LIFE SPAN AND CAUSES OF DEATH OF ALBINO RATS AFTER PROLONGED INTERMITTENT FEEDING WITH ETHYLENEDIAMINETETRAACETATE

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The periodic addition of the sodium salt of ethylenediaminetetraacetate to the diet of albino rats, starting when the animals have reached maturity, increases the mean and maximal life span although analysis of the causes of death revealed a higher incidence of neoplasms in these rats. The addition of the compound to the diet after the animals have reached a presenile age has no effect on the life span or morbidity of the rats.

KEY WORDS: life span; ethylenediaminetetraacetate.

Ions of the transition metals readily form nonspecific complexes with proteins and nucleic acids, leading to the formation of both intra- and intermolecular bonds. Their role in the processes of cross linkage makes metals entering the body a possible factor in aging [1, 5, 8]. To test this hypothesis experimentally, it has been suggested that chelating agents of low toxicity, and in particular, ethylenediaminetetraacetate (EDTA), which accelerate the metabolism of metals, be administered to animals [1, 2]. By binding metals present in the blood serum and promoting their more rapid excretion with the urine, EDTA stimulates the release of metals from the tissues into the bloodstream. If cross linkages in macromolecules are ruptured under these circumstances, the aging process would be expected to be delayed.

Previous experiments showed that the intermittent addition of EDTA to the diet of rats, starting when the animals had reached maturity (from the age of 320 days), leads to an increase in the mean life span [3].

In this investigation the survival of rats was compared after early and late administration of EDTA and the causes of death of the animals were analyzed on the basis of the results of pathomorphological investigation.

EXPERIMENTAL METHOD

Virgin female noninbred albino rats aged 320 days (series I) and 583 days (series II) were used. The disodium salt of EDTA was added to the diet as a 5% solution in a dose of 50 mg per rat per day for 10 days (course), with an interruption of 2 days in the middle of the course. Each subsequent course began 6 weeks after the end of the previous course. The scheme of administration of the compound was drawn up on the basis that prolonged, continuous administration of this dose induces appreciable changes in metabolism in the rats and, in particular, leads to baldness [9]. Intact rats of the same age, kept under identical conditions with the experimental animals, acted as the control. The animals were weighed before each course. Observations were made on 208 rats; 58 in the control and 56 in the experiment in series I; 48 and 46, respectively, in series II. The data of death was recorded precisely; some of the dying animals were autopsied and their internal organs examined histologically.

The criterion of Student and Fisher (χ^2) was used for the statistical analysis.

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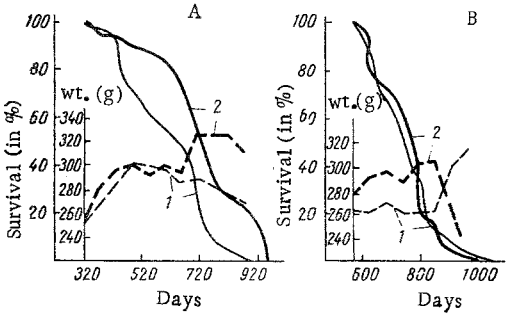

Fig. 1. Survival (continuous lines) and weight (broken lines) of rats fed intermittently with EDTA starting from maturity (A) and presenile (B) age: 1) control; 2) experiment.

TABLE 1. Effect of EDTA on Mean and Maximal Life Span of Rats (in days)

Series	Group of animals	Mean life span		Maximal life span
		mortality 50%	mortality 100%	
I	Control	486±15	571±20	769±29
	Experimental	610±23	678±27	903±22
		$P<0,001$	$P<0,001$	$P<0,05$
II	Control	676±11	756±15	949±24
	Experimental	689±13	763±14	931±27

EXPERIMENTAL RESULTS

Daily inspection revealed no pathological changes in the external appearance and behavior of the experimental rats.

Curves of the survival and dynamics of the body weight of the rats in the two series of experiments are shown in Fig. 1. The shift to the right of the curve of survival of the experimental rats in the experiments of series I indicates better survival of the animals receiving EDTA. Judging from the dynamics of the curves, there was no difference between the survival rate of the control and the experimental rats in series II.

A similar pattern was found when the mean and maximal life spans were compared (Table 1). The maximal life span was taken to be the mean life span of the longest-living individuals (the last 10% in each group). In the experiments of series I, both the mean and the maximal life span were significantly higher in the experimental rats (Table 1).

Analysis of the frequency of diseases causing death (Table 2) was based on the accepted classification of diseases in albino rats [7, 10]. Since the rats of series I and II were under observation from different ages, data on the morbidity of the rats in series I starting from the presenile age (over 540 days) are included in Table 2.

The commonest diseases were infectious and inflammatory: acute and chronic inflammatory diseases of the respiratory tract, evidently mycoplasmosis [4]; unidentified diseases of the ulcerative–necrotic type such as noma in the region of the neck; peritonitis secondary to pyogenic abdominal abscesses.

Benign tumors were represented by fibroadenoma of the mammary gland. The commonest of the malignant neoplasms were carcinomas in various situations (lungs, liver, small intestine, mammary gland); lymphatic leukemia was found in two rats (experimental in series I and control in series II).

Degenerative diseases include cirrhosis of the liver, hepatitis, glomerulonephritis, and cardiovascular failure.

It will be clear from the results shown in Table 2 that in series I there was a statistically significant difference between the control and the experimental groups as regards the frequency of neoplasms. In series II there was no statistically significant difference between the control and experimental groups for the frequency of any diseases.

Intermittent feeding with the sodium salt of EDTA, starting from the age of maturity, thus improves the survival rate and lengthens the life span of albino rats, whereas feeding with the compound starting later in life has no such action. If the beneficial effect of EDTA is in fact attributable to its interference with the aging process, the role of age discovered in this investigation evidently means that EDTA, when given in this way and in these doses, does not act on established senile changes but can retard their appearance.

The higher frequency of neoplasms in rats receiving EDTA from the age of maturity (compared with the corresponding control) calls for an additional study of the preparation, although careful investigations [6] did not reveal any carcinogenic properties of the calcium salt of EDTA when added to the diet of rats in doses of 50–250 mg/kg, i.e., close to those used in the present experiments.

The higher frequency of tumors is possibly an unfavorable aspect of the positive effect of EDTA on the life span. Since the experimental rats of series I lived longer than the controls, the probability of appearance of malignant neoplasms in these animals was higher, for tumor growth is closely connected with

TABLE 2. Frequency of Diseases Leading to Death in Rats Receiving EDTA Intermittently

Series	Group of animals	Total No. of rats autopsied	Infectious and inflammatory diseases (of the respiratory tract in paren.)	Neoplasms (malignant in parentheses)	Other degenerative diseases and senile wasting (in parentheses)
I	Control	17	12 (4)	1 (0)	4 (2)
	Experimental	20	9 (2)	6 (5)	5 (3)
			$\Sigma\chi^2 = 6{,}84$ for d. f. $= 2$ $P<0{,}05$		
II	Control	22	13 (4)	4 (2)	5 (5)
	Experimental	30	18 (12)	5 (4)	7 (6)
			$\Sigma\chi^2 = 0{,}06$ for d. f $=2$ $P>0{,}95$		

age. Comparison of the results obtained in the two experimental series confirms this hypothesis. In fact in series II, where there was no difference between the control and experimental groups as regards life span, there was likewise no significant difference in the incidence of neoplasms. Additionally, the higher mean age of the rats in series II was combined with a higher frequency of tumors.

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