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VITAMIN E—ITS SIGNIFICANCE IN MOUSE AGEING*

Summary

A small colony of C_3H/He and LAF_1 mice was set up with 50% of all stock being given a dietary supplement of 0.25% w/w vitamin E to study the range of ageing variables over which anti-oxidant administration has an effect. An increase in mean but not maximum lifespan with vitamin E was attributable to fewer fatalities early in life. This may have been due to low anti-oxidant levels in the controls. Lower fatal tumour incidence in both strains and a decrease in collagen content of LAF mice were noted. Lipofuscin levels in heart tissue were, as expected, reduced but the significance of lipid peroxidation to ageing of the organism is questioned.

Introduction

All the prerequisites for the autoxidation reaction are present in the tissues of any living organism. Polyunsaturated lipids (PUFA), which form the bulk of intracellular membranes, contain localized high electron densities in the carbon–carbon double bonds. They are thus liable to interact with omnipresent oxygen via a free radical pathway, leading ultimately to tissue damage (Harman 1956). The destruction of lipids in this manner is seen as producing waste matter which accumulates as age pigment (lipofuscin) but more importantly as inducing the decline in efficiency characteristic of an ageing system.

Although this scenario has not always met with approval (Green 1972), supporting evidence has been provided by the experimental administration of anti-oxidant chemicals which has been shown to reduce lipid waste accumulation in mice (Chio & Tappel 1969, Chow et al. 1973, Harman, 1972, Hochschild 1973). More controversially, the extrapolation of this hypothesis to include effects on ageing has been indicated by the experimental prolongation of the mean or maximum lifespan of mice by antioxidant supplementation (Harman 1957, 1961, 1968, Comfort et al. 1971). Such findings, however, have not been confirmed by other workers (Berg 1959, Kohn 1971, Tappel et al. 1973) and the suggestion has been made that these effects are dependent upon the normal lifespan of the strain of mouse being utilized (Kohn 1971).

The objectives of the current work were not only to study the effects of a dietary anti-oxidant (vitamin E) on the viability and longevity of mice but also to investigate the range of systems over which effects are apparent.

The purpose of this paper is to bring together in a skeletal form the many facets of our work in an attempt to present, in so far as is possible, a holistic view of the significance of vitamin E in mouse ageing.

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Materials and Methods

Ninety-six mice of the inbred strain C_3H/He and a similar number of the inbred cross LAF_1 were taken at the age of 5 weeks. Half the stock were fed a standard pelleted mouse diet (Oxoid) while the other half were fed a similar diet to which vitamin E had been added (0.25% w/w DL-α-tocopherol applied to the pellets in etherial solution).

Samples of the stock were culled at seven age points between 2.5 and 28 months to provide material for the tests outlined below.

Data for survival curves were obtained from individuals found dead or considered moribund. Allowances were made for the relatively greater importance of an individual dying later in the project when the populations had been reduced.

The tests carried out at each age point were as follows:

An *activity* rating was obtained over a 10-minute period prior to culling, utilizing an open field trial similar to that used by others (Wimer & Fuller, 1965).

A *vitamin E* assay was carried out on 0.5 g samples of liver tissue using a modified form of the method of Bieri (1967).

Lipofuscin levels in heart tissue were estimated by a fluorimetric technique following that of Fletcher et al. (1973).

The *collagen content* of skin samples was extrapolated from the hydroxyproline content (Newman & Logan 1950) which was determined by auto-analysis (Grant 1964) after hydrolysis (Woessner 1976).

The *post-stress recovery of skin samples* was derived from measurements taken before, during and after subjecting the samples to a 50 g load.

The *thermal shrinkage* of tail tendon fibres was studied by suspending individual fibres in a small bore Perspex chamber through which water of increasing temperature was passed. The shrinkage temperature was recorded together with the maximal degree of shrinkage prior to denaturation, expressed as a percentage of initial fibre length.

Results

As most of the stock was culled to provide samples for analysis, only a few points (between 8 and 14) were available for the construction of each survival curve (Fig. 1A).

Points shown on Fig. 1 B to H are the mean values for the mice culled at each age point. Although space does not allow data to be tabulated here, scatter within groups was generally high, which, despite casting doubt on the significance of individual points, nevertheless enables trends within groups and relationships between groups to be studied.

Survival

The survival curves of LAF_1 and C_3H (Fig. 1A) mice do not differ greatly due to the lack of the expected high tumour-susceptibility in the C_3H substrain utilized.

Although it is not possible to observe any dietary effect on maximum longevity, there is an apparently beneficial effect of vitamin E on viability earlier in life. Before the age of 24 months the LAF_1 controls showed eight fatalities as against four for supplemented stock, whereas the C_3H showed nine fatalities against four. This is a significant reduction in mortality over the first 24 months.

In later life a lower incidence of fatal tumours was found in the supplemented stock (four against 12) but this effect on survival was masked by the overall increase in death rate.

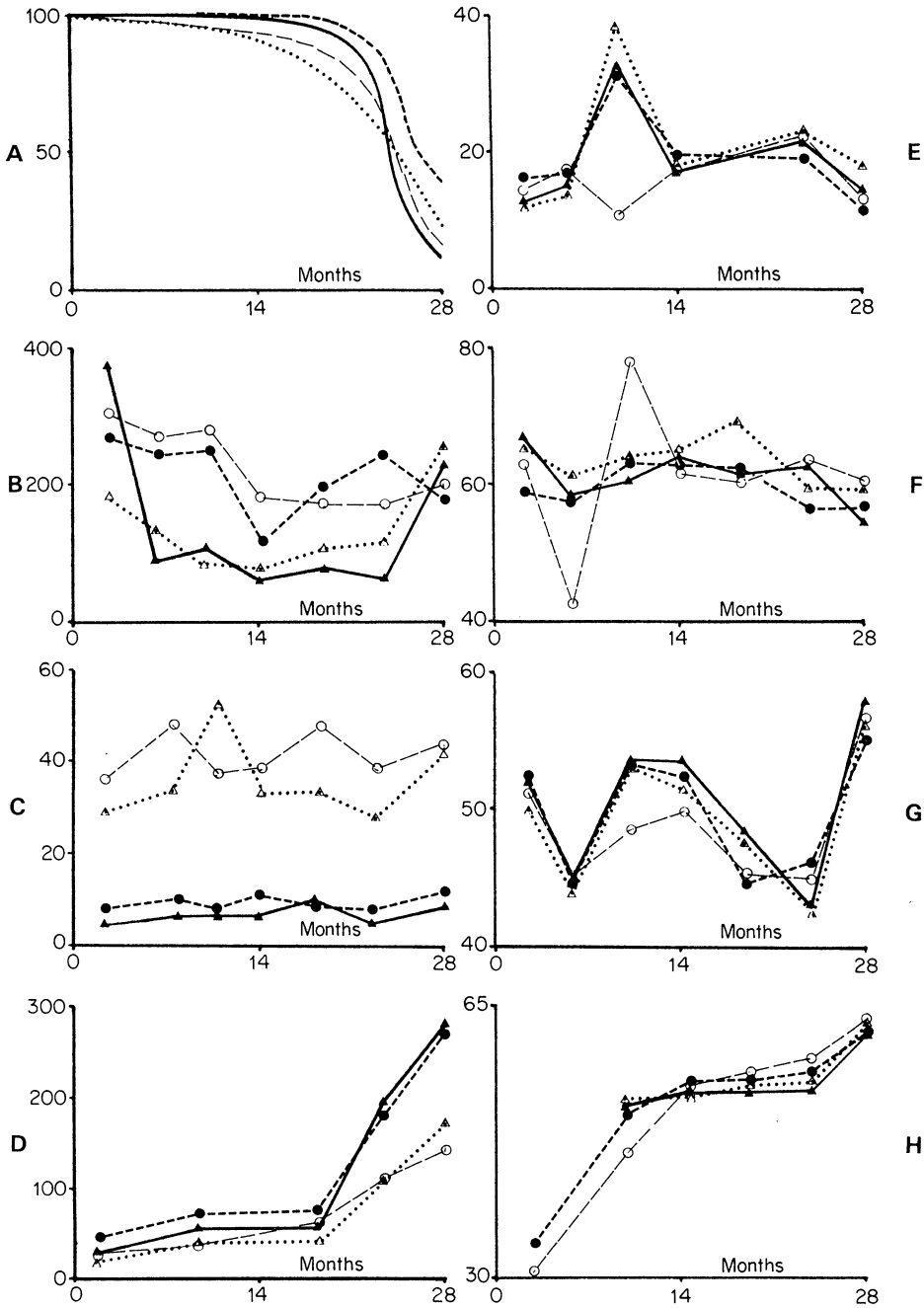

Fig. 1. A. Percentage survival. B. Activity score. C. Liver vitamin E (μg/g). D. Heart lipofuscin (arbitrary units). E. Percentage collagen in skin. F. Percentage recovery of skin. G. Tendon fibre shrinkage temperature (°C). H. Maximal degree of tendon fibre shrinkage (percentage of initial). C_3H control ▲———▲; C_3H supplemented △· · ·△; LAF_1 control ●– – –●; LAF_1 supplemented ○— — —○.

Activity

The activity scores with age (Fig. 1B) show a marked difference in distribution with strain but no significant effect due to vitamin E.

Vitamin E

That the values for supplemented stock are consistently higher than those for control stock (Fig. 1C) is as expected. It is evident therefore that the dietary anti-oxidant is being absorbed in the gut and disseminated throughout the tissues. The fluctuations throughout life are without pattern.

Lipofuscin

The fluorescence levels found for heart muscle (Fig. 1D) show a dramatic rise with age in both of the experimental strains indicating an accumulation of lipid debris throughout life.

The effect of vitamin E on this parameter is most marked, resulting in lower values for fluorescence at all age points. The average ratio of these values shows that vitamin E leads to a fluorescence level which is 72% of that for control C_3H mice and 64% of that for control LAF mice.

Connective tissue

The general pattern of the distribution of the collagen content (Fig. 1E) shows a maximum at 10 months followed by a levelling off and ultimate decline. Although the vitamin E appeared to exert no effect upon the collagen content of C_3H mice, a most unexpected result showed the 10-month peak to be absent from supplemented LAF mice.

The graphs showing skin recovery (Fig. 1F) show a similar anomaly in the response of LAF samples at 10 months and also at 6 months.

The shrinkage temperature (Fig. 1H) shows the type of rise late in life that would be expected from the increasing cross-linkage of collagen. The peak in mid-life is probably due to the increasing collagen content at this age (Fig. 1E) which also accounts for the much smaller peak shown by LAF supplemented mice.

The maximum shrinkage of tendon fibres (Fig. 1H), which is unfortunately incomplete for C_3H mice, shows a dramatic rise both early and late in life with a more stable phase between. The initial rise in supplemented LAF mice appears to be retarded.

Discussion

As no increase in survival into the latter phase of the lifespan was brought about by vitamin E it would appear that there is no evidence for a retardation of ageing per se. The beneficial effect of vitamin E supplementation earlier in the lifespan would seem to result from the counteraction of a condition leading to reduced viability of the control stock. The nature of this condition remains obscure, but the mice which died at an early age showed signs of a generalized debility. It cannot be ruled out that this was brought about by a low anti-oxidant level in the control diet which was merely compensated for by supplementation. Perhaps the normally accepted anti-oxidant level, whilst being adequate to prevent the classic deficiency syndrome, is nevertheless sub-optimal.

The observation of increased levels of lipofuscin with age, alleviated to a degree by vitamin E administration, is in accord with the Free Radical Hypothesis of Ageing (Harman 1956) but the lack of concomitant effects on other age-associated variables does not support extrapolation from lipid degradation to ageing of the organism as a whole.

Changes in the various connective-tissue parameters with age can be seen as functions of two basic variables. One is collagen content, which has been shown to peak early in life and then decline; the other is cross-linkage of the collagen molecule which increases throughout life. That an alteration in collagen content appears to have been induced in one strain at one point in life is interesting, but by virtue of its very selectivity is unlikely to prove of great consequence.

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