endogenous spleen colony assay were in the range of -0.5 to -0.6 Gy^{-1} , or Do = 0.54 to 0.77 Gy (Table 3). These results are similar to those of Ainsworth and Larsen (1969); Do = 0.66 Gy and Smith *et al.* (1966); slope = -0.56 Gy^{-1} . Some authors have reported higher Do

values than those presented here for endogenous CFU. Till and McCulloch (1963) reported a Do of 0.95 Gy. These variations are not completely understood but may in part be due to the rate at which dose is administered. Puro and Clark (1972) have demonstrated a change in slope, and hence in Do and extrapolation number n with varying dose rates. Dose rates of 0.03 to 1.05 Gy/min were given to groups of mice. The authors report a changing Do, or slope, with a change in the dose rate from 0.94 to 0.74 Gy min^{-1} respectively. The dose rate used in all my experiments was approximately 0.30 + 0.02 Gy/min . Another explanation for the discrepancy existing in the values of slopes of endogenous SCA may arise from the use of different mouse strains. A literature search did not reveal any endogenous SCA data for CD-1 mice. Although CD-1 mice have been used for CFU studies (Monette and Demers 1982), no dose response curves for endogenous CFU were presented.

Exogenous Spleen Colony Assay

The general form of the dose-response curves of the exogenous SCA for the transplanted bone marrow cell agrees well with data presented by other authors (Till and McCulloch 1963, Puro and Clark 1972). Although the slopes and extrapolation number (n) of bone marrow CFU are well documented, less data is available for transplanted spleen

CFU. Reports that are available suggest that spleen CFU may respond similarly to the bone marrow CFU (Siminovitch et al. 1965, Guzman and Lajtha 1970, Schofield 1970) with respect to the exponential part of the curve, Do being close to 1.0 Gy in both cases. The extrapolation number however is larger for the bone marrow cells (1.5 compared to that of spleen cells (0.8-1.0). The present results are consistent with the published data (Table 5). The Do for both spleen and bone marrow CFU in the present study is approximately 0.9-1.0 Gy. The extrapolation number for spleen is lower than that of bone marrow, n = 1.0 and n = 1.5 respectively for groups receiving no further treatment after the i.v. transplant and subsequent irradiation.

Extrapolating the exponential part of the curve back to zero dose yields the extrapolation number (n) where the curve intersects the ordinate (Figure 6, 7). The extrapolation number is thought to indicate an accumulation of repairable sublethal damage (Elkind and Sutton 1960, Alper 1979). An increase in the shoulder of such curves results in a larger n, hence a greater capacity for the cell to accept sublethal damage. It was though the influence of tocopherol on irradiated CFU may result from an increased capacity to accept and repair sublethal damage. This would appear as an extended shoulder in the survival curves of CFU in the tocopherol-

treated groups. However, as illustrated in Figures 6, 7 and Table 5 this is not the case. The shoulder in the case of the bone marrow survival curves was not modified by tocopherol, and where none existed in the control spleen CFU survival curves, none appeared in treated groups.

An interesting comparison may be made between the spleen and bone marrow CFU. Whereas the Do are similar for the two cell types, no shoulder appeared in the spleen derived cells. This would suggest that the hematopoietic stem cells originating from the spleen have a reduced capacity at repairing or accepting damage at the low dose region. The final slopes however are similar suggesting that repair capacity in the two cell types, as measured by radiosensitivity is similar at the higher dose range.

The finding that shoulder width is not increased in the case of bone marrow stem cells, nor is one induced in stem cells originating from the spleen, suggests that the observed tocopherol effect in reducing animal lethality is not due to a stimulation of repair at the low dose region. This is consistent with the results obtained from the thirty-day survival experiments (Figure 2, Table 1) and the endogenous spleen colony experiments (Figure 3). The effect of tocopherol appears to be negligible at the lower radiation dose range where the cell's capacity for repair is presumably more or less intact. The tocopherol effect appears to be at the higher

dose range where repair is diminished. Examination of the survival curves of CFU originating from bone marrow (Figure 7) suggests a probable slope change in the two control curves at doses greater than 4.3 Gy. There is a significant difference between mean colony counts of tocopherol-treated groups and control as well as non-injected groups at the higher dose range for bone marrow CFU (Table 6). Whereas the slopes of the non-injected and vehicle-injected groups appear to become more steep at doses greater than 4.3 Gy, that of tocopherol remains constant. The reason for the slope change is unknown. There may be a breakdown in the repair capacity of the cell at the higher dose range (Alper 1979). It has been suggested that such survival curves are actually not straight but curvilinear approaching linearity (Alper 1977), the slope increasing with increasing dose. It is conceivable that tocopherol is acting to stimulate or to protect the repair capacity of the cell. If the effect is linked to repair, I would suggest that the slope of the tocopherol-supplemented group would remain approximately linear up to a certain dose beyond which damaging events would override the protective effect of tocopherol. At this point, the slope of the vitamin E group would also increase as did the vehicle-injected and non-injected groups.

Doses could not be increased beyond the range of

3.4 Gy in the spleen CFU study as the number of cells required to achieve sufficient colony number on the spleen was so large that most animals died within minutes after being injected. As fewer cells were required to achieve sufficient colonies in the bone marrow groups, 10 CFU/10^5 cells in bone marrow (Appendix, Tables 12-17), and 1.5 CFU/10^5 cells in the spleen (Appendix, Tables 9-11), higher doses could be used. The largest dose that could be administered with the present protocol is 5.0 Gy for bone marrow cells. A larger animal model may prove more practical for evaluating CFU survival at higher doses. Rats have been used for CFU studies (Comas and Byrd 1967, Comas 1968). A large animal would not only yield a greater number of CFU as a donor, but would also be able to accept a larger transplant as a recipient. This would enable higher dose ranges to be used.

Spleen Microenvironment

Some authors have suggested that colony formation in the spleen can be increased by various agents that enhance the implantation of CFU due to an improved spleen microenvironment (Mori 1973,Mori and Kitamura 1976, Roszkowski *et al.* 1980).

Tocopherol was found to increase the number of colonies appearing in the spleen in the endogenous SCA (Figure 3) when administered immediately post-irradiation.

This may be due to an increase in the ability of the spleen to accept CFU in its tissue due to an improvement in its microenvironment. To test this hypothesis, samples of bone marrow or spleen cells were removed from healthy donor mice and injected into lethally irradiated recipients 24 hours after irradiation. Recipient mice were injected with tocopherol, vehicle, or were not injected immediately after the transplant. The seeding efficiency (CFU/10^6 cells) was similar within the three groups. (Table 7). This is consistent with the results of Roy et al. (1982) who showed no change in seeding efficiency in lethally irradiated mice receiving tocopherol immediately post-irradiation and the bone marrow transplant 20-24 hours later. Therefore, it does not seem probable that increases observed in the endogenous spleen colony counts were simply due to an improved microenvironment.

Split-Dose Assay

The kinetics of the 'Elkind-type' recovery established in the curves of the split-dose experiments generally agree with those published by other authors (Till and McCulloch 1963). The first dose given in the split-dose assay serves to damage the hematopoietic stem cells and initiate recovery. A certain fraction of CFU will have lost their proliferative capacity, while another fraction will have received a sublethal dose. The

sublethally-irradiated group, if given sufficient time, can completely repair the damage inflicted by the first dose (Elkind and Sutton 1960, Till and McCulloch 1963, Alper 1977, 1979). The induction of sublethal damage gives rise to the phenomenon of 'Elkind-type' recovery seen as the reappearance of the shoulder in dose response curves. One can ascertain whether a population of cells having received a dose of X-radiation has completed recovery by giving a second dose of radiation at various time intervals after the first dose. If the surviving population has in fact completed recovery, the original dose-response curve of the surviving, fully recovered, irradiated population will yield a curve with a shoulder and slope identical to that of the original unirradiated population (Elkind and Sutton 1960, Alper 1979).

The kinetics of recovery after a first dose follow well established patterns (Elkind and Sutton 1960, Till and McCulloch 1963, Alper 1977). In the present split-dose assay, a maximum is reached at approximately 7 to 9 hours followed by a minimum at 13 hours for CFU irradiated endogenously of CD-1 mice receiving two fractions of 3.4 Gy (Figure 8). The results are consistent with those of Till and McCulloch (1963) who have shown for C57B1/Ha and C3H/Ha mice receiving two doses of radiation, 3.0 - 4.0 Gy, the maximum recovery appears at 5 hours followed by a minimum at 11 hours. Fractionation recovery curves have

also been obtained from other cell types (Elkind and Sutton 1960, Hornsey and Silini 1962) with similar results.

A comparison of the three curves (Figure 8) of the split-dose assay suggests that the three groups of CFU may not be equally sensitive to the two doses of 3.4 Gy. This is indicated by the generally higher yield of colonies in the tocopherol-injected groups and the vehicle-injected groups as compared to the non-injected groups. The difference between mean colony counts at the various time intervals with respect to tocopherol-injected and non-injected groups is significant at 0, 1, 3, 5, 7, 9, 16 and 24 hours (Table 8). The vehicle shows some protective effects at the 1, 16 and 19-hour intervals. Although tocopherol-treated groups appear to have an increased number of proliferating CFU compared to the non-injected controls, the magnitude of the difference is not significant when tocopherol groups are compared with those of the vehicle-injected groups. The two exceptions are at 0 and 7 hour intervals. Tocopherol administration appears to increase the recovery peak in the region of maximum repair.

The exact interpretation of split-dose survival curves is not completely agreed upon. Sinclair and Morton (1964) interpret the maximum survival as being due to a combination of cell recovery and cell division kinetics.

Their reasoning is based on results of synchronized Chinese hamster cells growing in culture. These cells were found to have differing radiosensitivities depending on the phase of the cell cycle. Cells in S phase were far more radioresistant compared to those of G phase. The first dose of radiation of an asynchronous population, for example asynchronous CFU, will preferentially destroy cells in the more radiosensitive phase of the cell cycle. The population of cells is then somewhat synchronized. The second dose of radiation may be delivered to the remaining fraction of the cells at the more resistant part of the cycle resulting in an apparent reduced effect of radiation damage. This may be interpreted as cell recovery (repair) where none existed.

Elkind (1967), however, suggested that the first dose of radiation arrests cell division and results in the initiation of repair. At this point the cells in the more sensitive phase of the mitotic cycle have been destroyed, while those surviving are partially synchronized in the more radioresistant phase of the cycle. At some point after recovery is complete, (interpreted first as repair by Elkind and Sutton 1960, Elkind 1967) the synchronized population reenters division. This portion of the split-dose curve is characterized by the peak appearing at 7-9 hour interval in the present experiment (Figure 8). A second dose of radiation after the recovery period will

strike the cell cohort in a more sensitive phase of the cell cycle characterized by a reduction in mean colony counts. The cell population reaches a maximum sensitivity then passes through the cycle to more sensitive phases as illustrated by the minimum appearing at 13 hours (Figure 8). At this point, the population becomes less and less synchronized and eventually reaches a plateau of general response of an asynchronous population of cells. Evidence for this hypothesis was put forth by Elkind (1967). Cultures of Chinese hamster cells were kept at 37 °C or 24 °C. Dose-fractionation experiments were carried out at both temperatures. Both cell populations showed maximal recovery at two hours. The cells kept at 37 °C, capable of normal division, went through the normal kinetics of split-dose recovery. The cultures stored at 24 °C, incapable of cell division, reached a plateau at two hours. This is taken as strong evidence that the initial increase is due to cell recovery whereas the decrease appearing later is largely a phenomenon of cell division.

Vitamin E has been shown to increase cell proliferation in the spleen (Corwin et al. 1981, Corwin and Gordon 1982) and of smooth muscle cells in culture (Corwell et al. 1979, Miller et al. 1980). At present no studies have been published on the effect of tocopherol on cell division in hematopoietic colony forming units.

Results of the present experiments suggest that division is taking place in the tocopherol-injected group prior to that in the vehicle-injected or non-injected groups. The peak mean colony counts for tocopherol groups in the dose-fractionation study are at approximately 7 hours and 16 hours. The peaks were at 9 hours and 19 hours respectively for both the vehicle-injected and non-injected groups (Figure 8). This suggests that there is a probable decrease in the time required for recovery and initiation of cell division after the first dose of 3.4 Gy in the fractionated-dose assay. Due to the loss of synchrony, the split-dose response was only studied for 24 hours. If CFU could be induced to divide at a greater rate than controls, one would expect a greater number of stem cells (CFU) to be available for spleen colony production.

The mechanism of action by which tocopherol improves whole animal and CFU survival when administered post-irradiation is obsure. A large variety of data is available suggesting an antioxidant effect of tocopherol when present in the cell at the time of radical production (Bieri and Farrell 1976, Konings et al. 1979, Horwitt 1980, Konings and Osterloo 1980, Dougherty 1981, Gavino et al. 1981, McCay et al. 1982). A mechanism for the protection by tocopherol was suggested by Packer et al. (1979). According to this model, a radical may be reduced by Vitamin E resulting in the production of a

Vitamin E radical. This newly formed radical may then be reduced by vitamin C which in turn is reduced by NADH. The process would result in the regeneration of the antioxidant capacity of vitamin E.

A requisite for the antioxidant theory of tocopherol action is its presence at the time of radical production. Oxidizing radicals, and excited molecules, once produced, last from 10^{-14} to 10^{-3} sec (Coggle 1971). To have an effect, an antioxidant must be present during this short time period. Addition of an antioxidant shortly after radical production would not be expected to show any radical scavenging or neutralizing effect. In the present experiments tocopherol was added post-irradiation, hence post radical production and radical degeneration. However, an ameliorating effect was still observed.

Some authors have suggested post-irradiation hypoxia, brought about by decreased oxygen content in blood may be radioprotective (Whitfield and Brohee 1965, Rixon and Baird 1968). Rixon and Baird (1968) have shown that injections of serotonin after total body irradiation of rats, decreased animal lethality. The authors suggested that hypoxia induced by serotonin resulted in a reduced rate of cellular respiration which may in turn delay the manifestation of the radiation lesion. This delay may allow the cell's repair capacity to repair the damage before it is expressed.

/Sakamato and Sakka (1973) suggest that radioprotective effects of vitamin E may be due to an ability to reduce oxygen tension in the blood and tissues. Studies on oxygenation of tumor cells however have not shown any tocopherol effects on oxygenation in irradiated or un-irradiated tumors (Kagerud *et al.* 1978, 1981 a, 1981 b). Experiments on lymphoma cell survival after X-irradiation (Fonck and Konings 1978) have shown that tocopherol-supplemented cultures have the same slope of the survival curves as the non-supplemented cultures (Do = 1.0 ± 0.1 Gy). For supplemented or non-supplemented hypoxic cultures, the Do was 2.2 ± 0.25 Gy. If tocopherol was inducing hypoxia, the slope of the survival curves of the supplemented groups would be intermediate between the aerated control and the hypoxic cells. An interesting observation in these results was the appearance of a shoulder in the survival curves of the aerated or hypoxic cells receiving tocopherol supplementation. There was no shoulder in the non-supplemented groups. This suggests an increased capacity for repairing sub-lethal damage in the lymphoma cells as a result of tocopherol treatment.

Other compounds offer protection when administered post radical production. Injection of leukotrophin (cell-free thymus extract) post-irradiation has been found to increase endogenous colony counts and increase $LD_{50/30}$ in total-body-irradiated mice (Uray *et al.* 1980). The

authors also report an increase in the uptake of ^{3}H-thymidine in bone marrow and ^{59}Fe uptake in bone marrow and spleen. The authors suggest that the observed radioprotective effect may be due to an enhanced lymphopoieses and erythropoiesis. Takeda *et al.* (1981) report an increased survival in animals receiving an injection of purified ginseng extract 2.5 hours post-irradiation. They also observed an apparent increase in myelopoiesis and erythropoiesis. Studies with tocopherol injection post-irradiation suggest that the vitamin may not increase erythropoiesis. Roy *et al.* (1982) report an injection of tocopherol post-irradiation did not increase Fe uptake in the spleen or in bone marrow.

The enzyme superoxide dismutase (SOD) is known for its protective properties against the O_2^- anion. Injection of the enzyme post-irradiation to mice increased the $LD_{50/30}$ (Petkau *et al.* 1976). After irradiation, the levels of SOD fell and reached a minimum at 3-5 days. The levels returned to normal at day 7 (Petkau 1978). The superoxide anion, however, is not only produced as a product of irradiation but is also generated through the cell's metabolic processes. An injection of exogenous bovine SOD helped protect the cell against further damage from O_2^- production until the endogenous SOD concentration reached normality at day 7.

Antioxidant levels have been shown to decrease

after acute radiation exposure (Glavind et al. 1965, Dawes and Wills 1972, Wills 1980). Radical production from normal metabolic processes continues even in the absence of exogenous agents (Copeland 1978, Rosen et al. 1981, Pryor 1982). It is therefore possible that the administration of tocopherol post-X-irradiation results in the replenishing of depleted antioxidants and an arrest of further endogenous radical damage. If this were the case, other antioxidants administered post-irradiation would be expected to demonstrate some degree of radioprotection. However, Ben-Hur et al. (1981) have shown that no radioprotective effects are observed upon the addition of the antioxidants BHT,BHA and PG (propyl gallate) post-irradiation of Salmonella typhimurium. However, addition of the antioxidants prior to irradiation did show protection.

Administration of cysteamine 15 minutes post-irradiation protects liver cells from chromosomal aberrations (Gil'Yano and Malinovskii 1979). The authors suggest this may be due to an activation of the repair system, perhaps associated with repair of potentially-lethal damage in the form of chromosomal aberrations. Some data is available on the influence of tocopherol on chromosome damage. Patterson et al. (1981) reported a reduction in dimethyl sulfoxide (DMSO) induced chromosomal alternations in Chinese hamster ovary cells when

supplemented with Vitamine E. Alekperov and Akhundova (1974) showed a decrease in spontaneous and radiation induced chromosome aberrations by tocopherol administration prior to germination of Allium fistulosum (onion) and Vicia faba (broad bean). Tocopherol has also been shown to decrease chromosomal aberrations induced by potassium bichromate in bone marrow cells in the rat (Bigalieo and Elemesova 1978). Recently, Kalinina et al. (1979) reported tocopherol to be effective in reducing the mutation rate in two strains of the bacterium Salmonella TA 1535 and TA 1950, caused by N-nitroso-N-methylmonourea (NMU). Tocopherol was effective if administered to the cultures at 14 days, 7 days or 1 day prior to NMU. Interestingly, addition of tocopherol post-NMU also resulted in a decreased yield of mutations. This decrease was not due to tocopherol's direct action on NMU in the culture. Before addition of tocopherol, the cells were washed free of the mutagen. This suggests that vitamin E is capable of reducing chromosome damage, perhaps by stimulation of repair, even when added after the damage, or initiation of the damage, has taken place.

The observed effects of tocopherol in this study cannot be readily explained by its established properties as a radical scavenger during radiation stress (Sakamoto and Sakka 1973, Konings and Drivjer 1979, Srinivasan and Weiss 1981). The evidence of increased recovery in the

tocopherol-supplemented group of the split-dose assay, as well as the increased number of endogenous colonies in mice injected with tocopherol immediately after irradiation, suggest the observed tocopherol effects are mediated during the recovery period of the hematopoietic stem cell. This is further confirmed by the lack of tocopherol effect in increasing endogenous CFU when the vitamin is administered after the recovery period. The results of the endogenous SCA and the split-dose assay are consistent with reports of possible tocopherol involvement in the modification of cellular repair (Fonck and Konings 1978, Malick *et al.* 1978, Kalinina *et al.* 1981, Roy *et al.* 1982). The lack of increased shoulder, however, in the exogenous SCA of bone marrow derived and spleen derived hematopoietic CFU, suggest that tocopherol does not modify sublethal repair, or low level radiation damage of hematopoietic stem cells. This is consistent with the lack of increased exogenous and endogenous CFU, and the lack of increased animal survival, in the lower radiation dose-range of bone marrow syndrome. The post-radiation tocopherol effect appears to be mediated at the higher dose-range. Whether this effect is indeed related to modification of cellular repair requires further experimentation.

APPENDIX

TABLE 9

SURVIVAL OF EXOGENOUS HEMATOPOIETIC COLONY FORMING UNITS (CFU) ORIGINATING FROM THE SPLEEN OF DONOR MICE AND TRANSPLANTED INTO LETHALLY IRRADIATED RECIPIENT MICE. CFU WERE IRRADIATED IN VIVO IN THE RECIPIENTS.[C]

Dose (Gy)	0	0.22	0.43	0.86	1.29	2.58	3.44
# Cells inj X 10^5	3.0	3.3	3.3	4.2	5.1	17.1	56.8
Sample size	13	14	13	16	15	9	15
Mean colony count ± SD	6.7±2.8	6.1±2.4	6.2±2.5	4.6±2.6	4.0±2.0	3.8±2.1	5.2±2.4
CFU/10^6 cells	22.3	18.4	18.9	11.0	7.8	2.2	0.92
Surviving fraction X 10^{-2}	100	82.5	84.8	49.3	35.0	9.9	4.1

C Mice received no further treatment after irradiation.

TABLE 10

SURVIVAL OF EXOGENOUS HEMATOPOIETIC COLONY FORMING UNITS (CFU) ORIGINATING FROM THE SPLEEN OF DONOR MICE AND TRANSPLANTED INTO LETHALLY IRRADIATED RECIPIENT MICE. CFU WERE IRRADIATED IN VIVO IN THE RECIPIENTS.[V]

Dose (Gy)	0	0.22	0.43	0.86	0	1.29	2.58	3.44
B Cells inj X 10^5	5.0	6.6	6.6	8.3	5.0	10.0	34.0	112.0
Sample size	13	12	9	10	15	12	12	12
Mean colony count ± SD	6.9±2.8	7.5±2.8	5.2±1.3	5.7±3.4	7.0±4.2	4.2±2.2	4.6±1.6	5.2±3.8
CFU/10^6 cells	13.8	11.4	7.9	6.9	15.6	4.2	1.4	0.46
Surviving fraction X 10^{-2}	100	82.6	57.3	50.0	100	26.9	9.0	3.0

V Recipients were injected with the vehicle post-irradiation.

TABLE 11

SURVIVAL OF EXOGENOUS HEMATOPOIETIC COLONY FORMING UNITS (CFU) ORIGINATING FROM THE SPLEEN OF DONOR MICE AND TRANSPLANTED INTO LETHALLY IRRADIATED RECIPIENTS. CFU WERE IRRADIATED IN VIVO IN THE RECIPIENTS.[e]

Dose (Gy)	0	0.22	0.43	0.86	0	1.29	2.58	3.44
# Cells inj X 10^5	5.0	6.6	6.6	8.3	5.0	10.0	34.0	112.0
Sample size	7	13	10	12	13	9	9	10
Mean colony count ± SD	6.9±2.4	8.4±2.8	6.4±3.3	5.8±2.4	7.9±4.6	4.4±1.3	4.3±3.3	5.8±2.5
CFU/10 cells	13.7	12.7	9.7	6.0	15.8	4.4	1.3	0.52
Surviving fraction X 10^{-2}	100	92.7	70.8	43.8	100	27.8	8.2	3.3

e Recipients were injected with tocopherol post-irradiation.

TABLE 12

SURVIVAL OF EXOGENOUS HEMATOPOIETIC COLONY FORMING UNITS (CFU) ORIGINATING FROM THE BONE MARROW OF DONOR MICE AND TRANSPLANTED INTO LETHALLY IRRADIATED RECIPIENT MICE. CFU WERE IRRADIATED IN VIVO IN THE RECIPIENTS.[C]

Dose (Gy)	0	0.22	0.43	0.86	1.29	2.58	3.44
# Cells inj X 10^5	1.9	2.1	2.1	2.6	3.2	10.8	36.0
Sample size	14	14	14	14	10	11	13
Mean colony count ± SD	14.9±4.5	15.9±3.9	14.0±3.5	12.4±4.2	11.5±2.8	11.2±3.6	11.8±5.4
CFU/10^6 cells	78.2	75.5	66.7	47.3	35.7	10.4	3.3
Surviving fraction X 10^{-2}	100	96.6	85.3	60.4	45.7	13.3	4.2

C Mice received no further treatment after irradiation.

TABLE 13

SURVIVAL OF EXOGENOUS HEMATOPOIETIC COLONY FORMING UNITS (CFU) ORIGINATING FROM THE BONE MARROW OF DONOR MICE AND TRANSPLANTED INTO LETHALLY IRRADIATED RECIPIENT MICE. CFU WERE IRRADIATED IN VIVO IN THE RECIPIENTS.[C]

Dose (Gy)	4.3		4.7		5.0	
	Control	Irradiated	Control	Irradiated	Control	Irradiated
# Cells inj X 10^5	2.0	60.0	0.87	125.0	2.2	97.0
Sample size	11	8	11	9	12	6
Mean colony count	17.7±5.1	12.4±4.4	11.4±1.6	11.7±5.5	16.7±6.6	2.8±2.3
CFU/10^6 cells	88.7	2.1	130.9	0.93	75.9	0.29
Surviving fraction X 10^{-2}	100	2.3	100	0.71	100	0.37

C Mice received no further treatment after irradiation.

TABLE 14

SURVIVAL OF EXOGENOUS HEMATOPOIETIC COLONY FORMING UNITS (CFU) ORIGINATING FROM THE BONE MARROW OF DONOR MICE AND TRANSPLANTED INTO LETHALLY IRRADIATED RECIPIENT MICE. CFU WERE IRRADIATED IN VIVO IN THE RECIPIENTS.[v]

Dose (Gy)	0	0.22	0.43	0.86	1.29	2.58	3.44
# Cells inj X 10^5	1.9	2.1	2.1	2.6	3.2	10.8	36.8
Sample size	12	13	15	11	12	13	12
Mean colony count ± SD	17.6±5.8	18.3±6.7	15.7±4.3	17.2±3.1	14.9±3.1	13.9±4.4	12.8±5.0
CFU/10^6 cells	92.8	87.2	74.8	65.3	46.3	12.9	3.5
Surviving fraction X 10^{-2}	100	94.0	80.6	70.4	50.0	13.9	3.8

v Recipients were injected with the vehicle post-irradiation.

TABLE 15

SURVIVAL OF EXOGENOUS HEMATOPOIETIC COLONY FORMING UNITS (CFU) ORIGINATING FROM THE BONE MARROW OF DONOR MICE AND TRANSPLANTED INTO LETHALLY IRRADIATED RECIPIENT MICE. CFU WERE IRRADIATED IN VIVO IN THE RECIPIENTS.[v]

Dose (Gy)	3.9		4.3		4.7		5.0	
	Control/Irradiated		Control/Irradiated		Control/Irradiated		Control/Irradiated	
# Cells inj X 10^5	2.0	30.0	2.0	60.0	0.87	125	2.2	97.0
Sample size	8	7	8	11	7	9	15	11
Mean colony count	19.2±8.1	7.3±3.6	19.2±8.1	7.7±3.4	11.3±3.0	5.4±5.8	14.6±5.4	1.8±1.8
CFU/10^6 cells	96.3	2.4	96.3	1.3	130.0	1.2	66.4	0.19
Surviving fraction X 10^{-2}	100	2.5	100	1.3	100	0.95	100	0.29

v Recipients were injected with the vehicle post-irradiation.

TABLE 16

SURVIVAL OF EXOGENOUS HEMATOPOIETIC COLONY FORMING UNITS (CFU) ORIGINATING FROM THE BONE MARROW OF DONOR MICE AND TRANSPLANTED INTO LETHALLY IRRADIATED RECIPIENT MICE. CFU WERE IRRADIATED IN VIVO IN THE RECIPIENTS.[e]

Dose (Gy)	0	0.22	0.43	0.86	1.29	2.58
# Cells inj X 10^5	2.5	2.8	2.8	3.5	4.3	14.4
Sample size	12	13	12	11	15	15
Mean colony count ± SD	20.8±4.8	19.3±3.8	19.7±2.0	18.3±4.9	18.8±3.9	16.7±3.4
CFU/10^6 cells	80.0	68.9	70.4	52.2	41.9	11.6
Surviving fraction X 10^{-2}	100	86.1	88.0	65.2	52.3	14.5

e Recipients were injected with tocopherol post-irradiation.

TABLE 17

SURVIVAL OF EXOGENOUS HEMATOPOIETIC COLONY FORMING UNITS (CFU) ORIGINATING FROM THE BONE MARROW OF DONOR MICE AND TRANSPLANTED INTO LETHALLY IRRADIATED RECIPIENT MICE. CFU WERE IRRADIATED IN VIVO IN THE RECIPIENTS.[e]

Dose (6y)	3.4		4.3		4.7		5.0	
	Control/Irradiated		Control/Irradiated		Control/Irradiated		Control/Irradiated	
# Cells inj X 10^5	1.9	36.0	2.0	60.0	0.87	125	2.2	97.0
Sample size	12	8	9	12	12	10	12	11
Mean colony count ± SD	16.7±4.4	5.3±6.3	18.0±6.3	13.8±5.3	11.2±2.7	20.1±4.6	3.9±4.8	6.7±4.2
CFU/10^6 cells	87.7	4.2	90.0	2.3	129.6	1.6	63.2	0.69
Surviving fraction X 10^{-2}	100	4.8	100	2.6	100	1.2	100	1.1

e Recipients were injected with tocopherol post-irradiation.

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