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The Effects of Vitamin E on Mouse Fitness and Survival

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Abstract. A small colony of C_3H/He and LAF_1 mice, of which 50% were receiving a diet supplemented with vitamin E (0.25%, w/w, *dl*-α-tocopherol), was set up for investigation of the reported action of antioxidants on increasing longevity. It was found that vitamin E exerted no effect on maximum longevity, but increased the numbers of both genotypes surviving to 24 months. The beneficial effects appeared to act by reducing the incidence of fatal tumours late in life and by counteracting a debilitating condition early in life. It is suggested that this debility may have resulted from low antioxidant in the control diet. The relevance of free radicals to ageing is questioned.

It is now well over 20 years since the first research paper was presented which showed a relationship between antioxidant supplementation and longevity (*Harman,* 1957), and yet the causation, even the validity, of this relationship remains a matter of dispute.

The free radical theory of ageing (*Harman,* 1956) has been proposed as a theoretical background to this work. Highly reactive free radicals, after having been generated from the peroxidation of polyunsaturated lipids within the cell membranes, are seen as causing non-specific cellular damage.

The initial research (*Harman,* 1957) was carried out on C_3H and AKR mice which were found to have lifespans under control conditions of 14.25 and 7.5 months, respectively; both rather below average. Of the antioxidant chemicals administered to this stock all but one (ascorbic acid) increased the mean lifespan of AKR mice, whereas 5 out of the 8 treatments actually resulted in a shorter average lifespan for the C_3H mice. These somewhat inconclusive results were, however, sufficient to allow *Harman* to conclude that antioxidants prolong the lifespan by neutralising free radicals and were sufficient to stimulate various other authors to follow up his experiments.

Soon afterwards, *Berg* (1959) published some contradictory results from an experiment involving the administration of 100 mg/week of vitamin E to rats in the form of oral drops. Despite this high level of an-

tioxidant, he found no effect at all on either longevity or chronic disease pattern.

A later work by *Harman* (1961) using C_3H, AKR and Swiss mice apparently showed that antioxidants acted positively on the longevity of C_3H and AKR stock, but not on Swiss mice. The Swiss mice, however, showed a reduction in lifespan comparable with the prolongation exhibited by the other strains. When a longer-lived hybrid, LAF_1, was used (*Harman,* 1968), the pattern was found to be rather different with no increase in maximum lifespan, but an increase in numbers surviving into later life.

Kohn (1971) working with $C_{57}BL$ mice, concluded that antioxidants have no effect on stock showing full life-span, but are capable of prolonging life in strains normally showing a shorter lifespan than average. Other workers, after having produced an increase in both mean and maximum lifespan for C_3H mice (*Comfort* et al. 1971) suggested that this might be due to a lower dietary intake in the case of treated stock, since this itself could increase longevity (*Stoltzner,* 1977). A similar voluntary dietary restriction was shown by CD-1 mice, to whom antioxidants were administered, but in this experiment it was not correlated with any effect on longevity (*Tappel* et al., 1973).

Antioxidant supplementation has, therefore, at various times been shown to increase mean lifespan, increase maximum lifespan, reduce lifespan or to have no effect. It would appear that the choice of antioxidant, the normal lifespan of the stock and the conditions under which they are kept are all potential factors in determining the outcome of the experiment.

Irrespective of effects on ageing, antioxidants have been shown to counteract deleterious occurrences, such as radiation damage (*Black and Chan,* 1975; *Nesterenko* et al., 1974) tumours (*Black and Chan,* 1975; *Dzhioev and Balanski,* 1974; *Pliss* et al., 1974) and haemolysis (*Dayton* et al., 1965; *Tsen and Collier,* 1960). Such beneficial effects of antioxidants could obviously increase maximum longevity in short-lived strains or increase mean longevity in longer-lived strains without affecting the ageing process per se. The present study was aimed at determining whether this is in fact the case, or if antioxidants are capable of retarding 'true' ageing by the mechanism postulated by the free radical theory.

Materials and Methods

96 mice of the inbred strain C_3H/He and a similar number of the hybrid cross LAF_1 were purchased as weanlings from a commercial breeder (Mess. Banting and Kingman, Aldborough). The stock, comprising equal numbers of each sex, were housed in wire-topped polythene cages with soft-wood shavings covering the floor. Water and food pellets (Oxoid-pasteurised breeding diet) were available ad libitum. Artificial lighting for 12 h/day was provided in addition to daylight, the mean temperature was 20 °C.

From the age of 5.5 weeks, 50% of the stock were given a dietary supplement of the natural antioxidant, vitamin E (*dl*-α-tocopherol, Sigma), at a level of 25 mg/kg diet. This was administered as a coating on the pellets, being sprayed over the food as a solution in 'AR' diethyl ether, (BDH). The solvent was given sufficient time to evaporate prior to utilisation of food.

Samples of stock were culled at ages between 2.5 and 28 months to provide data for various physical and biochemical tests (*Blackett,* 1979). Sickly animals were not culled preferentially as each individual was assigned to an age point at the outset, but dead animals together with any appearing moribund were removed for examination to ascertain the cause of death/illness. At no stage were any of the animals re-grouped.

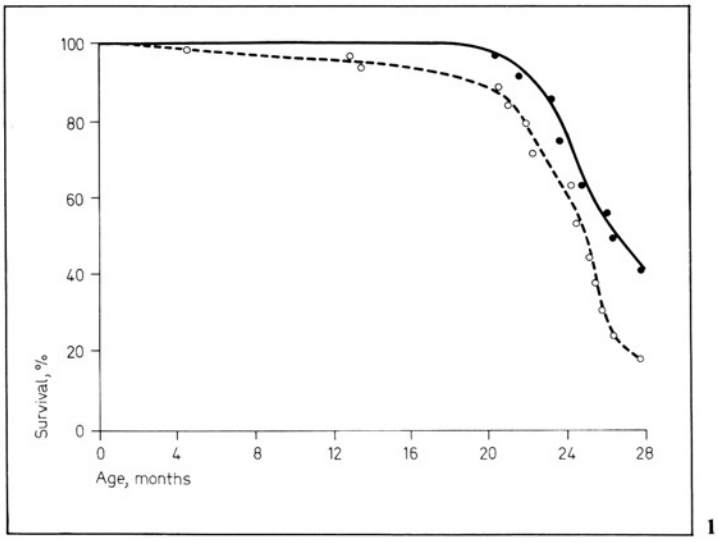
Data for survival curves was provided by those mice found dead or considered to be moribund. As the

total population was reduced at each scheduled culling point, the relative importance of each mouse increased throughout the experiment, a factor compensated for in the construction of the curves.

All culled animals were examined and measurements were taken of body and tail length, together with the weights of the body and of various organs, namely heart, kidneys, spleen, liver, and testes.

Results

The mortality data for the stock is expressed in the survival curves shown in figure 1 for the LAF_1 mice, and in figure 2 for the C_3H mice. Both genotypes exhibited a high age for 50% population survival;



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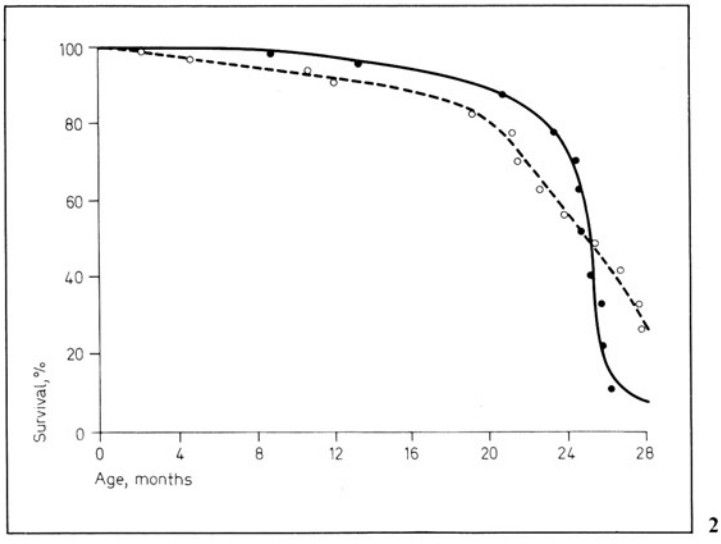

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Fig. 1, 2. Mortality data for LAF_1 mice (1) and C_3H mice (2), both sexes. ● = + Vitamin E; ○ = controls.

25 months for LAF_1 controls, 26.6 months for LAF_1 supplemented stock and 24.25 months for both groups of C_3H mice. No figures for maximum lifespan were obtainable due to the format of the experiment and its termination at 28 months.

Whilst the longevity of the LAF_1 mice was compatible with the findings of previous workers (*Goodrick,* 1978; *Lorenz* et al., 1955), the C_3H showed a greater lifespan than might have been expected from previous studies (*Comfort* et al., 1971; *Russell,* 1966). The normally accepted short lifespan of C_3H mice is attributable to a high incidence of mammary tumours (*Festing and Blackmore,* 1971; *Storer,* 1966) to which this substrain were evidently not susceptible, only two such occurrences being observed in all.

It is notable in both survival curves that the vitamin E-supplemented stocks appear to show enhanced viability over the first 2 years of life. After this point, the advantage appears to become less important; with the C_3H controls actually surviving longer than the supplemented group. Numbers of mice had, however, declined to very low levels by this phase of the lifespan due to culling, and the significance of results in these terminal portions of the survival curves is severely limited. Differential mortality between dietary groups is, however, significant in the earlier phase of the lifespan where deaths, as indicated by the individual points on the graphs, can be seen to be much more common in the control stock.

LAF_1 controls suffered 3 deaths in the first 20 months and 7 in the first 24 months; the corresponding figures for supplemented stock being 0 and 3. Similarly, C_3H controls suffered 5 deaths within 20 months and 9 within 24 months as against 2 and 4 deaths, respectively, in the supplemented population. After 24 months, the increase in mortality shown by all groups obscured any differential effect of diet which may have been present.

Cause of death in the young control mice was not attributable to any single specific effect, the carcases showing signs of generalised debility with such symptoms as alimentary haemorrhage, muscular degeneration and haemolysis; which were presumably secondary manifestations of the primary cause of death.

In older rats, tumours were found to be more common, either as the cause of death or in otherwise healthy culled animals. The incidence of tumours as shown in table I shows that neither of the genotypes studied was particularly susceptible to any one form of cancer.

The proportion of tumours found in supplemented animals was 40% of all tumours discovered, but this included only 25% of all tumours which resulted in a fatality. The vitamin E treatment would, therefore, seem to have been effective in reducing tumour incidence or at the very least in reducing the severity of the consequences of the cancers.

One kind of tumour, termed 'mesenteric' and probably of pancreatic origin, was actually more common in the vitamin E-supplemented stock, especially in the LAF mice. That this normally infrequently occurring tumour shows this abnormal distribution is interesting, although the small numbers preclude statistical significance.

The physical parameters which were measured showed the expected strain and sex variations, but no differences whatsoever between dietary treatments. The most important result here, in the light of previous findings, is the complete failure to observe any reduction in body weight in the supplemented stock. This indicates that these ani-

Table I. Incidence of tumours as distributed between mice of differing strains, sexes and diets

	LAF/C_3H	Male/Female	Vit. E/Controls	Total	Total fatal
Mesenteric	5/2	3/4	5/2	7	3
Hepatic	4/2	5/1	3/3	6	3
Ovarian	0/5	0/5	1/4	5	3
Mammary	2/2	0/4	1/3	4	4
Renal	3/0	0/3	0/3	3	3
Total	14/11	8/17	10/15	25	16
Total fatal	9/7	3/15	4/12	16	–

mals were not exposed to any self-imposed dietary restriction and removes this possible influence from the interpretation of the results.

Discussion

In the planning of this experiment it had been hoped that under control conditions C_3H mice would exhibit a shorter lifespan than the LAF mice, thus permitting a comparison of the effects of antioxidant administration on stocks of differential longevity. That this was not the case illustrates the great differences that can be found between substrains as regards longevity and reiterates the importance of a predisposition towards malignancy in reducing the average lifespan. Any such factor which acts to increase the rate of mortality will decrease the mean lifespan of a population and if its effect is great enough or the population small enough, it will also decrease the maximum lifespan. Removal of such a factor will result in increased longevity without affecting the rate of ageing.

Administration of antioxidants appears to parallel the above model in that when applied to long-lived mice (current work and *Harman,* 1968) an effect on mean lifespan is noted, whilst when applied to short-lived mice (*Comfort* et al., 1971; *Harman,* 1961) an effect on mean and maximum lifespan is noted. This pattern is compatible with the antioxidant counteracting a deleterious force which had initially reduced the expected longevity of the short-lived strains and was responsible for a slight reduction in viability of the more robust long-lived strains. If the effect was directly on the ageing process, then an increase in lifespan of all groups would surely be expected.

In the mice studied in this experiment, vitamin E appears to have had two separate effects. First, it removed the condition which resulted in a slight wastage of stock during the first part of the lifespan. Secondly, it reduced the incidence of fatal tumours during the latter part of the lifespan. Despite these obviously beneficial effects, the supplemented stock ultimately succumbed to the deteriorations of age without any advantage over the controls other than the fact that more of them were able to reach the terminal portion of the lifespan. In short, vitamin E was shown to improve the viability of the mice over the first part of the lifespan; it was not shown to affect ageing as such.

As to the nature of the condition which resulted in early deaths in the control stock, no definite diagnosis can be afforded. It is, however, possible that what is being observed is a sub-clinical form of vitamin E deficiency sufficient to lower the resistance of certain individuals to the point where they are no longer viable. Vitamin E levels in standard laboratory diets are often low, although high enough to prevent the classic deficiency syndrome. It may well be that above this limit there is a further level resulting in depletion, but not in deficiency and producing the slight 'wastage' of stock which is usually regarded as inevitable and tolerable.

The action of vitamin E on reducing tumour incidence was not unexpected, but this suggestion would put into question the relevance of such an effect. Is it perhaps that the control stock are unnaturally open to mutagenic action, being unprotected due to antioxidant depletion? Does vitamin E supplementation merely restore the status quo?

Whether the dietary groups represent sufficiency versus supplementation or depletion versus sufficiency, the fact remains that vitamin E does afford protection against a deleterious force – most probably the action of free radicals. Thus far the free radical theory is substantiated. On the question of ageing, however, no support for the theory can be gleaned from this work. The benefits accruing from antioxidants are ultimately overridden by a more powerful and more fundamental force which results in indistinguishable lifespans for control and supplemented animals.

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