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CONNECTIVE TISSUE AGEING: THE INFLUENCE OF A LATHYROGEN (β-AMINOPROPIONITRILE) ON THE LIFE SPAN OF FEMALE C57BL/Icrfa^t MICE

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INTRODUCTION

CROSS-LINKING of collagen and elastin molecules is essential if these proteins are to fulfill their physiological function correctly. However, it has been suggested that cross-linking in excess of that required to produce optimum physiological function might impair the performance of tissues and thus, cross-linking of collagen and elastin might be an important factor in ageing (for discussion see Sinex, 1968; Bailey, 1968; Bornstein, 1970; Kohn, 1971; Hall, 1976; Schofield and Davies, 1978; Schofield and Weightman, 1978). Although numerous investigations have been carried out to examine changes in collagen and elastin with age, the results of these experiments have not so far provided conclusive evidence as to the relevance of cross-linking in these proteins to the ageing process. One approach that has been employed recently to test the relevance of cross-linking to ageing has been to study the effects of known inhibitors of collagen and elastin cross-linking reactions (lathyrogens) on the survival of rodents (LaBella, 1972). The results of some of these experiments have indicated that prolonged treatment of mice with such drugs may increase survival and this may be consistent with cross-linking in connective tissue proteins being a factor in ageing.

A number of papers have been published describing the influence of the lathyrogen β-aminopropionitrile on the lifespan of laboratory mice. LaBella and Vivian (1975, 1978) have shown modest to somewhat greater increases in the mean and maximum lifespans of LAF/J mice. Various doses of the drug were administered, ranging from 1 to 3 mg/ml in the drinking water, to young animals (2 months of age) for differing lengths of time. The maximum increase in mean survival was about 2 months, the mean survival of the control group being about 33 months. The shape of the survival curves and the correlation of increased mean lifespan with increased body weight in low dose BAPN-fed animals suggested that the drug influenced some fundamental part of the ageing process. These findings were put forward as extending and confirming those made in earlier reports (for reference see LaBella and Vivian, 1978).

We have been carrying out a long term experiment on the influence of BAPN on the lifespan, as well as other physiological parameters, with respect to C57BL/Icrfa^t mice. The data presented in the study show clearly that several different doses of BAPN fed to young mice of this strain, produced decreases in both mean and maximum lifespans.

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However, feeding this drug to animals which could be considered adult leads to no significant alteration in the lifespan when compared with controls.

MATERIALS AND METHODS

Animal stocks

Female C57BL/Icrfa[1] mice were maintained under constant conditions in the Animal Unit of the University of Manchester. Groups of up to 25 females were housed in plastic boxes with stainless steel tops and fed the drug BAPN in the drinking water at a variety of doses, both the drug and food was applied *ad libitum*. The maintenance conditions for this colony have been published previously (Davies and Fotheringham, 1980; Schofield, 1980).

Life tables

Six groups of animals were established for the experiments and three dose regimes of the drug were employed over two different periods of the lifespan. A control group was fed water and food *ad libitum*. The experimental design is summarised in Table 1. Each of the groups of animals was fed the drug *ad libitum* in the drinking water at dose rates indicated, from either 3 or 9 months of age. Deaths of individual animals within the cages were recorded. Animals which were deemed grossly unfit were killed in order to comply with legislation governing the conduct of experiments on animals, although these occasions were rare (these animals were not included in the survival curves).

TABLE 1. DESIGN OF EXPERIMENTS TO DETERMINE THE EFFECT OF BAPN ON THE SURVIVAL OF C57BL MICE

Group	Age at start of BAPN experiment (days)	Age at end of BAPN experiment (days)	Concentration of BAPN in drinking water mg/ml	Number of animals	Mean* expectation of life
1	104	475	0.5	36	447
2	108	487	1.0	34	424
3	90	540	1.0	36	440
4	111	659	1.0	48	453
5	87	452	2.0	49	426
6	267	632	1.0	38	856
Controls	—	—	0.0	94	883

*Deevey (1947).

RESULTS

The survival curves presented in Figs. 1–7 clearly show that those groups of animals maintained on the drug BAPN from three or four months of age display a considerable reduction in survival. For the control group the mean expectation of life was about 880 days and the maximum lifespan was approx. 1100 days (Fig. 7) whereas for the groups started on the BAPN treatment between 87 and 111 days the mean expectation of life was about 440 days and the maximum lifespan about 820 days (Figs. 1–5). The results of these experiments also indicate that there were no dose–response relationships for the group started on BAPN at approx. 100 days of age since there is no significant difference in the median and maximum lifespans among these groups. In contrast, the survival of the mice fed the drug from 267 days of age onwards showed a survival curve essentially similar to the control group (Figs. 6 and 7).

It is also evident that differences between the age at the start of treatment and the mean expectation of life or the maximum lifespan are also quite different for animals started on the treatment at approx. 100 days of age and those started at around 260 days of age (see Table 1). For example, the difference between age at start of treatment and 50% survival value for the younger animals started on BAPN treatment is about 350 days,

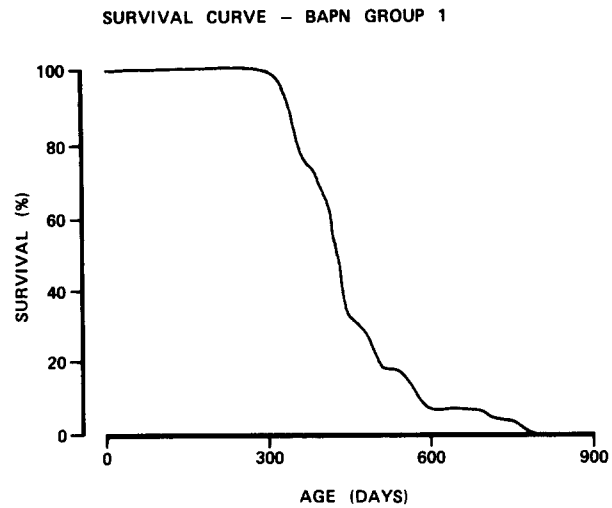

FIG. 1. Survival curve for mice in experimental Group 1. BAPN dose of 0.5 mg/ml in drinking water. See Table 1 for details.

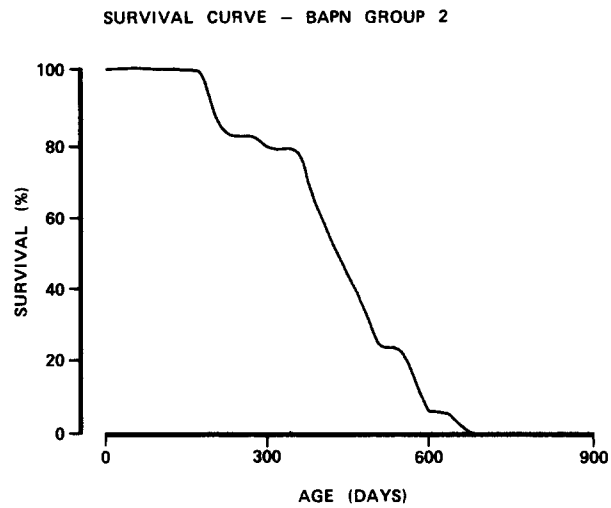

FIG. 2. Survival curve for mice in experimental Group 2. BAPN dose of 1 mg/ml in drinking water. See Table 1 for details.

whereas this difference for animals started on the treatment later in the lifespan is about 610 days. The differences between age at start of treatment and maximum lifespans are about 510 days and 830 days for the 100 day and 260 day groups respectively. Thus, the survival curve for the group started on the drug treatment late in the lifespan is not merely shifted to the right, with 260 days of age fortuitously being chosen so that the curves for this group coincide with the control group. Rather, the data shows that this group responds to the BAPN treatment quite differently from those started on the drug at around 100 days of age. However, the results indicate that none of the treatments produces any prolongation of life in the study and most, in fact had a profound life-shortening effect.

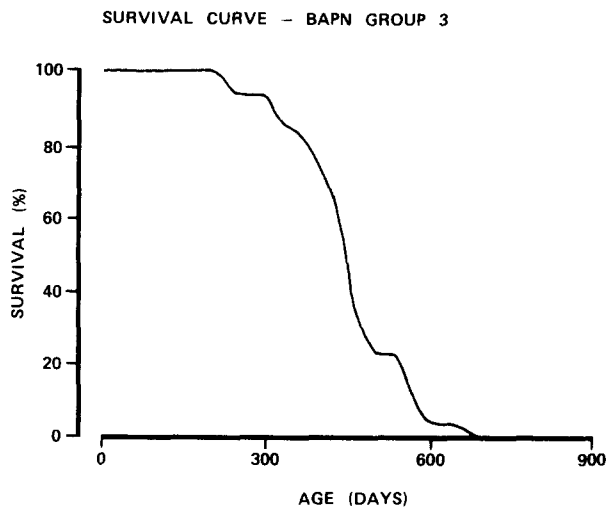

FIG. 3. Survival curve for mice in experimental Group 3. BAPN dose of 1 mg/ml in drinking water. See Table 1 for details.

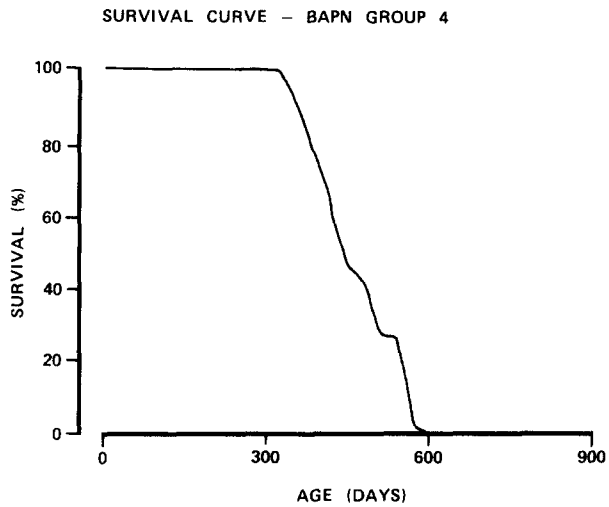

FIG. 4. Survival curve for mice in experimental Group 4. BAPN dose of 1 mg/ml in drinking water. See Table 1 for details.

DISCUSSION

The initial stage in the formation of cross-links between collagen molecules is the oxidative deamination of the lysine or hydroxylysine residues catalysed by the enzyme, lysyl oxidase. The aldehydes produced by this enzymic reaction then condense with reactive groups, predominantly the ε-amino group of hydroxylysine residues in adjacent molecules, to form inter-molecular bonds of the aldimine type. As an animal matures and ages, these aldimine-type bonds are modified, by as yet incompletely understood mechanisms, such that the cross-links become chemically more stable and the collagen fibres become increasingly resistant to solubilisation procedures. Thus, the aldimine-type bonds may be

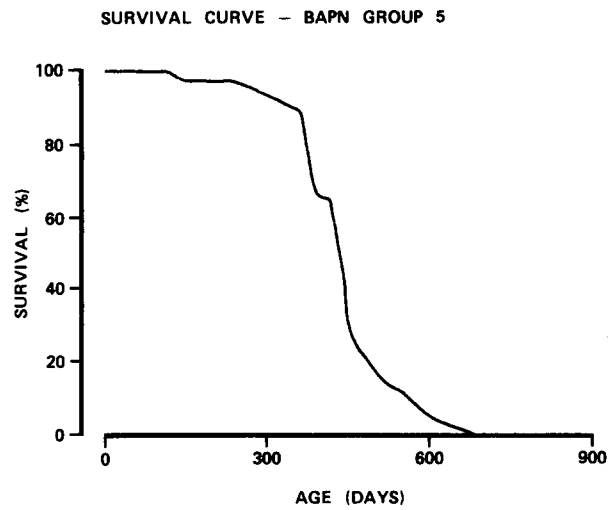

FIG. 5. Survival curve for mice in experimental Group 5. BAPN dose of 2 mg/ml in drinking water. See Table 1 for details.

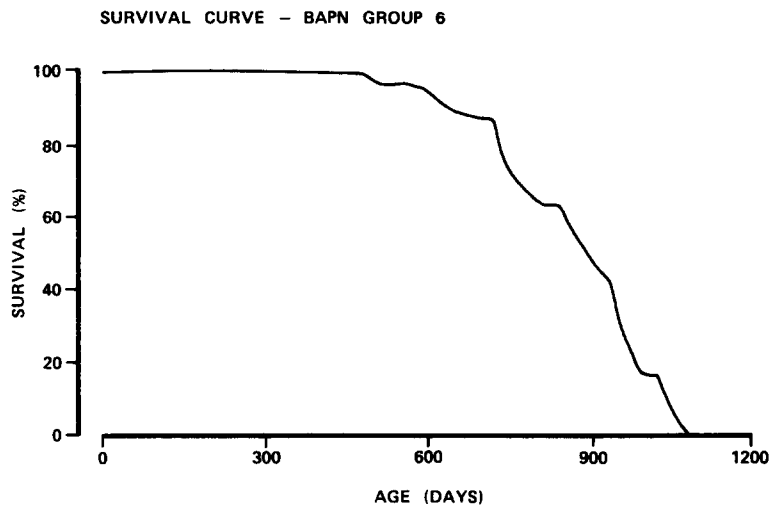

FIG. 6. Survival curve for mice in experimental Group 6. BAPN dose of 1 mg/ml in drinking water. See Table 1 for details.

considered as intermediates in the cross-linking process. The chemistry of elastin cross-linking is more complex, but, as in collagen, the cross-links are initially formed from aldehyde moieties produced by oxidative deamination of lysine residues. Details of the chemistry and formation of cross-links in collagen and elastin may be found in several reviews (Piez, 1968; LaBella, 1971; Gallop *et al.*, 1972, 1972; Bailey and Robins, 1973, 1976; Bailey *et al.*, 1974; Sandberg, 1976; Tanzer, 1976; Bailey, 1978).

It is well known that nitriles such as BAPN prevent the formation of cross-linking in collagen and elastin by non-competitive inhibition of the enzyme, lysyl oxidase, thus preventing the formation of the aldehyde cross-link precursors (Barrows *et al.*, 1974;

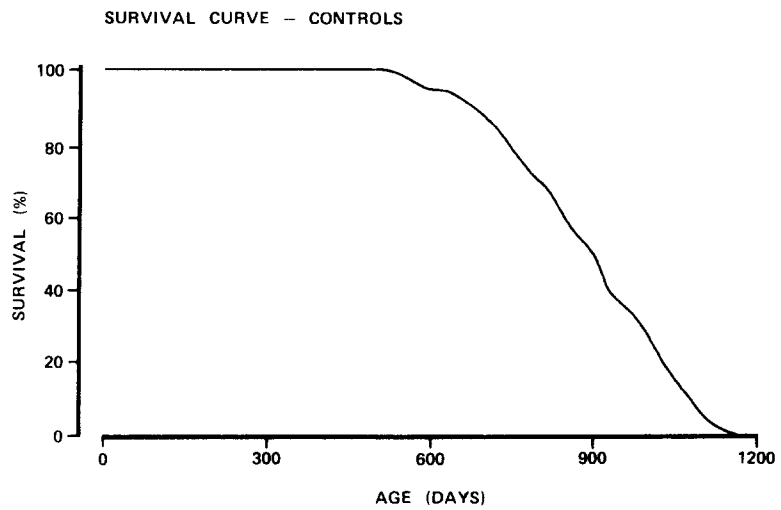

FIG. 7. Survival curve for control animal group.

Nimni, 1975). Other lathyrogens, for example penicillamine, may also prevent cross-link formation by binding to the aldehyde groups themselves. The rationale for instituting the lathyrogen treatment as a possible inhibitor of the ageing process is based on the hypothesis that these compounds may be delaying age-related, deleterious cross-linking processes (LaBella, 1972). The results of work carried out in LaBella's laboratory have indicated that chronic administration of lathyrogen, BAPN, does increase survival of LAF/J mice significantly (LaBella and Vivian, 1975, 1978). This work also indicates that the lathyrogen-sensitive period occurs at a relatively early stage in the lifespan but that the mechanism by which the lathyrogen treatment brings about these beneficial effects has not been established. The finding that the lathyrogen-sensitive period occurs early in the lifespan, while development and growth is occurring at a rapid rate, would be consistent with an effect on collagen and/or elastin cross-linking since it has been known for some time that lathyrogens have a far greater effect on the connective tissues of young, growing animals than on those of mature or old animals (Tanzer, 1965; Barrows *et al.*, 1974).

In contrast with the results obtained by LaBella's group, we observed that for female C57BL/Icrfa^{t} mice, chronic administration of BAPN in the drinking water, at concentrations between 0.5 and 2 mg/ml, starting early in the life-span, resulted in a dramatic decrease in survival (Table 1, Figs. 1–5). LaBella and Vivian (1975, 1978) used similar experimental conditions and when BAPN was incorporated into the drinking water at concentrations similar to those used here, significant increases in survival were observed. In our experiments, the young animals show no dose response to BAPN, suggesting that some quite fundamental lesion is being caused under these circumstances. Kohn and Leash (1967) also carried out BAPN administration experiments with rats, but these workers were unable to find any effect of the lathyrogen treatment on survival.

Chronic administration of BAPN to adult animals (approx. 260 days of age) did not produce such dramatic changes in survival as it did for young animals. Examination of the survival curve (Fig. 6) indicates that, when BAPN treatment was started later in the lifespan, the survival of these animals was essentially the same as that of the controls (Fig. 7). The difference between the rates of decrease in survival for animals started on BAPN treat-

ment early and late in the lifespan, and the difference between the age at the start of treatment and mean expectation of life or maximum survival for the two groups shows that the response of the young adult animals to the drug is quite different from that of the older animals. Thus, although survival was affected differently in our experiments compared with LaBella's experiments, the two pieces of work do agree in showing that the lathyrogen-sensitive period occurs early in the lifespan.

There are obviously marked strain differences in the response to chronic BAPN administration between the C57BL mice used in our experiments and the LAF/J mice used in LaBella's work. We are now constructing a variety of experiments to discover the lesion causing accelerated deaths to our animals under these experimental conditions. Initial observations suggest that the weight gain of the treated C57BL mice is similar to that of controls, but that the treated animals show a considerable behavioural hyperactivity compared with control mice. A possible explanation for the different responses of C57BL mice and LAF/J mice could be in terms of differing levels of sensitivity to the drug (i.e. a much higher dose rate might be required to produce increases in survival in LAF/J mice than in C57BL mice and/or C57BL mice might be much more susceptible to some toxic effect of the drug). However, there is no information available at present to assess these possibilities.

If it can be shown eventually that the increased survival of LaBella's BAPN-treated LAF/J mice is indeed due to inhibition of collagen and/or elastin cross-linking, this would raise the interesting possibility that cross-linking might not, in fact, be at an optimal level in the tissues of mice of this particular strain, but rather might be in excess of that required for optimal physiological functions. BAPN treatment might thus prevent such excessive cross-linking and this might result in increased survival, although the results are obviously border-line. On the other hand, cross-linking of collagen and elastin might be optimal in C57BL mice and any reduction in the extent of cross-linking in this strain might be detrimental to survival. There is little or no information to the extent of cross-linking of these two proteins in the tissues of animals of different strains but within the same species. The relationship between the extent of cross-linking and physiological performance is also unclear and in view of this lack of information it is not possible to determine the validity of this explanation for the differences in susceptibility to BAPN between the two strains.

The mechanisms by which the intermediate aldimine-type cross-links are stabilised are not yet fully understood and neither is the physiological significance of these stabilisation reactions (Bailey, 1978). However, there is little evidence to suggest that these modification reactions could be inhibited by BAPN and there remains a possibility that they could play a role in the ageing process. Furthermore, cross-linking mechanisms have been discussed that do not involve preliminary oxidative deamination of lysine residues (Bornstein, 1970; LaBella, 1971; Hall, 1976). These reactions also may not be susceptible to interference by compounds such as BAPN, although at present there is little experimental evidence in support of their existence, let alone on the effects they may have on ageing. A more complete knowledge of the chemistry of collagen and elastin cross-linking should allow a more realistic assessment of the role of cross-linking in these proteins in the ageing process, and it should also allow a more rational appraisal of the most effective way of controlling these processes.

SUMMARY

The influence of chronic administration of the lathyrogen β-aminopropionitrile (BAPN), in drinking water, on the survival of female C57BL/Icrfa^{t} mice has been determined.

We observed that BAPN treatment, at several different dose rates, resulted in dramatic decreases in survival when the treatment was initiated at approx. 100 days of age; the reduction in survival was independent of dose rate when concentrations of BAPN in the drinking water of 0.5–2.0 mg/ml were used. Thus, the mean expectation of life for the controls was about 880 days and the maximum lifespan was about 1100 days, whereas the mean expectation of life for the groups treated with BAPN from approx. 100 days of age was about 440 days and the maximum lifespan was about 620 days. These results are in marked contrast to those obtained previously with male LAF/J mice when fed BAPN under similar conditions. When BAPN treatment was initiated at approx. 260 days of age, however, the survival of the treated animals was essentially the same as that of the control, untreated animals. That the response of the younger animals was different from that of the older animals was indicated by differences in the further survival of the animals after commencement of the lathyrogen treatment. The lathyrogen-sensitive period, therefore, occurs early during life.

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