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INCREASED LONGEVITY OF KINETIN-FED *ZAPRIONUS* FRUITFLIES IS ACCOMPANIED BY THEIR REDUCED FECUNDITY AND ENHANCED CATALASE ACTIVITY

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Summary: Kinetin, a cytokinin plant growth hormone, retards senescence in plants, delays aging in human cells in culture, slows down development of insects and prolongs their lifespan. We have now observed that the increased longevity of Kn-fed *Zaprionus* fruitflies was accompanied by an increase in the specific activity of catalase during developmental stages and in adult insects. In addition, the egg laying capacity of Kn-fed fruitflies was reduced drastically as compared with those kept on a normal diet. These results support the view that improved maintenance of the soma and prolongation of its life is achieved at the cost of decreased reproductive activity.

Key words: aging, anti-aging, lifespan, kinetin, cytokinin, antioxidants, insects

INTRODUCTION

Anti-aging effects of kinetin (Kn), a cytokinin plant growth hormone, have been reported for several plants and normal human diploid fibroblasts in culture (1, 2). Previously, we had reported that Kn retarded aging and prolonged the lifespan of the fruitfly *Zaprionus paravittiger* when these insects were fed with 25–125 ppm Kn added in their diet medium (3). Kn also slowed down the developmental processes and prolonged the larval and the pupal stages of the developing insects (3). The anti-aging effects of Kn were not merely due to the slowing-down of development, but were mainly due to a reduction in the age-specific death rates throughout the adult lifespan. This implies that Kn has certain protective effects on the body, which reduce the rates of aging and death in these insects. Therefore, we undertook studies in order to find out what other physiological and biochemical alterations occur in Kn-fed animals, which may explain their increased longevity and improved maintenance of the body.

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In this study, we report the effects of Kn feeding on the egg laying capacity and catalase activity of *Zaprionus* fruitflies. The reasons for studying these two parameters are based in modern theories of aging and longevity. For example, an inverse relationship between reproductive activity and lifespan is predicted by the so-called disposable soma theory, according to which an extension of lifespan and improved maintenance of somatic cells will reduce resources available for the germ line (4). We have tested this proposition by studying the effects of Kn on the fecundity of these insects.

Another way in which the life-prolonging effects of various compounds have been observed is by an increase in the activities of antioxidative enzymic defence systems. This is because antioxidants constitute a crucial component of the normal maintenance and repair network which assures the growth, development and survival of an organism (5). A large number of studies have shown that an extension of lifespan by chemical treatments, nutritional modulation and gene manipulation is almost always accompanied by stimulation of various antioxidant enzymes, such as superoxide dismutase, glutathione and catalase (6). Here we report the stimulatory effects of Kn on the activity of catalase during development and adult life of *Zaprionus* fruitflies.

MATERIALS AND METHODS

Zaprionus indianus (Diptera; Drosophilidae), previously referred to as *Z. paravittiger,* were reared on standard corn meal agar medium, as described earlier (3). After starting the stock culture, the flies were taken through four generations of inbreeding in order to obtain a relatively homogenous population. Stock cultures of flies were maintained in culture flasks (about 250 flies per flask) containing diet medium in an incubator at 26 ± 2 °C with a light:dark cycle of 12 h each. The flies were transferred to the fresh medium after every 5 days.

For lifespan studies, several hundred freshly emerged flies were distributed in glass vials (2.5 cm diameter) so that each vial contained 5 male and 5 female flies. Stock solution of Kn (highest grade from Serva, Germany) was prepared by first dissolving Kn in 0.1 N NaOH followed by its addition to the diet medium at final concentrations of 6.25, 12.25, 25, 50 and 100 ppm (mol. wt. of Kn is 215). Pilot experiments with various amounts of NaOH equivalent to those present in the Kn medium did not show any harmful effects on the growth, survival and lifespan of the fruitflies. Effects of Kn on the duration of developmental phases and the adult lifespan were determined as described previously (3).

Fecundity of adult flies fed on the Kn-supplemented medium was determined by counting the number of eggs laid everyday. For this purpose, a large number of vials were set with newly emerged (0–24 h old) one male and one female fly per vial, which were fed with various concentrations of Kn as described above. The number of eggs laid were counted every 24 h and the flies were transferred to new vials until one member of the pair died.

Catalase (EC 1.11.1.6) enzyme activity was determined in whole body homogenates of fruitflies as described previously (7, 8). Briefly, the homogenates (0.2% w/v) were prepared from the flies at various stages of development and

centrifuged at 7000 x g for 10 min at 4°C. The extracts thus prepared were assayed for catalase activity by measuring the amount (μmol) of H_2O_2 degraded per 100 mg tissue per min at 25°C. The remaining undegraded H_2O_2 was titrated out with 0.1 N solution of $KMnO_4$ until a light pink colour appeared.

RESULTS

Feeding of *Z. indianus* with various amounts of Kn had dramatic effects on its development, aging and longevity. Table 1 shows the median and maximum lifespans for male and female *Z. indianus* in the presence or absence of various concentrations of Kn in their diet medium. In this series of experiments, the median (LT_{50}) and the maximum (LT_{100}) lifespans of the control male flies were 40 ± 3 days and 67 ± 2 days, respectively. Similarly, the median and maximum lifespan of the control female flies were 37 ± 1 days and 59 ± 4 days, respectively. The addition of various amounts of Kn in the diet medium affected the lifespan of *Z. indianus* (Table 1). Between the doses of 12.50 and 50 ppm, Kn-fed fruitflies had their median and maximum lifespans increased by 60% and 25%, respectively. At 6.25 ppm concentration, although the Kn-fed insects had about 20% extension of maximum lifespan, their median lifespan was not affected significantly. Kn at 100 ppm and above was life-shortening for *Zaprionus* fruitflies, and their median and maximum lifespans were reduced by 30–50%.

The effects of Kn on the developmental period and duration of various phases were also observed. The post-zygotic developmental period of *Z. indianus* is comprised of three larval instars (L_1, L_2 and L_3), two pupal stages pre-pupa (P_1)and pupa (P_2), followed by the emergence of adult flies. As reported previously for *Z. paravittiger,* the developmental period of *Z. indianus* in the Kn-containing diet medium increased from 141 h to 173 h at 25 ppm Kn and to more than 200 h at 50 ppm Kn and above (data not shown).

Extended longevity of Kn-fed fruitflies was accompanied by a reduction in their egg laying capacity. Figure 1 shows the average number of eggs laid per day by *Z. indianus* females during their adult life. Kn-fed insects had not only significantly reduced overall fecundity, but also their active period of egg laying was reduced along with a shift towards an early age for maximum activity. For example, whereas the control fruitflies laid a total of 170 eggs in 21 days with a maximum egg laying on the day 6, the flies fed with 25 ppm Kn (which was the optimum concentration for lifespan extension) laid only 56 eggs in 17 days, and had a maximum egg laying activity of 12 eggs on the day 4. Similar alterations in fecundity can be seen at all levels of Kn concentrations tested in this series of experiments (Fig. 1).

Table 1. Median (LT_{50}) and maximum (LT_{100}) lifespans (mean ± SD) of *Zaprionus indianus* reared on various concentrations of kinetin

Kinetin (ppm)	Median lifespan (days) male	Median lifespan (days) female	Maximum lifespan (days) male	Maximum lifespan (days) female
0	40±3	37±1	67±2	59±4
6.25	39±3	35±1	80±2**	79±2**
12.50	46±4**	49±3**	71±12	69±8*
25.00	64±1**	59±4**	84±2**	72±1**
50.00	43±7	43±4**	84±3**	73±7**
100.00	31±1**	35±3*	40±1**	54±3*

* = $p < 0.01$ and ** = $p < 0.001$ significantly different from the control.

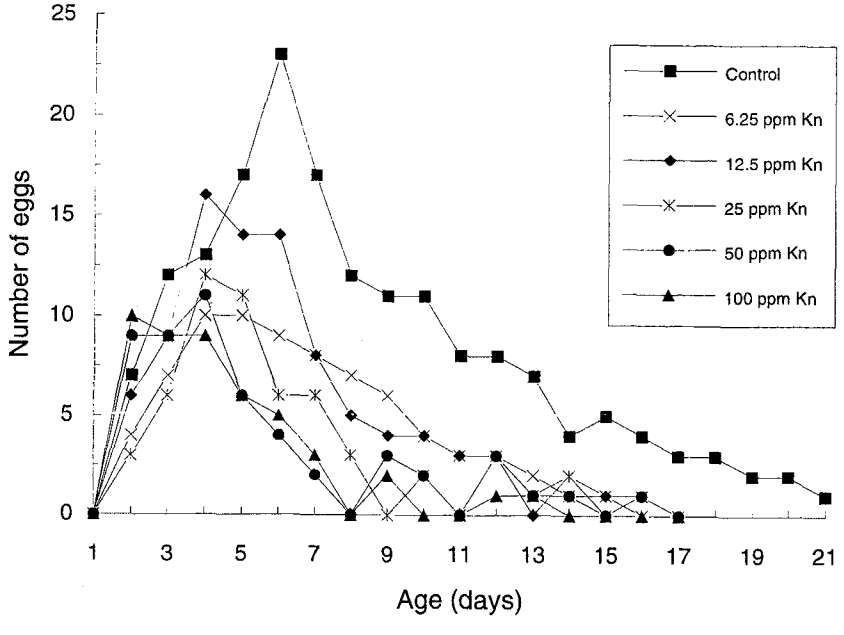

Figure 1: Average number of eggs laid per day by Z. indianus females fed on various concentrations of kinetin during their adult life.

The differences in catalase activity in developing larvae, pupae and freshly emerged adult *Z. indianus* fruitflies cultured on a diet medium in the absence or presence of various concentrations of Kn are shown in the table 2. At all stages of development, Kn-fed fruitflies had higher levels of catalase activity than control flies. For example, at the optimum concentration of 25 ppm Kn, there was an increase of more than 57% in the catalase activity of Kn-fed L_2 larvae as compared with controls. This difference became maximum (2.2 fold) at the pupal stage P_2 (Table 2). Freshly emerged adults also had significantly different levels of catalase activities in Kn-fed and control flies. Although these differences were not as big as those during developmental stages, Kn-fed adult males had between 5% and 84%, and females had between 8% and 92% higher levels, depending upon the concentration of Kn in the medium (Table 2).

DISCUSSION

We had previously reported that 25–50 ppm Kn in the diet medium of *Zaprionus* fruitflies prolonged their developmental period and increased their lifespan (3). We have now extended these studies and checked even lower concentrations of Kn in

Table 2. Effect of kinetin on the specific activity* (mean ± SD) of catalase in *Zaprionus indianus* during development and adulthood

Developmental stage**	Kinetin concentration (ppm) in the diet				
	Control	12.5	25	50	100
Larva L_1	380±4	427±40^b	431±7^b	384±6	388±1^b
Larva L_2	228±40	323±8^b	375±6^b	383±1^b	298±2^b
Larva L_3	93±8	182±7^b	178±2^b	133±1^b	218±2^b
Pre-pupa P_1	86±1	95±1^b	188±1^b	187±2^b	204±4^b
Pupa P_2	88±8	134±5^b	191±2^b	218±1^b	302±5^b
Adult male flies	146±6	168±8^b	151±2^a	169±2^b	269±1^b
Adult female flies	138±2	168±4^b	149±2^a	165±2^b	266±2^b

* µM of H_2O_2 decomposed/100 mg tissue/min at 25° C. **see main text for explanation of various developmental stages. ^{a} = $p<0.01$ and ^{b} = $p<0.001$ signficantly different from the control.

terms of its effects on development and lifespan, and have observed additional effects on the fecundity and antioxidant enzyme activities. In confirmation with our previous observations, 25 ppm Kn in the medium appears to be the most effective anti-aging and life-prolonging dose for fruitflies. Higher concentrations, especially 100 ppm and above, have life-shortening and toxic effects on these insects. This is not a surprising observation since it is known that the anti-aging effects of Kn on human cells are best seen within a concentration range of 40–200 μM, which is equivalent to about 8–40 ppm, above which Kn begins to inhibit cell growth (2). One of the reasons that the insects could tolerate concentrations of Kn somewhat higher than those tolerated by human cells in culture may be that the cells *in vitro* were directly exposed to the hormone present in the culture medium, whereas the insects ingested it through food. However, at present nothing is known about the kinetics of uptake, absorption and accumulation of Kn *in vitro* and *in vivo* in any cells and organisms.

An analysis of detailed life-tables of Kn-fed and control flies shows that the life-prolonging effects of Kn are not merely due to a slowing down of the developmental stages, but are mainly due to the reduction in age-specific death rates and slowing-down of aging. This suggests that Kn prevents early deaths either by maintaining the efficiency of various repair and defence systems or by reducing the accumulation of metabolic defects and debris that may have harmful effects on cellular physiology and biochemistry.

Since it has been suggested that Kn has antioxidant properties (2, 9), we have tested this possibility by measuring the levels of an antioxidative enzyme catalase in the larval, pupal and adult stages of *Zaprionus* flies. A significant enhancement of catalase activity was observed in Kn-fed flies as compared with controls. If the catalase activity-enhancing effects of Kn on *Zaprionus* flies are compared with those of other antioxidants, Kn is a more potent antioxidant than propyl gallate (22% enhancement) (7) and vitamin E (20% enhancement) (8), but is less potent than methionine (2-fold enhancement) (10). Interestingly, maximum enhancement of catalase activity by Kn was observed at 100 ppm concentration which does not have any life-prolonging effects and actually reduces the lifespan of these insects. These results once again underline an important biological rule that increasing the levels of the so-called "good" things may not always have positive effects over the long term.

Our observations that the increased longevity of Kn-fed fruitflies is accompanied by a significant reduction in their fecundity support the main prediction of the disposable soma theory of aging. According this theory, there is an inverse relationship between reproductive activity and lifespan in the sense that an extension of lifespan and improved maintenance of somatic cells reduces resources available for the germ line (4). Various lines of evidence from studies on life-extension of

insects and other animals by either nutritional modulation, chemical treatments or genetic selection of natural or induced mutants show that an increase in longevity is almost always achieved at the cost of reduced fecundity, fertility and total reproductive activity (11). Although the biochemical and molecular mechanisms of this effect are not known at present, results obtained in our studies give further support to this relationship. Clearly, additional studies are required in order to elucidate the ways in which Kn brings about its various biological effects, including the slowing-down of development, delaying the onset of aging, reducing the reproductive activity and prolonging the lifespan of *Zaprionus* fruitflies.

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