THE EFFECT OF NUTRITIONAL REGIMES UPON COLLAGEN CONCENTRATION AND SURVIVAL OF RATS

Z. Deyl, M. Juřicová and E. Stuchlíková

Czech. Acad. Sci. and Fourth Med. Dept. Charles Univ.

Prague, Czechoslovakia

It is generally believed that food restriction of experimental animals in the adult age does not alter the expected lifespan of the animals. Already the early experiments of McCay and coworkers (1) showed that no further increase in the lifespan could be brought about by restricting the food intake in adult animals, namely in rats beyond one year of age. Some other data on the same topics were published by Barrows and Roeder (2) who reported that rats that were subjected to limited food intake in the adult age did not survive longer than their ad libitum fed counterparts. Similar effects were observed also in insects. So for instance Kopeć (3) was unable to prove the increase in the lifespan of the imago of Drosophila if the imagos were subjected to reduced dietary intake. In connection with this latter experiment Northrop (4) proved that the overall increase in lifespan of Drosophila can be accounted for by the changes which occur during the larval stage. In a series of more recent experiments Barrows (5) studied the overall lifespan of some rotifers using different types of restricted diet. This author observed that in some cases animals which were food restricted throughout their whole lifespan lived shorter than those which were kept on a food restricted regime after cessation of egg production. These latter animals increased their lifespan by approximately one quarter. Barrows (5) concludes that under certain conditions the lifespan of animals can be increased even when dietary restriction is imposed later in their life. In order to explain this latter statement, Barrows (5) proposes that our present knowledge due to the paucity of data regarding the effect of the degree of dietary restriction and the age at which the dietary restriction is imposed to alter the lifespan can hardly offer a univocal statement about life prolongation. Another problem which is connected with the increase of the lifespan of animals deals with the mechanism and proposals that

could be offered to explain the increased lifespan of food restricted animals. It is possible that the programmed genetic events which occur throughout the life of an organism are influenced by sequential synthesis of specific regulatory RNA and proteins and that the regulation of the genetic information transfer therefore may possibly be controlled by the synthesis of specific RNA and proteins at various times throughout the lifespan of an organism (5). From this presumption he proposes that a possible way to delay senescence especially if it results from the genetic expression would be to decrease the synthesis of specific RNA or protein during the life of an organism. From this point of view one could expect that restricting of the dietary intake would delay the occurence of these specific events during the early life and consequently result in life prolongation. It should be said, of course, that this is a conceivable speculation but nothing more than speculation since there are no direct experimental data that would prove this statement.

Among the different specific events which occur during the individual lifespan and can be considered as markers of aging is the rate of accumulation of fibrous proteins of the intercellular matrix. In a previous paper we have been able to show (6) that there is a distinct increase in the collagen concentration in lung, liver and kidney in rats around 15 months of age. In organs investigated in that paper no distinct organ specificity was observed. In undernourished animals by which we mean fifty per cent food-restricted individuals the same phenomenon was delayed until the age of 25 months. The starting point of collagen accumulation is followed by an evident increase in the mortality rate. The shift of the starting point of collagen accumulation between control animals and food restricted animals is exactly the same as shift of the average lifespan of undernourished and fully fed animals.

MATERIALS AND METHODS

Male rats of the Wistar strain, golden hamsters and mice were used throughout this investigation. All animals were kept in separate cages populated with a single animal only. Undernourished animals were given exactly one half of the amount of food consumed by controls. The experiment was divided into four parts. Control animals were fed ad libitum throughout their whole life (Group No. 1). In the group No. 2 animals were food restricted throughout the whole life. In the group No. 3 animals were kept on full diet when young and food restricted when adult and in group No. 4 the regime was reversed. Animals were food restricted when young and fully fed when adult and old. Each experiment started with 100 animals except for rats which were 25 at the beginning. Survivals and in the case of rats weights were registered throughout the whole life and results are summarized in the Results.

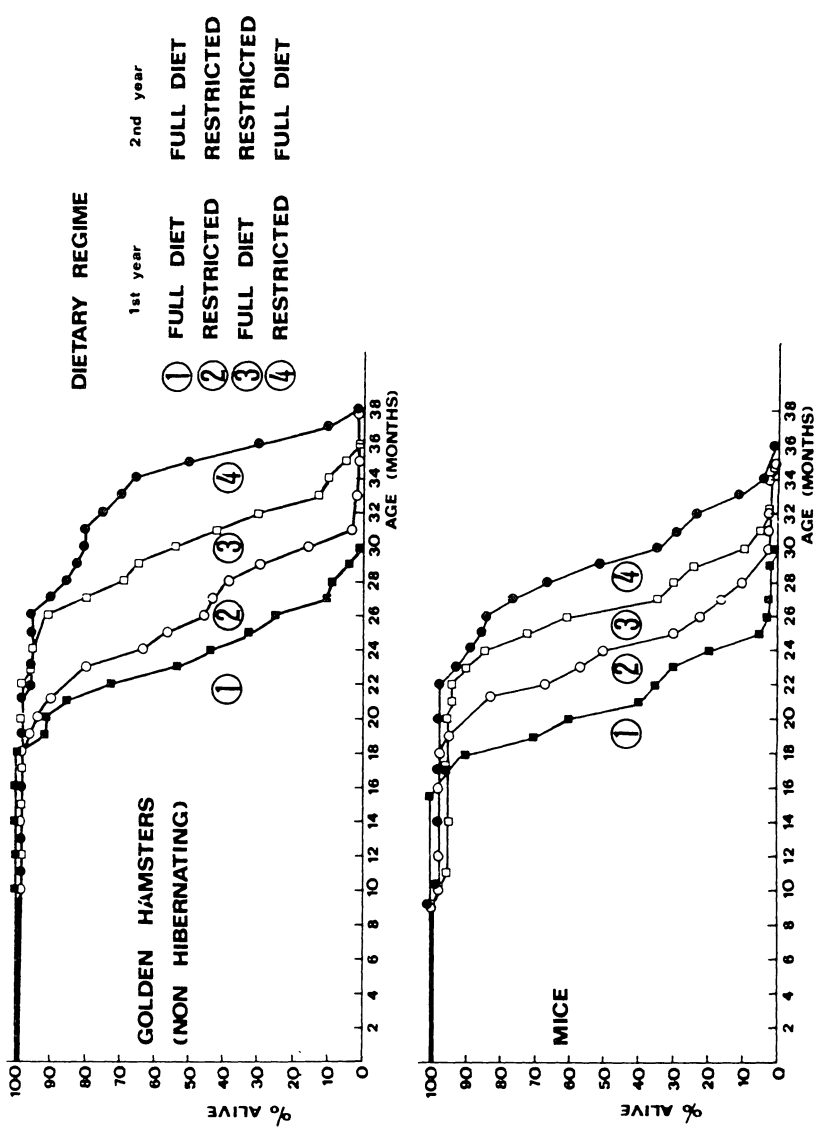

Fig. 1a. Survival curves for golden hamsters, mice and rats kept on different dietary regimes as indicated.

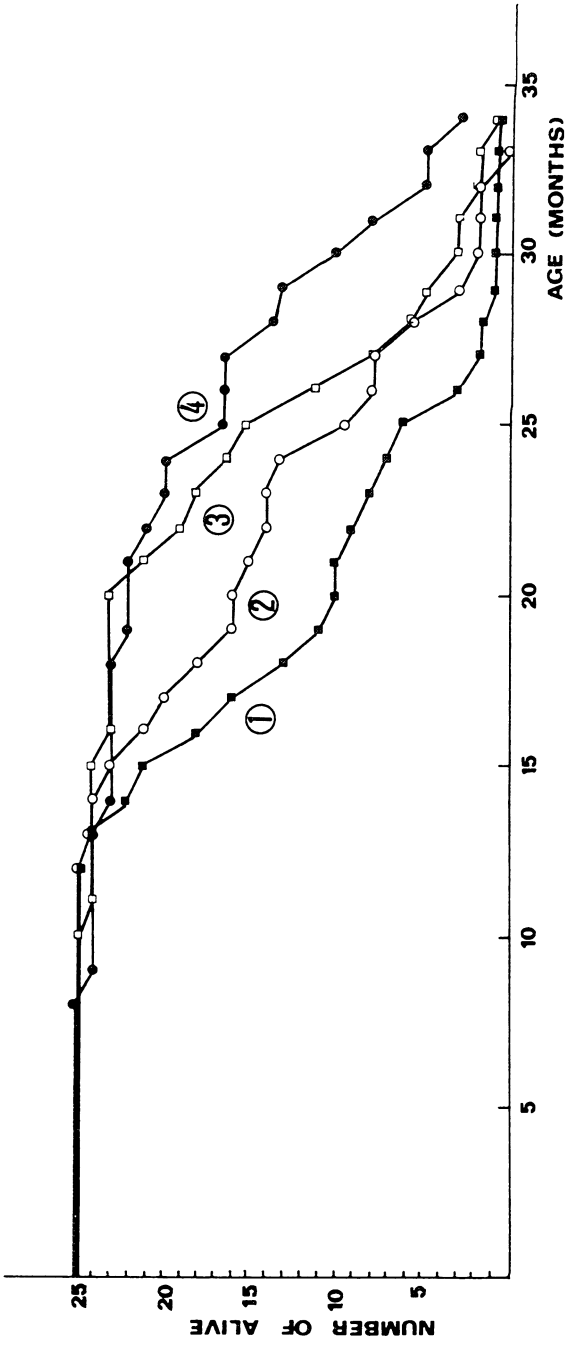

Fig. 1b. Survival curves for golden hamsters, mice and rats kept on different dietary regimes as indicated.

Another series of rats divided into groups (100 animals each) one fed ad libitum and the other food restricted during the first year and fully fed thenafter was used for the estimation of collagen concentration in kidneys and liver. Animals were killed at definite intervals (always three randomly selected individuals) and the collagen level in the respective organs and their weights were estimated. For collagen determination the procedure published by Stegeman (7) was used.

RESULTS AND DISCUSSION

As it is evident from Figure 1, food restricted animals had always a better survival than animals kept on a full diet. In agreement with previously published data no substantial increase in the maximum survival was observed in any of the three studied species. However, this is due to the fact that some few of the ad-libitum fed animals survived much longer than the vast majority of the population.

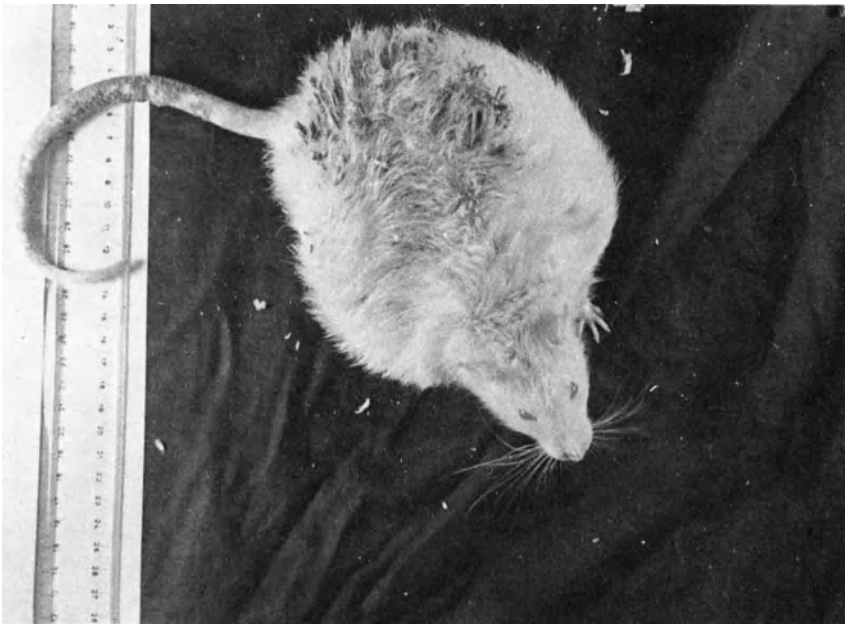

Fig. 2. A typical portrait of a rat food restricted during the first year of life and fully fed thereafter. The individual was 28 months old when the picture was taken.

TABLE I. 50% Survivals in Golden Hamsters, Mice and Rats Kept on Different Dietary Regimes. Numbers in Parenthesis Indicate the Increase in the Age of 50% Survivals in Months.

	Age of 50% survival (months)				Relative increase to fully fed (%)		
Regime 1st year 2nd year	Full diet Full diet	Restricted Restricted	Full diet Restricted	Restricted Full diet	Restricted Restricted	Full diet Restricted	Restricted Full diet
Golden hamsters	23.3	25.5 (2.2)	30.3 (7.0)	35 (11.7)	9.44	30.1	50.1
Mice	20.5	24 (3.5)	26.4 (5.9)	28.4 (7.9)	26.8	28.8	38.5
Rats	18.3	24.2 (5.9)	26.1 (7.8)	29.4 (11.1)	32.2	42.6	60.6

The average lifespan and 50% survival in undernourished animals was increased quite distinctly. Though the data of the relative increase of the 50% survival are somewhat different when comparing rats, mice and hamsters as indicated in Table I, the general order of individual dietary regimes in all species investigated is the same. By far, the best results with respect to survival were observed in animals which were food restricted during the first year of life. In all cases the shortest survival was in animals which were fully fed throughout the whole life. The increase in the average lifespan of those animals which were food-restricted when adult and old is about thirty per cent. Those animals which were food restricted when young and fully fed when old, developed a certain degree of hyperphagia when they were allowed unlimited amounts of food. These individuals grew up very fat as indicated in the attached figure (Fig. 2). Their average weight after one month of realimentation was over 700 g and in this experimental group there were animals (rats) which approached the total body weight of one kilogram (Fig. 3). This extreme body weight caused of course a considerable restriction in their ability to move, but in spite of this, these animals did very well from the viewpoint of survival. The increase in 50% survivals was always over 35%. In a preliminary experiment which followed these long term studies just reported, we have used two different types of reducing agents in order to elucidate the question whether the food restriction is somewhat related to the oxidative free radical attack on long chain macromolecules. Both half per cent (0.5% w/w) addition of ethoxyquin to the standard diet and reduced diet and similar addition of tert-butyl hydroxytoluene resulted in a considerable increase not only of the average lifespan but also of the maximum lifespan. In control animals which were offered free access to the antioxidant enriched diet the increase in maximum lifespan was almost two hundred days and was somewhat lower in 50% survival. In animals which were subjected to some of the food restricted regimes as indicated in Figure 4 the effect of reducing agents was quite similar. To our opinion several conclusions can be drawn out from the above reported experiments. It can be concluded that from the environmental factors nutrition is quite important and it is clearly demonstrated that the onset of food restriction is always very efficient. It is also demonstrated that the effect of undernutrition is not identical with that of reducing agents since combination of both induced an additive increase in lifespan.

When comparing the collagen concentrations in kidneys and liver of fully fed animals and those which were fully fed during the first year and food restricted thereafter the onset of the increase in collagen concentration has been postponed accordingly so that it appeared near the age of 30 months while in controls it could be observed between 10 and 15 months of age. In accordance with our previous experiments (6) this onset appears roughly ten months before 90% of the population dies out.

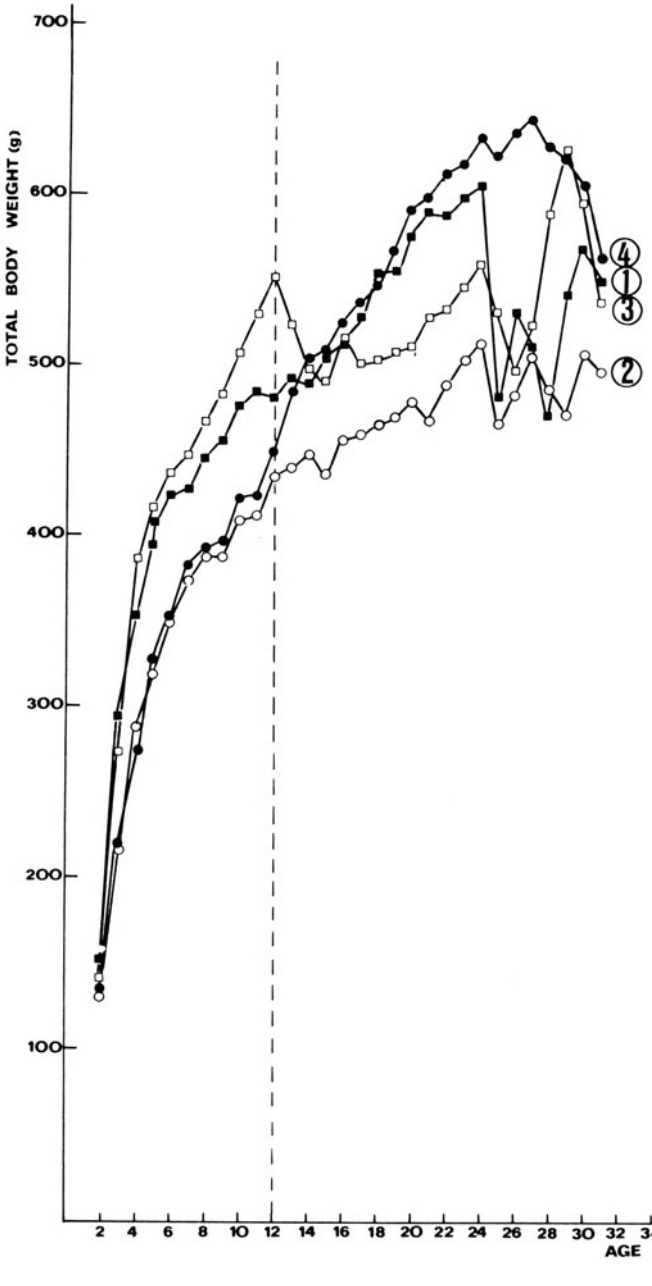

Fig. 3. Average body weights of rats kept on different dietary regimes.

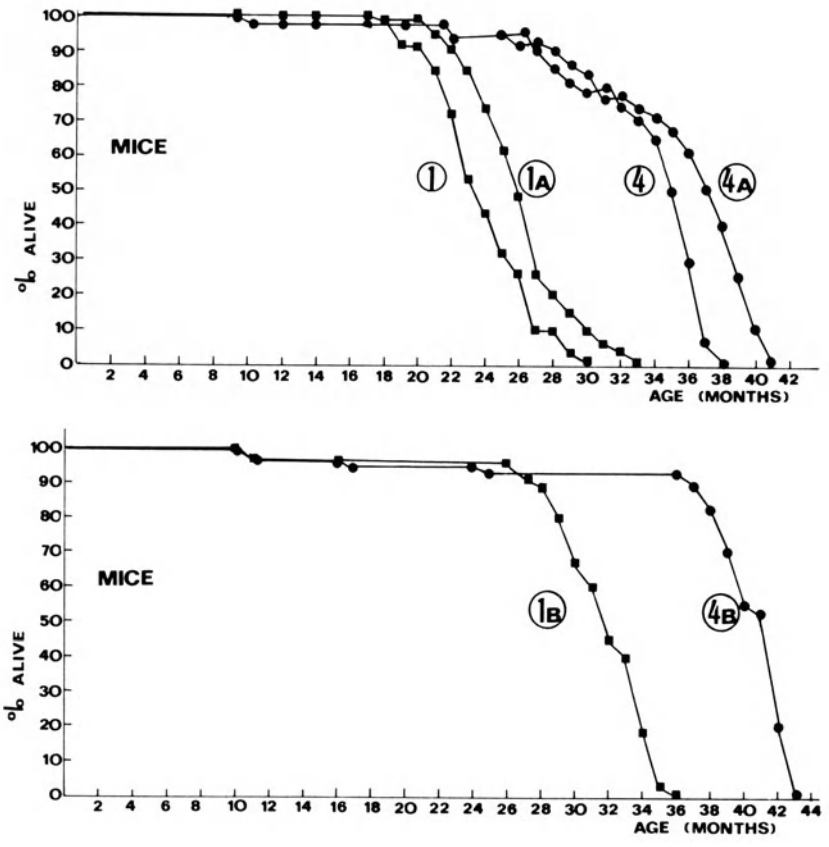

Fig. 4. Survival curves of mice kept on full access to food (1) and restricted during the first year of life (4) in comparison with animals kept on the same regimes to which 0.5 w/w per cent ethoxyquin (indicated by A) and the same amount of tert-butyl toluene (indicated by B) was added.

The data accumulated throughout this work are indicative of the fact that a slowdown in metabolic processes, deprivation of redundant energy and possibly more effective use of energy supplies including more efficiently used transcriptions at the DNA-RNA level are the most easily accessible ways of life prolongation in experimental animals. However, in order to be fair with the published data, no data have been accumulated which would allow a rational explanation of the observed phenomena.

SUMMARY

It has been demonstrated that food restriction put upon animals at any stage of the individual's life, if chronic, produces a distinct increase in the lifespan. This can be effected in youth and still have a distinct effect in old age even if the food restriction is cut down and for the rest of the life the animal is allowed food ad libitum. Since the effect upon the aging of the animal is delayed on the time scale, it is obviously an effect which is stored somewhere and it is suggested that this storage occurred somewhere along the DNA-RNA pathway. Also the effect of undernutrition is not identical with that of oxidizing free radical blocking agents and therefore it is concluded that the food deprivation does not minimize the attack of the free radicals on the long chain macromolecules and as a matter of fact it seems that the proportion by which free radicals contribute to the changes in the average lifespan in undernourished and fully nourished animals is small. It has been also demonstrated that the addition of reducing agents to normal diet and to the diet of food restricted animals increases the average and maximum lifespan in both cases practically to the same extent, which supports the idea expressed before. This feeding effect has been observed in three different species of rodents and no extrapolation has been done to other types of mammals. Due to the data published on this topic and dealing with rotifers and some insects (2) it is conceivable to conclude that the effect of undernutrition is general and is not limited to the food restriction in the early stages of development only.

Collagen starts to accumulate in the kidneys and liver of experimental animals roughly ten months before 90% of the population dies out. Thus an increase in collagen concentration can be indicative of involutional changes in the organ (and perhaps organism). These data are i- good agreement with previously published results on the relation between collagen accumulation and chronic food deprivation in rats (6). It can be also concluded that food deprivation induced in the adult, though not so effective in life prolongation as the food restriction during early development, still can increase survival in experimental animals to a high degree.

REFERENCES

1. McCay, C.M., Crowell, M.F. and Maynard, L.A. 1935. Effect of retarded growth upon length of life-span and upon ultimate body size. J. Nutr. 10: 63.

2. Barrows, C.H. Jr. and Roeder, L. 1965. The effect of reduced dietary intake on enzymatic activities and lifespan of rats. J. Gerontol. 20: 69.

3. Kopeć, S. 1928. Influence of intermittent starvation on longevity of imaginal stage of Drosophila melanogaster. Brit. J. Exp. Biol. 5: 204.

4. Northrop, J. 1917. The effect of prolongation of the period of growth on the total duration of life. J. Biol. Chem. 32: 123.

5. Barrows, C.H. Jr. 1972. Nutrition, aging and genetic program. Amer. J. Clin. Nutr. 25: 829.

6. Deyl, Z., Juřicová, M., Rosmus, J. and Adam, M. 1971. The effect of food deprivation on collagen accumulation. Exp. Geront. 6: 383.

7. Stegeman, H. 1958. Mikrobestimmung von Hydroxyprolin mit Chloramin-T und p-Dimethylaminobenzenaldehyd. Hoppe-Seylers Zeitschrift für Physiologische Chemie 311: 41.

QUESTIONS TO DR. DEYL

Dr. Martin: If I read the slides correctly, there was an increase in mean survival for the rat with your various regimes. There did not appear to be an increase in the maximum lifespan in contrast to the findings with mice and hamsters.

Dr. Deyl: I would say that the only effect is the increase in the average lifespan. The difference in the slides I showed is based on the fact that in a population of animals you always find a single one which is long lived. When drawing survival curves you always come to a stage when you are waiting for this last animal to die and it keeps living. You can not circumvent these effects and they spoil the appearance of survival curves a little bit.

Dr. Cristofalo: What do the animals die from? Do they die from the same reason? Are you familiar with the work of Ross in which he could modify, by dietary manipulation, the incidence of diseases with age? Does B.H.T. have an anorexic effect on your animals?

Dr. Deyl: I have no idea what the animals die from. As far as B.H.T. is concerned our animals ate everything that they were offered.