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THE EFFECT OF SIMULTANEOUS ADMINISTRATION OF TOCOPHEROL AND SUNFLOWER OIL ON THE LIFE-SPAN OF FEMALE MICE

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INTRODUCTION

THE FREE radical theory of ageing has been known for a number of years. It has been demonstrated that free radicals shortened the life-span of experimental animals, but that antioxidants led to an increase in survival. Harman (1969) found that 2-mercaptoethylamine increased substantially the mean life-span of rats fed a powdered commercial base diet, whereas the life-span was less increased in the case of those treated with butylated hydroxytoluene. The anti-oxidative effect of tocopherol has been investigated; a survey was reported by Barber and Bernheim (1967).

The effect of free radicals is undoubtedly connected with the metabolism of lipids. Highly unsaturated free fatty acids in the diet readily undergo peroxidation, free radicals are generated, and thus, malondialdehyde occurs as a secondary product of lipoperoxidation. Malondialdehyde has been shown to alter mitochondrial membranes (Horton and Packer, 1970), nucleic acids and some proteins (myosin, bovine serum albumin, ribonuclease, cytochrome *c*). In connective tissue, it enhances the formation of desmosine cross-links in elastin, if the maturation is influenced during the intrauterine life (Ledvina *et al.*, 1975); collagen is altered as well (Švadlenka *et al.*, 1973).

Fatty diet in experimental animals leads to results which contradict to a certain degree the commonly accepted recommendation for the composition of human dietary lipids; Harman (1969) found the maximum life-span in rats fed with lard (with a very low content of polyunsaturated fatty acids), but the shortest life-span was demonstrated in the rats fed with olive oil. In accordance with this observation Harman *et al.* (1976) and Eddy and Harman (1977) found that the peroxidation of dietary fat supplemented with highly unsaturated fatty acids (e.g. 22:6ω3-docosahexanoic acid) had an adverse effect on the function of the central nervous system in the rat. The authors suppose that this effect of dietary fat may be produced, at least in part, by enhancing the level of more or less random free-radical reactions throughout the brain. The result of Kohn (1971) indicate that neither butylated hydroxytoluene nor 2-mercaptoethylamine alter the 50% survival time in the long-lived strain C57BL/6J of mice, and only in the case where survival was suboptimal was a certain insignificant lengthening of the life-span achieved. Harman (1971) observed that the mean life-span in C3H female mice markedly decreased with the amount or degree of unsaturation of dietary fats. The decrease in the rats and in other mouse strains was insignificant.

Free radicals presumably play an important role in the mechanism of the effect of ionizing radiation, and are connected with oxygen toxicity. An increased amount of free radicals is associated with tocopherol deficiency (Zalkin and Tappel, 1960), and it influences the maturation of connective tissue (Greenwald and Moy, 1979).

The aim of our experiments was to ascertain how the administration of tocopherol might influence the life-span of female mice saturated sufficiently throughout their entire life-span by the permanent addition of sunflower oil with a high iodine number. The results were compared with a group of mice of the same strain fed the commercial base diet supplemented only with sunflower oil; a control group were given the commercial diet only.

METHODS AND MATERIALS

Animals

Females of the mouse strain C3 obtained from the breeding center at Třebeš were used. They were kept five per cage (43 × 28 × 25 cm clear plastic cages with stainless steel tops) at a temperature between 21 and 23°C. Food and tap water were provided *ad libitum*. Actual food consumption is given below (Fig. 1). The body weight curves in the individual groups are also illustrated in Fig. 1. The mice were weighed, counted, and the presence of any tumor recorded.

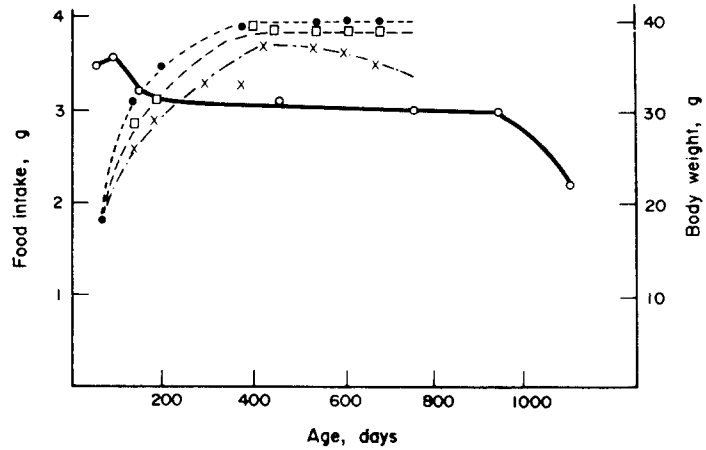

FIG. 1. Food intake and body weight of control and experimental mice. ○ = food intake. Body weights: × = tocopherol + sunflower oil; □ = sunflower oil; ● = controls.

Arrangement of the experiments

Young mice weighing approx. 16 g at the age of 46 days were divided into three groups:

Experiment* I—*addition of tocopherol and sunflower oil. Twenty-seven mice were used in the experiment. The usual commercial pelleted diet (Velaz, Lysá n.L.) was supplemented with α-tocopherol dissolved in sunflower oil. Sixty-seven and a half grams of the pellets were moistened with water, and α-tocopherol solution, soaked thoroughly into them by means of a syringe, was prepared as follows: 300 mg of tocopherol acetate in 1 ml of sunflower oil per injection (Erevit *forte pro injectione*, Spofa, Prague) were diluted by 1 ml of ether and then by 1 ml of ethanol. The pellets were air-dried and used on the following day and later on. The prepared diet was stored at 4°C for 3–4 days, in the last phase of the experiments not more than 14 days.

Thus, 1 g of the prepared pellets contained 4.4 mg of tocopherol acetate and 13.63 mg of sunflower oil. The iodine number of the sunflower oil was 125–136, spec. gravity 0.917–0.924, saponification number 188–194, peroxide number max. 15, and acidity number max. 0.3.

Experiment II—*addition of sunflower oil only.* The same number of mice (27) were taken into the experiment. The pellets were saturated with sunflower oil (*Oleum helianthi*, Pharmacopea Bohemoslov. 3) by the method used in Experiment I. Sixty-seven and a half grams of the pellets were supplemented by 1 ml of the oil (= 0.92 g). The iodine number of these preparations determined by means of the Hanuš iodine bromide volumetric method (Winton and Winton, 1947) was 127–136; therefore the values were comparable with those in the Erevit injections. No attempts were made to prevent tocopherol deficiency.

Controls. Twenty-five mice were fed only commercial pellets without any additions.

RESULTS

Food consumption of the mice varied with age, as shown in Fig. 1. The maximum intake was seen on the 90th day; from the 500th day onwards constant values were observed (about 3.1 g/day/mouse) for a long period. In very old mice, the consumption was substantially reduced. There were no significant differences in the food consumption between the control and experimental animals.

From this curve, a calculation of actual drug amount supplemented to the diet can be made. Thus, in the period between the 46th and 130th day, average amounts of 15.4 mg of tocopherol and 38.25 mg of sunflower oil were consumed per day in Experiment I and 46.55 mg of sunflower oil in Experiment II respectively. In later periods (after the 130th day) the corresponding amounts were lower (13.6 + 33.64 mg in Experiment I and 41.23 mg in Experiment II).

The body weight curves of the experimental and control mice are also demonstrated in Fig. 1. The differences observed between the body weight curves are slight. Even at the time intervals where the difference was most marked no significant difference among them was calculated.

Figure 2 shows survival of the mice of groups I and II, and of the control group in percentage calculated from the initial number. The differences between the curves were evaluated by statistical methods.

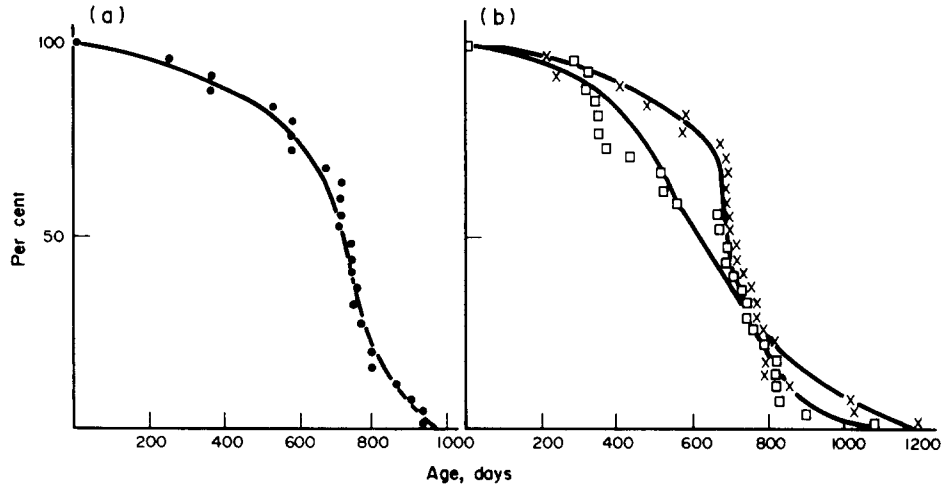

FIG. 2. Per cent of surviving mice. a = control group, b = diet supplemented with tocopherol + sunflower oil (×, Exp. I), with sunflower oil only (□, Exp. II).

Mean life-span of group I (tocopherol + sunflower oil) was 704.2 ± 209.1 days (± 1 S.D.), maximum life-span 1200 days. Group II (supplemented with sunflower oil only) revealed a mean value of 620.4 ± 217.2 days and a maximum value of 1078 days. Mean life-span in the control group was 690.4 ± 168.1 days, maximum life-span 933 days. Comparing the values given here by means of the Student test, no significant differences were detectable: group I: group II—$t = 1.417$ ($p > 0.10$); control group: group II—$t = 1.300$ ($p > 0.10$), control group: group I—$t = 1.365$ ($p > 0.10$). The results remained unchanged if the median was used instead of the mean value.

A few premature deaths in the control and experimental groups were observed. To differentiate premature deaths due to morbidity from senescence-related deaths, only the last 20 surviving mice of each group were evaluated (as in the paper of LaBella and Vivian, 1978). Of course, the mean values were then higher—group I: 790.5 ± 135.4 days, group II: 720.6 ± 152.8 days, controls: 756.4 ± 99.4 days. In this case, the difference between groups I and II was slightly significant (t value = 1.720), but only at p = 90%. No differences were found between the median values. The differences between the survival curves in the experimental groups were also evaluated by means of another statistical method—the Kolmogorov–Smirnov nonparametric test. The value found was 0.86 (critical value 1.36). The differences were shown to be not significant.

DISCUSSION

In order to evaluate the influence of diet on the longevity of mice, we were obliged to check if the body weight was a decisive factor or not. Drug treatment is often accompanied by a reduction in the body weight, and thus food intake, is diminished. As generally accepted, caloric restriction and longevity go in parallel. This was another reason why the body weight was investigated. Food intake in the groups investigated revealed slight variations, but these insignificant differences could scarcely explain the survival curves.

Antioxidant agents (α-tocopherol) obviously influence the capture of free radicals. The amount of free radicals must increase after the ingestion of lipids with a higher content of polyunsaturated fatty acids. The question remains of how longevity may be altered by a higher or lower content of free radicals. One plausible mechanism is the formation or inhibition of protein cross-links. This was designated by LaBella (1966) as the "cross-linking theory of ageing". The influence most thoroughly studied is that on cross-linking elements of macromolecules of connective tissue—collagen and elastin. These cross-linkages are represented not only by lysine-derived cross-linking amino acids, such as desmosines in elastin and recently discovered pyridinoline in collagen (Fujimoto *et al.*, 1978), but they are also formed by further secondary reactions resulting in an increase of connective tissue pigmentation with age; thus quinoid substances are formed. LaBella (1974) demonstrated that aromatic compounds might play a cross-linking role in mammalian connective tissue proteins. Tyrosine-derived cross links can consist of dityrosine (Malaník and Ledvina, 1979), and of hypothetical binding between aromatic rings called "tanning process" as well (LaBella, 1974).

LaBella and Vivian (1978) proved that β-aminopropionitrile, a known lathyrogen, inhibits not only the synthesis of collagen and elastin cross-links, but also increases longevity in mice by approximately two months; nevertheless no direct evidence is available that the effect, i.e. the prolongation of life, is due to an influence on connective tissue.

In our experiments not only the influence of α-tocopherol on longevity of female mice has been studied. The experiments followed the effect of antioxidant agents under the condition when the body was saturated with polyenic fatty acids throughout the entire life-span, with the amount of generated free radicals substantially exceeding the normal values.

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SUMMARY

A diet supplemented with sunflower oil (rich in polyunsaturated fatty acids) was administered to female mice throughout the entire lifespan. The effect of this diet on the longevity of mice was compared with a group of mice provided with α-tocopherol plus sunflower oil, and with a control group as well. The addition of antioxidants caused a slight prolongation of maximum life-span and mean life-span values, whereas median values remained unchanged; no significant difference was found between the two survival curves. The diet enriched only with sunflower oil (generating higher amounts of free radicals) had no effect as compared with the control group.

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