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BETA-AMINOPROPIONTRILE PROMOTES LONGEVITY IN MICE*

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INTRODUCTION

IN A PREVIOUS study we showed that BAPN added to the drinking water over periods from 3 to 12 months significantly increased survival of mice (LaBella and Vivian, 1975). We now present data from another study in which two dose levels of the drug were used, the duration of treatment extended, and body weight monitored. Again, BAPN significantly increased mean survival.

METHODS AND MATERIALS

Seven groups of 24 mice each were used as control or treated with either 1 or 3 mg/ml BAPN (beta-aminopropiontrile fumarate was obtained from the Aldrich Chemical Company) starting at two months of age for durations of 6, 12 or 18 months. LAF/J mice, strain 0305 were obtained at approximately 6 weeks of age from Jackson Labs., Bar Harbor, Maine, and started on BAPN two weeks later. Mice were randomly assigned to a control and six test groups and housed 12 to each plastic cage. There was both vertical and horizontal randomization of the positions of the cages within the temperature and light controlled segregated animal room. The mice were provided with a standard pelleted diet and tap water, both supplied ad libitum. The BAPN was administered in the drinking water. Stock solutions of the drug were made up fresh each week, kept refrigerated, and diluted with fresh drinking water each day.

RESULTS AND DISCUSSIONS

In Fig. 1 survival data are presented for the control and drug-treated groups. The curves are characteristic of a population undergoing senescence, with approximately half of the population attaining 80% of the maximum lifespan. Morbid processes were evident in some of the cages, however. One of the cages for the group treated with 1 mg BAPN for an 18 month duration had a severe early episode of fatalities which markedly distorted the survival curve for that group. A few premature deaths in the control group were also evident. To differentiate premature deaths due to morbidity from senescence related deaths, only the data boxed in the solid-line (Fig. 1) were considered for further analysis.

Table 1 presents mean and maximum survivals for the various groups of mice. Five of the six BAPN-treated groups showed an increased mean survival of approximately two months over the control group. There was a statistically significant overall effect of the BAPN, but no significant differences were found between the two doses of BAPN or among different durations of treatment. Nor was there significant interaction between doses and duration.

At the 1 mg BAPN/ml level, for all treatment durations, body weights were, in fact, greater than those of control animals for all or most of the lifespan (Fig. 2). Body weights of mice given 3 mg BAPN/ml, however, did tend to be lower, suggesting drug toxicity. There were no significant differences in mean lifespan among five of the six treated groups—the

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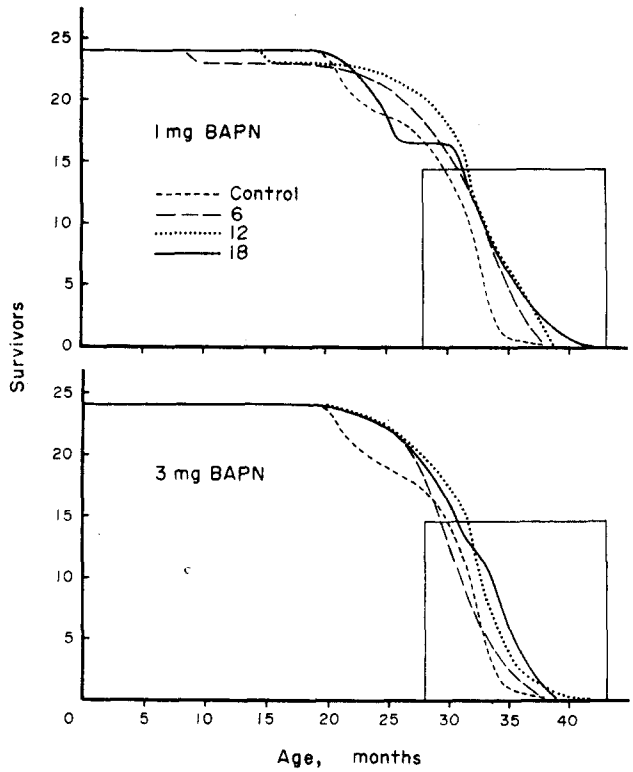

FIG. 1. Survival curves of male LAF/J mice given BAPN in the drinking water for 6, 12 or 18 months. Drug treatment was begun at age 2 months. Upper panel: 1 mg BAPN/ml. Lower panel: 3 mg BAPN/ml. To ensure an equal number of replicates for each treatment group and thus facilitate statistical analysis, the last 15 surviving mice of each group were selected. The data obtained from area of the curves boxed by the solid line were used to calculate mean lifespan.

three groups given 1 mg/ml and two of the three groups given 3 mg/ml. Mice receiving 3 mg/ml for the 6-month period did not show greater survival over control. Several animals in one of the cages housing this group died prematurely (probably due to infectious disease) thereby lowering the mean survival time of the group.

Two significant facts are to be noted in these studies. Firstly, the survival curves of the LAF/J mice maintained in our laboratory approach that of an idealized curve for a population which experiences senescence (Comfort, 1964) (Fig. 1), insofar as there were very few deaths until approximately 2 years of age, with a precipitous mortality thereafter. In populations where significant mortality occurs even during the pre-senescent period, agents with salutary effects on survival may act to suppress specific morbid processes rather than to inhibit the aging process directly.

Secondly, drug treatment is often accompanied by a reduction in body weight, a non-specific consequence of chronic toxicity resulting in diminished food intake. Caloric restriction is a well-established means of increasing survival (McCay *et al.*, 1935; Berg, 1960) and is often a real or suspected confounder in longevity studies. Therefore, the relationship between body weight and longevity was examined. Weight curves for all groups were

TABLE 1. MEAN AND MAXIMUM SURVIVAL OF MICE AS A FUNCTION OF DOSE AND DURATION OF TREATMENT WITH BAPN

	Mean survival (months)	Maximum lifespan*
Control	32·8	38·6
BAPN 1 mg/ml		
6 mos.	34·3†	38·1
12	34·7†	38·3
18	34·7†	41·2
BAPN 3 mg/ml		
6	32·8	38·0
12	34·3†	41·7
18	34·6†	38·5

* Age at death of the last survivor.
† Significantly different from control $p < 0.05$.

Factor analysis

Source	DF			
Effect of BAPN	1	27·07	27·07	4·25‡
Effect of dose level	1	9.87	9.87	1·55 NS
Effect of duration	2	22·90	11·45	1·80 NS
Interaction: dose × duration	2	7·15	3·58	1 NS
Error	98	624·33	6.37	
Total	104	691·35		

‡ $p < 0.05$.
NS $p \geq 0.05$.

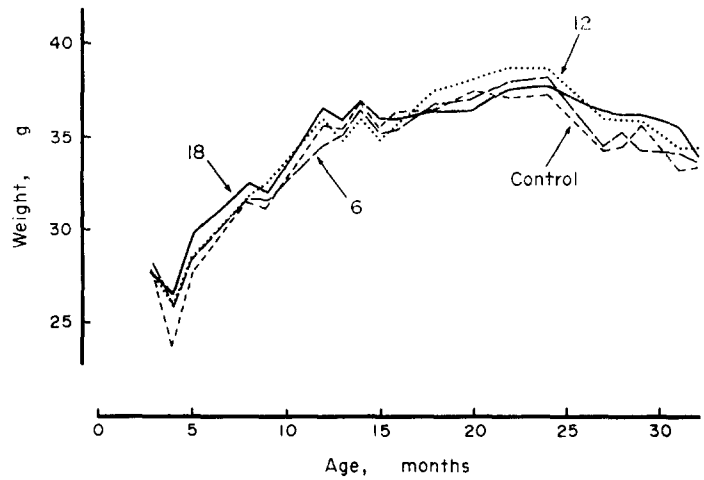

FIG. 2. Average body weights of mice treated with 1 mg BAPN/ml drinking water for varying periods of time.

similar, showing continuous weight gain to about the 23rd month followed by progressive weight loss. There was little consistency between the treatments and body weights during the first two years. There is, however, a definite pattern during senescence (defined as the period of precipitous mortality). A test for correlation was made between body weights of treatment groups taken at 28 months, corresponding roughly with the start of the period of senescence, and lifespan of animals attaining senescence. A test for correlation was also made between weights of mice at three months, just prior to BAPN treatment, and the survival times of mice reaching senescence. The correlation was small, not statistically

significant, and negative ($r = -0{\cdot}47$). There was significant positive high correlation between weights and survival times ($r = 0{\cdot}79$). This result rules out caloric restriction as a contributing factor to the enhanced survival induced by BAPN. Thus, we believe the increased longevity in this experiment is not due to nutritional factors. Indeed, if the aging process itself has been retarded by the BAPN, parallel retardation of weight loss with age observed in our study, would be expected.

BAPN and other "lathyrogens" are known to prevent the formation of stable crosslinks between peptide chains in collagen and elastin (LaBella, 1971, 1972, 1974). BAPN inhibits an enzymatic process which converts lysine residues on these proteins to reactive species that condense with other lysine side chains. Our studies on BAPN in relation to its effect on longevity of rodents were initiated at a time when, although the inhibitory effects of the drug on connective tissue maturation were recognized, the chemical nature of the crosslinks in the structural proteins was unknown. The rationale for instituting lathyrogenic treatment as a possible inhibitor of the aging process was based on the hypothesis that the drug might delay age-related and deleterious crosslinking processes which continue in the post-maturation phase. Kohn (1965) administered BAPN to rats for the same rationale but has not reported increased survival for treated animals. The lathyrogen-sensitive crosslinks appear to be formed very early in life; thus, the basis for observed beneficial effects of BAPN on survival is yet to be established.

SUMMARY

Beta-aminopropiontrile (BAPN) was administered at 1 or 3 mg/ml in the drinking water to 2-month old male LAF/J mice for 6, 12 or 18 months. Five of the six treated groups showed an increase in mean survival about 2 months greater than the 33 months for the control group. The shape of the survival curve and the correlation of increased mean lifespan with higher body weight strongly suggest that BAPN influences fundamental aging processes. These findings extend and confirm those in a previous report.

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