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LONG-TERM OBSERVATIONS ON THE EFFECT OF POLYADENYLIC ACID IN MICE OF DIFFERENT AGES

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Abstract—In order to better life performance, polyadenylic acid (poly (A)) was given intraperitoneally to CBA/Ca mice for almost a two-year period. This substance, as one of the components of double-stranded polynucleotides (like poly A:U), is known to improve some immune responses of the aging organism. Five approaches (changes in body-weight, adaptation to cold stress, biological half-life of body proteins, mortality and pathology) were applied to test the effects of this substance on life performance. It was found that the beneficial effects of double-stranded polynucleotides cannot be mimicked by polyadenylic acid only, despite its anti-senescence effect, namely, it accelerates the apparent protein turnover, cf., biological half-life. Polyadenylic acid shortens life-expectancy (because of the higher mortality rate of mice). Possible mechanisms of these actions are discussed.

INTRODUCTION

NOWADAYS A very promising possibility is at hand, that the rate of aging can be mitigated to some extent through immunoengineering with advancing age. It seems as an effective approach to give adjuvants, as induction agents, which have potential immune enhancing activity in the aged organism (Boyse and Abbott, 1975; Braun, Yajima and Ishizuka, 1970; Jaroslow and Taliaferro, 1956; Johnson, 1973; Kent, 1977; Makinodan, 1979; Scheid, Hofmann, Komuro, Hammerling, Abbott, Boyse, Cohen, Hooper, Schuloff and Goldstein, 1973). The synthetic double-stranded polynucleotides, like poly A:U, poly C:G and poly I:C proved to be efficient in restoring some immune functions in old mice.

We must point out that the polyadenylic acid, poly (A), is one of the components of the double-stranded polynucleotides concerned and has a unique role in nuclear metabolism (Darnell, Jellinek and Molloy, 1973; Puckett, Chambers and Darnell, 1975; Bergmann and Brawerman, 1977). Some of these mean that blocking of poly (A) synthesis stops the appearance of labeled mRNA in the cytoplasm (Darnell, *et al.* 1973). In other words, polyadenylation and poly (A) chain extension may stabilize the mRNA by protecting it from degradation (Rose, Jacob and Kumar, 1979). The role of aging in the nuclear RNA manufacturing was studied by Yannarell, Schumm and Webb (1977) who concluded that a decreased poly (A) content of the putative mRNA might be relevant to the problems of cellular aging.

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In the light of the foregoing, the aim of the present paper is to call attention to how the polyadenylic acid influences some parameters, namely, body-weight, cold tolerance, apparent turnover, mortality and pathology of mice during aging.

MATERIALS AND METHODS

Our studies are based on the barrier-maintained inbred mice strain CBA/Calati, obtained from the Laboratory Animal Institute, (LATI) Gödöllő, Hungary. The SPF animals (whose pathology was monitored regularly) were male and monthly injected ip. with a 150/μg dose of polyadenylic acid (REANAL, Budapest) dissolved in physiological saline, pro animal. A total of 147 animals (between 28 and 33 g) were used and the mice were fed a standard laboratory chow (LATI) and supplied with tap water *ad lib.* Body-weights were regularly recorded in every month. Five approaches (changes in body weight, cold tolerance, ^{75}Se-selenomethionine biological half-life, mortality and pathology) were applied to test the effects of poly(A) on life performance. In order to test the mice for their adaptive capacity against acute cold stress, the rectal temperatures of the animals were measured three times in the periods concerned; i.e., before, just after the cold (−4−−2°C) and one hour later, respectively. ^{75}Se-selenomethionine turnover was used as an index to measure protein metabolism. This was done and evaluated according to Yousef and Johnson (1970 a,b); Yousef and Luick (1969). Using ^{75}Se-selenomethionine as a tracer, we must point out that its reutilization is comparatively high. Thus the present indication "apparent" (biological half-life) seemed to be essential.

For histologial examinations, small parts of the liver, spleen and kidney were fixed in 96% ethanol, embedded in paraffin. The 4 μ sections were stained with haematoxylin-eosin, Congo-red, gentiane-violette and PAS. For electronmicroscopical investigations, small pieces of the spleen were freshly fixed in cold glutaraldehyde, then in osmium tetroxide, finally embedded in Araldite. Ultrathin sections were prepared by LKB-Ultratrome III, contrasted with lead citrate and examined in a JEM 100 C electron microscope. The electronmicroscopical examinations were used for verification of the light microscopical observations. Data were evaluated by using the Student "t" test and the level of significance was chosen as $p < 0.05$.

RESULTS

Figure 1 shows the body weight changes of the untreated (control) and poly(A)-treated mice during the experiment. (The animals were treated with poly(A) from the 6th month

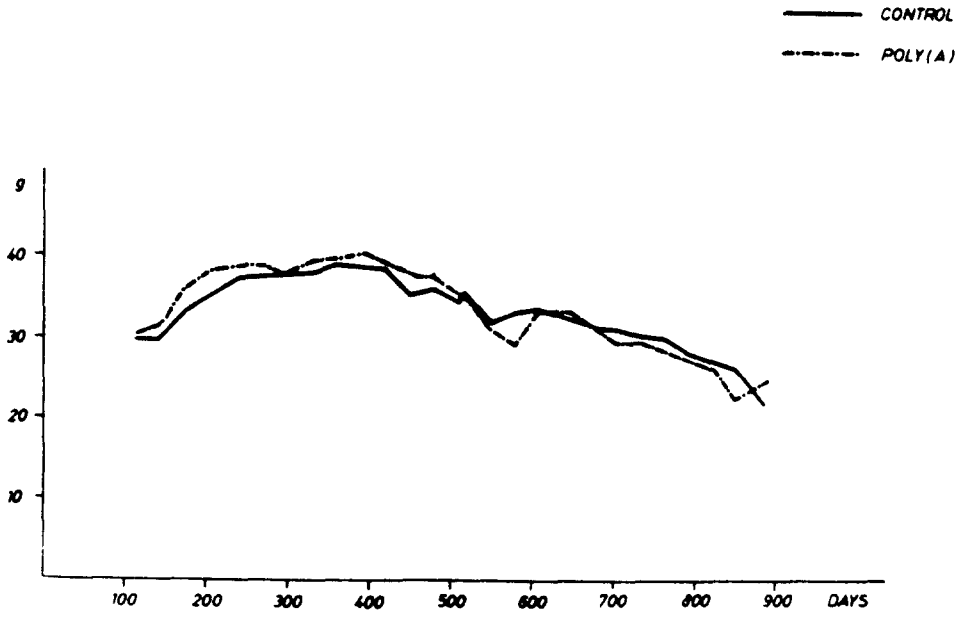

FIG. 1. Body weight changes of mice.

onwards.) The data indicate that there were no considerable differences among the body weights of the control and experimental groups.

Figure 2 demonstrates the changes in body (rectal), temperatures of the control and poly(A)-treated animals. One might observe that there is a decreasing trend of rectal temperature as time progresses (first columns of each age-group). As the figure shows, the experimental group reveals to some extent different temperature values, after the 1 hour cold exposure than that of the controls. (These temperature values are shown by the second and third column of each age-group. Comparatively similar standard deviations were measured in the poly(A) group as well; however, these are not drawn for the sake of better comprehension.) All the controls decreased (during cold) and increased (after cold) their rectal temperature significantly ($0.01 > p$). The response to the cold of the poly(A) treated animals were found more markedly in the last two age-groups ($0.05 > p$).

Figure 3 discloses the biokinetical data of the ^{75}Se-selenomethionine metabolism of the two groups. The data clearly demonstrate that the $T_{½}$ (biological half-life) values increase in all animals as time advances, but not at the same rate. In the case of the controls the half-life values elevated nearly linearly with increasing age, however, the augmentation of the $T_{½}$ values is lessened in the poly(A) treated mice. The difference between the controls and experimental group becomes more marked as time advances thus the variance proved to be significant ($0.05 > p$) at 23.2 month of the experiment. No significant changes were recorded at 27.8 month, possibly due to the relatively small number of animals.

The mortality curves are demonstrated in Fig. 4. The highest mortality was observed in the poly(A) group when the animals reached their age of roughly 1½ years. The percentile survivors of the controls were apparently more, namely by 10%.

These findings can partly be explained by the main pathological observations which are

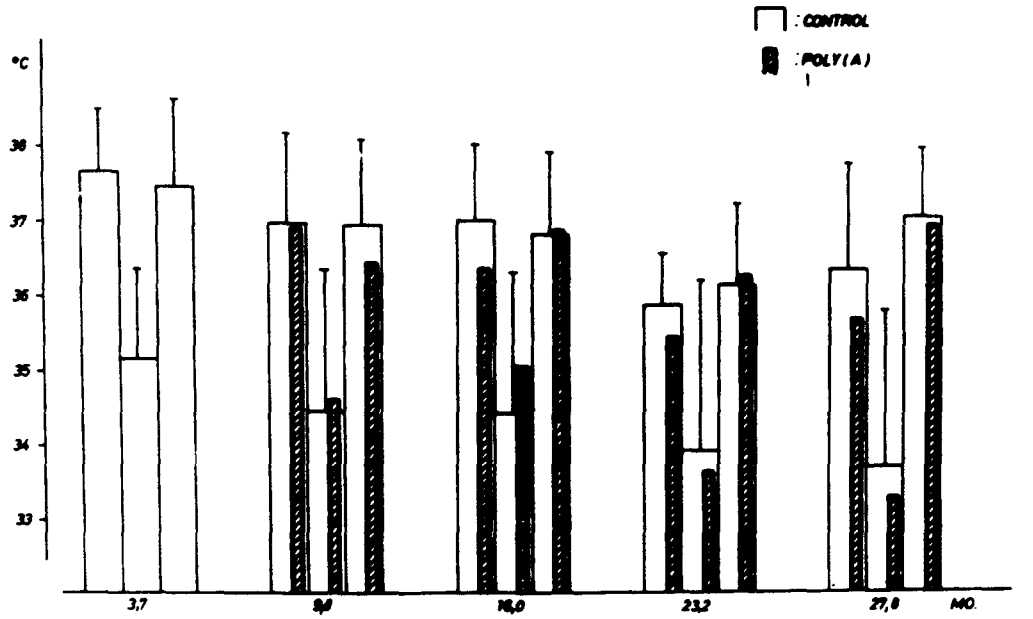

FIG. 2. Changes in body temperature (Mean ± SD).

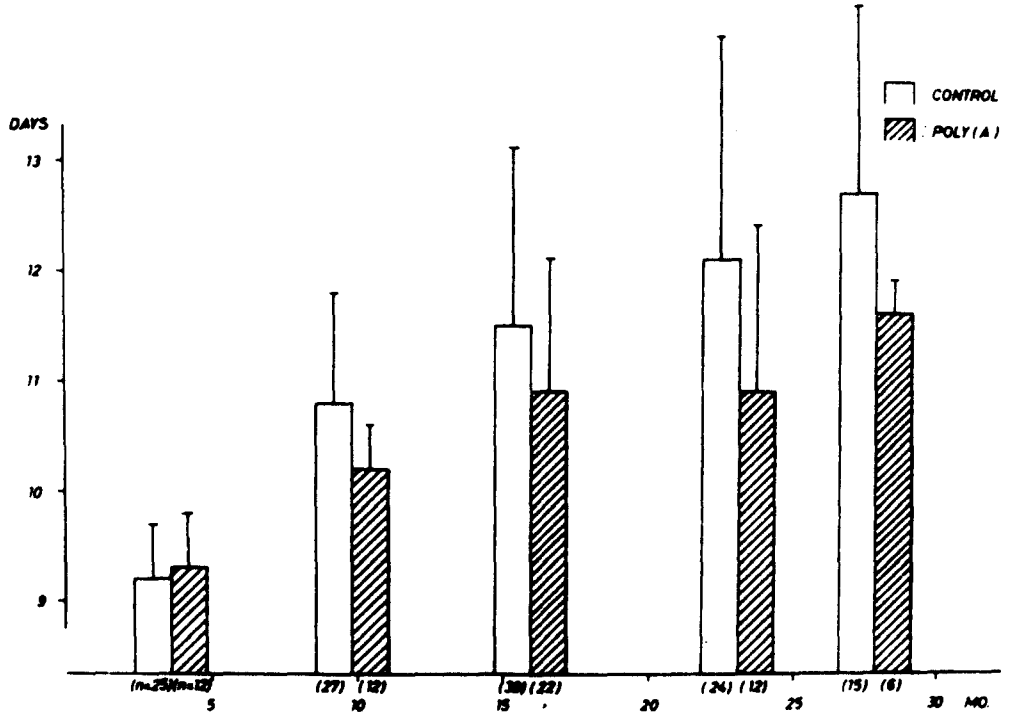

FIG. 3. Apparent biological half life (^{75}Se-selenomethionine $T_{1/2}$).

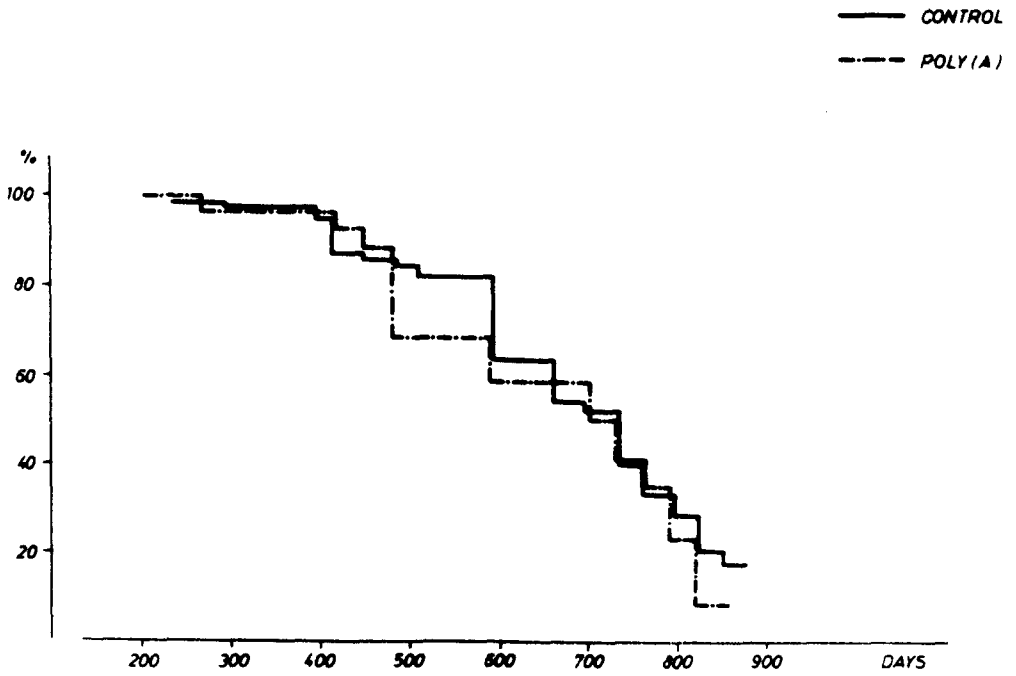

FIG. 4. Percent survivorship vs. age.

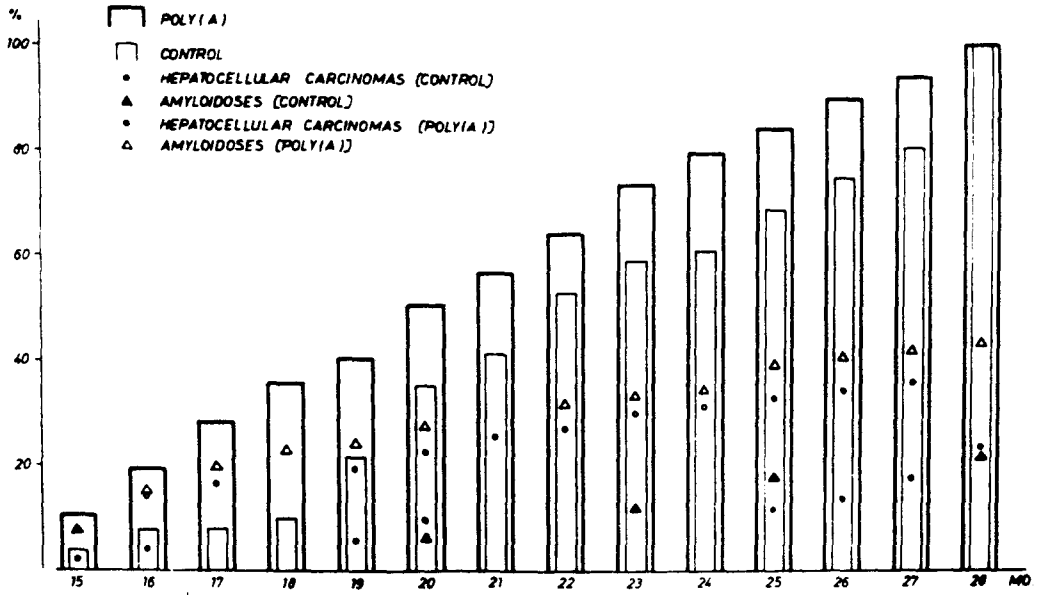

FIG. 5. Age-dependent pathological alterations.

shown in Fig. 5. The poly(A) group exhibited a significantly greater occurrence of age-dependent pathological alterations of which the proportion of hepatocellular carcinomas and amyloidoses proved to be considerably higher than that of the controls. On the other hand, these changes occurred much earlier than in the untreated, control mice.

DISCUSSION

The results show that the polyadenylic acid exhibited a controversial effect on the parameters investigated in the present study. In respect to the cold tolerance of the animals, all mice kept the rectal temperature in a similar way beyond the age of 93 weeks. In old age, there seems even a mild hypercompensation after the cold exposure. It should be added, however, that the poly(A) treated mice tolerated the cold stress more efficiently. According to the relatively low number of the experimental animals, the poly(A) treated mice were roughly half so many as that of the controls, the differences between the rectal temperatures were not so explicitly significant $0.02 > p$) than that of the untreated animals ($0.01 > p$). On the whole, the control mechanisms change as the animals age.

Furthermore, the data show that the biological half-life of ^{75}Se-selenomethionine increases (approx. by 40%) in the control ranging from 3.7 to 27.8 months of age. This is in accordance with the findings of Sobel and Bowman (1971) who established that following injection with ^{14}C-lysine the $T_{½}$ value increased more than 30% in male mice ranging in age from 320 to 750 days of age.

One of the main conclusions of this work is that the apparent protein turnover decreases most dramatically in young and young-adult mice up to 480 days of age and then remains relatively less changed beyond the ages of 16 months. This finding fairly harmonizes with the observations of Yousef and Johnson 1970(a) who established that protein metabolism, as measured by the ^{75}Se-selenomethionine turnover rate method, shows a

decreasing trend during lifespan of the rat. We have to add to these, that the greater apparent protein turnover corresponds to the rapid body weight gain of mice, roughly in the first one-third of the lifespan concerned. Comparatively less elevation in apparent biological half-life ($T_{½}$ in days) in poly(A) treated mice was observed during the course of the 830-day experimental period.

Poly(A) administration alone does not confirm the findings to better life performance as that of the beneficial effects of double-stranded polynucleotides. Despite its anti-senescence influence, in the sense of accelerating the apparent protein turnover, poly(A) shortens lifespan because of the higher mortality rates; i.e., increased aging is a by-product of increased metabolism. The present data thus confirm the fact that the metabolic factor can influence the onset and progress of age-dependent diseases (amyloidoses and hepatocellular carcinomas).

This metabolic change could partly be attributable to the altered condition of the thyroid. It is known that in senescence there is a decrease in thyroid function. According to Eleftheriou (1975) the constant decline in rectal temperature of inbred mice directly correlates to thyroid activity. The higher apparent protein turnover in the present case indicates that some intrinsic factors within the gland or a higher responsiveness to the levels of TSH even to TRH cannot be ruled out.

From the present observations it seems very probable that the characteristics already mentioned as well as the immune restoring actions previously described of one of the substances in question depend firstly on the double-stranded polynucleotide molecule as a whole and not on one component only, like polyadenylic acid.

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