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Chemical Geroprotectors and the Problem of Increasing the Life Span

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The information available in the literature about the applications of geroprotectors—chemical substances used in experimental gerontology to increase the life span and to retard ageing—are described systematically and analysed. The proposed mechanisms of ageing are discussed only in connection with the characteristics of the action of the geroprotectors.
The bibliography includes 100 references.

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I. INTRODUCTION

Practical steps have been taken in modern gerontology in the search for agents which retard ageing.[1-3] Experimental gerontology endeavours to develop methods for influencing the ageing process whereby one could alter the biological limit of the life span, which cannot be influenced significantly by progress in medicine and by improvements in living conditions. The increase in life span by the retardation of growth and development, of the kind achieved in the experiments of McCay et al.[4] on rats, which were fed an inadequate diet, is evidently unsuitable for humans. This stimulated the search for other ways of prolonging life. The first hopeful experimental results of the employment of chemical substances to retard ageing were obtained at the end of the 1950s, the period when the theoretical development of this problem also began[5-8]. The chemicals and their mixtures used to increase the life span have come to be called geroprotectors.

On the initiative of Academician N. M. Emanuel', a combined study of the biological activity of radical-reaction inhibitors was begun in the 1950s at the Institute of Chemical Physics at the USSR Academy of Sciences. The hypothesis that free-radical processes are important in the development of certain pathological processes (radiation disease, malignant growth), in ageing, and in damage sustained by interaction with the environment proved to be fruitful. It promoted a successful search for biologically active substances, including geroprotectors, among anti-radical preparations.

No claim is made that the present review is exhaustive. Extensive data on the application of geroprotectors for the retardation of the ageing of cell cultures, the discussion of which would require the analysis of specific mechanisms of the ageing of the cell outside the organism, have been deliberately omitted. Those interested in this problem will find in this review references to original papers.

II. BIOLOGICAL ACTIVITY OF GEROPROTECTORS

The available information about the biological activity of chemicals which increase the life span of laboratory animals is summarised in Table 1. The specific manifestations of ageing are extremely varied, but they all reflect the disorganisation of the vital functions of the organs and tissues. The ageing of the organism is frequently regarded as a consequence of the accumulation of molecular injuries in cells and primarily in the genetic material[30]. The ideas concerning the harmful effects of free radicals are consistent with several at superficially incompatible hypotheses concerning the biological manifestation of ageing.

Table 1. Biological properties of geroprotectors.

2,6-Di-t-butyl-4-methylphenol (ionol)	food antioxidant[9], antiradiation agent[10], antileukaemic and anticarcinogenic agent[11-14], inductor of microsomal liver enzymes[14,15], substance reducing the frequency of chromosomal aberrations[16]
2-Mercaptoethylamine	antiradiation agent
Vitamin E	biogenic antioxidant, substance reducing the frequency of chromosomal aberrations[16]
6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline	food antioxidant, inductor of microsomal liver enzymes[15], anticarcinogenic agent[13]
Nordihydroguaiaretic acid	antioxidant, inductor of microsomal liver enzymes[15]
3-Hydroxy-2-ethyl-6-methylpyridine.HCl	antiradiation agent[10], antioxidant[17]
2-(Dimethylamino)ethyl *p*-chlorophenoxyacetate (meclophenoxate)	substance preventing the accumulation of lipofuscin[18], stabiliser of biological membranes[19]
Dimethylaminoethanol	stabiliser of biological membranes[19]
Salicylates	stabilisers of biological membranes[19]
L-3,4-Dihydroxyphenylalanine	intermediate in the biosynthesis of adrenaline, used in the treatment of Parkinson's disease[20]
Sodium salt of ethylenediaminetetraacetic acid	prevents the formation of cross-links in macromolecules[21]
β-Aminopropionitrile	ditto (quoted after Comfort[2])
Corticosteroids	inductors of the biosynthesis of protein stabilisers of biological membranes[19], stimulators of the proliferation of cell cultures[22-24]
β-Diethylaminoethyl *p*-aminobenzoate (procaine)	active principle of the geriatric agent "Gerovital NZ", stimulator of the proliferation of cell cultures[27]
Ginseng and eleutherococcus extracts	substances influencing the secretory function of the adrenal cortex and the steroid hormone equilibrium[25], antioxidants, stimulators of cell division[26]
"Folcysteine U" complex	reactivates certain enzyme systems whose activity decreases wtih age, influences the SH/SS ratio[28]
"Decamevit", "Geroton", and "Amivit" polyvitamin complexes	used in geriatric practice, normalise metabolic processes, stimulate regeneration and haemopoiesis[29]

Free radicals play a significant role both in normal and in pathological vital processes. They have been detected in various tissues, subcellular structures, and in isolated

enzyme systems. It has been established that free radicals are active intermediate substrates in oxidation-reduction processes. It is suggested that about 60% of the overall concentration of free radicals in the cell, detected by ESR, is associated with electron transfer processes via the respiratory chain of mitochondria and with the formation of semiquinone radicals[31].

Free-radical states in other cell organelles have been inadequately investigated. About 20% of the overall concentration of free radicals is probably located in microsomes, where various extraneous substances are oxidised without phosphorylation and undergo detoxidation[32,33]. A correlation has been established between the amount of free radicals and the metabolic activity of the tissues[32,34]. Non-enzymatic oxidation processes involving organic molecules, mainly lipids, constitute another source of free radicals. Vitamin E, ascorbic acid, and other natural antioxidants regulate the normal level of these processes in the cell. Artificially created condition favouring non-enzymatic oxidation (irradiation, excess oxygenation) lead to pathological states[35]. Thus, following an increase in the amount and degree of unsaturation of fats in the diet, the life span of C3H mice decreased, and the incidence of mammary gland cancer rose, which may be due to the formation of toxic lipid peroxides. The introduction of α-tocopherol into the diet prevented these phenomena[36].

The possibility of intensifying free-radical reactions during ageing has been discussed in the literature, but systematic studies on these lines have not been undertaken. The concentration of free radicals in the organs of mice and rats of different ages has been investigated[37]; maximum concentrations have been recorded on the 12th and 43rd days of the animal's life, i.e. during the period of growth, after which there was a monotonic decrease. A comparative study of the organs of young (4 months old) and old (2 years old) rats of the "Vistar" strain revealed an increase with age in the concentration of free radicals in the liver and blood[38]. The free radical concentration in the brain tissue of 30 month old rats was higher than in 10 month old animals[39].

Numerous experimental data have shown that the physicochemical properties and functional characteristics of the molecules of information DNA and RNA change in the course of ageing[30,40-43]. Highly reactive free radicals of the type RO· and $RO_2^{\cdot}$ may play the role of agressive factors. The formation *in vivo* of lipid peroxides and unstable low-molecular-weight radical oxidation products, which may modify biological structures, causing, in particular, a decrease in the templet activity of DNA (quoted after Vilenchik[30]), has been demonstrated experimentally. Radical processes involving the oxidation of lipid components may be the cause of the breakdown of the integrity of membranes of subcellular particles. Lysosomes are present in the cytoplasm of almost all types of animal cells; they contain hydrolytic enzymes, which participate in the digestion of ballast substances, in the lysis of dead cells, etc. Damage to the lysosomal membrane *in vivo* can release the enzymes of the lysosomes into the cytoplasm and lead to the breakdown of its protein structures, damage to many important cell components, intensification of katabolic processes, and stimulation of the formation of connective tissue[19]. It is suggested that accumulation of the senile pigment lipofuscin is also associated with the degradation of lysosomes[44].

Inactive enzymes have been detected in the cells of old animals and in diploid cultures in the degradation phase[45-47], i.e. molecules retaining a specific structure which have lost their catalytic properties. The reason for the appearance of inactive enzymes may be structural defects in the templet, weakening of control by nuclear DNA over the synthesis of RNA[40], and postsynthetic modification of the proteins by the enzymes of lysosomes or free radicals.

Table 2. The life span of laboratory rodents.

Strain of animals	Sex. no. of animals	Median life span, weeks	Life span, weeks		Refs.
			average ± s.d.*	maximum	
LP	males, 77	107	103±2.6	142	63
	females, 51	102	99±2.7	127	
129	males, 54	126	117±4.2	154	63
	females, 95	111	104±3.1	148	
DBA/2	males, 67	86	82±3.7	125	63
	females, 51	85	81±3.2	118	
CBA	males, 17	111	107±3.9	125	63
	females, 33	94	91±2.9	116	
C57BL/10	males, 39	124	118±4.1	165	63
	females, 35	104	99±4.4	141	
C3H	males, 29,	112	113±5.3	157	63
C3H·K	males, 25	115	104±5.0	131	
(LP × 129) F_1	males, 54	120	122±3.1	169	63
	females, 77	118	118±2.5	158	
(LP×DBA/2)F_1	males, 42	123	121±4.4	163	63
	females, 43	95	93±3.7	152	
(129×DBA)F_1	males, 47	139	135±4.0	186	63
	females, 58	119	119±3.2	169	
(CBA×C57BL/10)F_1	males, 40	126	142±0.7	143	63
	females, 29	104	141±0.9	142	
(LP×C57BL/10)F_1	males, 42,	130	125±3.9	185	63
	females, 48	120	123±3.4	176	
(A×C57BL/10)F_1	males, 42	116	117±3.3	152	63
	females, 72	127	125±2.7	186	
(CBA×C3H·K)F_1	males, 21	111	109±3.7	133	63
(C3H×C3H·K)F_1	males, 36	122	120±2.7	147	63
CBA/J	males 73	—	75.0±2.5	—	64
	females, 75	—	75.0±2.0	—	
DBA/2	males, 75	—	101±3.2	—	64
	females, 80	—	102±3.0	—	
129/J	males, 79	—	97±2.9	—	64
	females, 78	—	92±3.2	—	
C57BL/6J	males, 74		96.5±2.9	—	64
	females, 75		99±2.9	—	
AKR/J	males, 39	—	46.5±2.0	—	64
	females, 72	—	39.4±1.2	—	
C3H	males, 40	—	78.7±3.4	101.5	65
	females, 40	—	77.3±4.7	103.0	
CD—1 **	males***, 86	74.3	74.5	—	66
	females, 86	99.5	92.8	—	
Sprague-Dowley rats	males	—	108	172	quoted after (Ref.61)
		101	—	167	
Ditto	?	—	99±31	—	61

*s.d. = standard deviation.
**non-inbred strain.
***germ-free conditions.

The influence of antiradical preparations on the level of oxidation-reduction reactions in the tissues of the organism can be inferred from the change in the antioxidant activity (AOA) of lipids, which characterises the ability of lipid extract to counteract oxidation and is caused by the presence of natural antioxidants. Measurements of AOA of mouse liver lipids during ageing showed that it steadily decreases with age and that the rate of its variation is not the same for different strains of animals[48]. In radiation sickness, stress states (starvation, supercooling), and poisoning a decrease of AOA is observed, as in ageing. The development of these processes may be retarded and even prevented by the introduction of substances which increase the level of anti-oxidant activity. Natural and

synthetic antioxidants (tocopherol, ionol, 2-ethyl-3-hydroxy-6-methylpyridine), certain hormones (hydrocortisone, adrenaline), and preparations belonging to the class of non-specific adaptogens (eleutherococcus) are able to alter the level of AOA of tissue lipids, i.e. they can influence directly or indirectly the intensity of radical oxidation processes in the organism [49-53].

The possibility of preventing or effectively retarding a wide variety of undesirable deviations, which inevitably occur in the living organism, and also the prophylaxis and liquidation of pathological states have a bearing on the problem of the prolongation of the life span. The increase of the incidence of illness in middle age, which may be associated with the activation of latent viruses, is considered as one of the manifestations of ageing [54]. Effective geroprotectors (antioxidants—ionol and ethoxyquin) have a set of favourable properties: anticarcinogenic activity [12-14], the ability to prevent the accumulation of chromosomal aberrations [16], and antileukaemic activity [11]. Ionol is used in the treatment of bladder tumours and radiation-induced and trophic lesions of the skin [56,57].

It is well known that the toxic effect of chemical agents and medicinal preparations increases with the recipient's age. The effects of ionol, nordihydroguaiaretic acid, propyl gallate, and ethoxyquin on the activity of oxidative enzyme systems of liver microsomes, which metabolise extraneous chemical compounds, medicinal preparations, poisons, etc., have been studied [15]. The introduction of ethoxyquin into the diet of rats aged 81–440 days increased by a factor of two the enzymatic activity of the microsomal fraction; ionol and nordihydroguaiaretic acid exhibited a somewhat lower inducing capacity; propyl gallate did not exhibit the properties of an inductor and did not increase the life span of rats. Preliminary administration of ethoxyquin to rats decreases the toxic effect of carbon tetrachloride, increasing the rate of its conversion into CO_2. [58] Possibly the induction of the detoxicating enzyme system in microsomes constitutes the molecular mechanism of the mobilisation of the vital forces of the organism against the harmful effect of the chemical components in the environment.

The set of favourable properties of antiradical preparations may serve as a basis for the future development of medicinal compositions designed to reduce the rate of ageing in man.

III. COMPARATIVE ASSESSMENT OF THE EFFECTIVENESS OF GEROPROTECTORS

The wide-scale employment of laboratory rodents in the study of ageing implies the assumption of a definite similarity between the ageing processes in these species and in man. To estimate the rate of the ageing process and the effectiveness of geroprotectors, mortality indices for the population are used. The following characteristics are usually analysed: the average and maximum life span; the "median" life span, i.e. the age corresponding to 50% mortality; the law of the frequency distribution of deaths; the relative death rate linked to age by a logarithmic relation (the Gompertz index); the "expected life span". [59]

A correct study of the percentage survival of laboratory rodents is possible when not less than 800–1000 animals are used. About 3000 mice of the SAS/4 strain of both sexes have been observed from birth to death [60]. The average life spans of the males and females did not differ, amounting to 103.8 weeks. The Gompertz relation held over the range 50–100 weeks, while at more advanced ages the mortality index increased more slowly. A logistic function of the type

$$\mu = 122 \cdot 10^{-5} + \frac{3.3 \cdot 10^{-5} e^{0.0632t}}{1 + 23 \cdot 10^{-5} e^{0.0632t}},$$

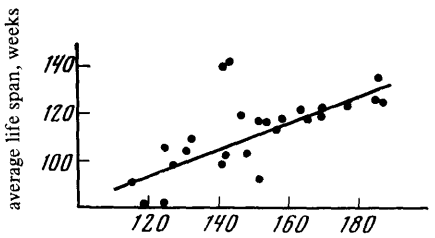
where $\mu = -(1/n)(dn/dt)$ is the relative death rate and n the number of living individuals whose age is t, is suitable for a complete description of the percentage survival curve. The distribution of life spans proved to be asymmetric.

The study of the percentage survival of laboratory Sprague-Dowley rats was continued for five years [61]; 747 animals were used in 18 experiments, 7 of which were performed in a germ-free medium and the remainder in the usual vivarium with careful maintenance of cleanliness and constant living conditions. The average life spans were virtually the same in all the experiments, the mean amounting to 692 ± 22 days. Analysis of the frequency distribution of deaths showed that, compared with the normal distribution, there is an excess of short-lived individuals. For old rats aged 800–950 days, the expected life span remains constant and the relative death rate decreases. The decrease of the Gompertz index by a factor of two at an age of 1060 days compared with an age of 450 days was observed also for CAF_1 hybrid mice [62].

In the majority of experimental studies groups of 100 and fewer animals are usually employed, which leads to appreciable differences between the percentage survival indices for animals of the same strains investigated in different laboratories and even in the same laboratory but at different times. Table 2 presents data for the life spans of mice belonging to 8 inbred strains and 8 first generation hybrid groups, one non-inbred strain of mice (CD-1), and Sprague-Dowley rats. Comparative analysis of the data in Table 2 permits the following conclusions: (1) the life span of male mice is greater by 10–12% than that of female mice, although there are exceptions [67,68]; (2) the life span of first generation hybrids is longer than for mice of the parent strains; (3) the average life span is close to the median value, i.e. the two parameters can be used to an equal extent; (4) the greatest differences between the life span are observed for the inbred strains 129 and AKR (by a factor of 2.7); (5) the average life spans obtained in different laboratories differ for strains 129, DBA/2, and the genetically similar strains C57BL/6, and C57BL/10 approximately by a factor of 20% and for strains CBA and CXH by 40%. A study of the life spans of C57BL male mice in four independent experiments in the same laboratory showed that the median and maximum life spans differ by 34 and 17%, and the source from which the animals had been obtained is significant [69].

In selecting the animals to be subjected to protracted survival tests, preference should be given to the following inbred strains with a stable genotype: 129, CBA, C57BL, C3H, C3HA, LP, BALB/c, and SWR; it has been shown that the incidence of malignant tumours in these strains or first generation hybrids is low [61,63,68,70]. The advantages of gnotobiology are not evident: a decrease of the life span of the animals under germ-free conditions is sometimes observed [66,71-73]. Since the average life span depends significantly on environmental and dietary factors, we must assume that the chemicals employed as geroprotectors really retard the ageing process only when an increase in the maximum life span is observed compared with the control group of animals of the same breed. The treatment of quantitative data for the percentage survival of mice of different inbred strains and first generation hybrid groups [63] showed that there is a linear relation

between the average and maximum life spans under optimal living conditions (see Figure). The regression equation is $y = 24.6 \pm 0.57x$, with a correlation coefficient $r = 0.85 \pm 0.055$. This relation implies that, when ageing is retarded, a decrease of mortality is to be expected for the entire population, which leads to a rightward shift of the percentage survival curve.



Regression line for the average and maximum life spans of mice according to Smith et al.[63]

The possibility, in principle, of increasing the life span of laboratory animals by means of certain chemicals as dietary additives in small amounts was demonstrated for the first time in a number of studies[5,6,8]. When inhibitors of radical reactions with the properties of antiradiation agents and antioxidants (2,6-di-t-butyl-4-methylphenol, ammonium diethyldithiocarbamate, 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline, and α-tocopherol) were added to the diet in amounts of 0.25–1 wt.%, the percentage survival in the test groups after 20 months amounted to 61.66, 74, and 13% compared with 9% in the control[74].

The results of subsequent investigations are combined in Table 3. The increase of the average and maximum life spans varies in the range 13–50% and 11–58%. The influence of the most effective chemicals is compared with the results obtained in studies by McCay[4], who used prolonged partial starvation of young rats as a factor retarding their growth and development and leading to an increase of their life span. In experiments carried out in 1934 the life span of test individuals exceeded by 78% the maximum life span of the control. In repeated experiments the increase of the life span varied from 22 to 32%. Nikitin and coworkers[82], who reproduced McCay's experiments, found that the average life span of test animals increased by 39% and the maximum life span by 47%.

Despite the fact that the search for chemicals retarding the ageing process has continued for about 20 years as a scientific discipline, the list of active compounds includes no more than 20 items. The principal cause of the slow development of this field is lack of knowledge of the primary causes and mechanisms of ageing. In selecting geroprotectors, the investigators were forced in most cases to employ more or less plausible hypotheses as a basis. The preferential use of laboratory rodents makes the experiments long, expensive, and laborious, and their results cannot be predicted as long as data for the mortality of a control group of animals have not been secured.

Attempts were made in a number of investigations to develop biological methods making it possible to follow the rate of ageing by procedures which are not based on mortality indices alone. Several tests have been proposed to characterise the biological age of rats. When the rate of ageing is accelerated by fractional irradiation with X-rays, comparison of the calendar and biological ages using the method proposed by the authors showed that the test group was "older" than the corresponding control group. The opposite findings were obtained when ageing was retarded by periodic feeding of Na_2-EDTA.[76,83,84] The fundamental studies[85,86] in which changes in enzyme induction associated with ageing are investigated as a biochemical expression of ageing are interesting and promising.

Table 3. Influence of chemical geroprotectors on the life span of laboratory rodents.

Compound	Dose	Strain of animals, sex, age at beginning of experiment	Difference relative to control, %		Refs.
			avergae life span	maximum life span	
2-Mercaptoethylamine.HCl	0.5 wt.%	LAF_1 male mice	12.8	–	75
"	1.0 wt.%	ditto	29.0	–	
2,6-Di-t-butyl-4-methylphenol (ionol)	0.25 wt.%	"	17.6	–	75
Ditto	0.5 wt.%	"	44.6	–	
6-Ethoxy-2,2,4-trimethyl--1,2-di-hydroquinoline (ethoxyquin)	0.5 wt.%	C3H male mice, 3 months	18.0	25.0	3,65
Ditto	"	C3H female mice, 3 months	20.0	26.0	
Na_2 salt of EDTA	50 mg day^{-1}	female rats, 320 ± 10 days	18.7	–	76
2-(Dimethylaminoethyl) *p*-chlorophenoxyacetate (meclophenoxate)	60 mg kg^{-1}	Swiss male mice, 8.6 months	27.3	26.3	77
Dimethylaminoethanol	7 mg kg^{-1}	A/J male mice, 604–664 days	49.5	10.9	78
L-3,4-dihydroxyphenylalanine	40 mg/g of diet	Swiss male mice, 4–5 weeks	34.0*	–	79
3-Hydroxy-2-ethyl-6-methyl pyridine-HCl	1.5 mg day^{-1}	SHK female mice, 2 months	34.0	12.5	80
Ditto	3 and 6 mg day^{-1}	SHK female mice, 8–8.5 months	23.2	58.0	80
Ginseng and eleuthero-coccus extracts	–	white rats	21 and 16	–	26
Polyvitamin complexes: "Decamevit"	–	rats	3.3	–	81
"Geroton" and "Vitavit"	–	"	13.3	–	81
Complex of polymicro-elements	–	"	23.3	–	81

*At 18 months of age.

Apart from laboratory rodents, populations of the fruit fly *Drosophila melanogaster* are frequently used to investigate the ageing process and to select geroprotectors. Its use has advantages in the sense that in the tissues of the adult insect there are no dividing cells, i.e. the death of the organism coincides with the death of its cells. The life span of the adult insect depends on the conditions in the medium, the population density, and the rate of preimago development[44,87,88]. The average life spans of various strains differ significantly: it is 40 days for the short-lived mutant strains Hk^1P and Sh^5,[89] while for wild species it is almost twice as long[19]. The older the

parents the shorter the average and maximum life spans of the offspring. The life span of the second generation hybrids is somewhat shorter than that of F_1 hybrids[90]. It is possible to use F_2 hybrids in those cases where a large initial population of individuals with the same life spans is required for the experiments.

As for laboratory rodents, the retardation of ageing should lead to a decrease of the mortality of the entire population, to an increase of the maximum life span, and to a rightward shift of the entire mortality curve. Among the 38 compounds of different classes which were added to the diet in different concentrations, positive effects were found for only a few (Table 4). The majority of the compounds tested were selected on the basis of their known or postulated ability to stabilise cell or lysosome membranes towards decomposition *in vivo* and *in vitro*[91]. A reliable increase of the average life span of males was achieved under the influence of corticosteroids, salicylic acid derivatives, and meclophenoxate. The greatest increase in the maximum life span (by 42%) was attained when a mixture of salicylsalicylic acid and aspirin was used.

Table 4. The influence of chemical geroprotectors on the life span of *Drosophila melanogaster*[19,92].

Compound	Dose, mg/100 ml of medium	Strain of insect, sex	Difference relative to control, %	
			average life span	maximum life span
		(*Canton S* × *Oregon 2S*)F_1		
Hydrocortisone acetate	10	males	38	16
		females	21	18
		(*Canton S*)		
Cortisone acetate	18.8	males	43	31
Deoxycorticosterone acetate	0.5	females	18	13
		males	21	21
Triamcinolone(9α-fluoro--16α-methylhydrocortisone)	4	males	18	5
		females	19	16
		(*Canton S* × *Oregon RS*)F_1		
Aspirin	250	males	22	27
		females	13	16
3:1 salicylsalicylic acid–aspirin mixture	490	males	24	18
Ditto	162	females	38	42
Salicylamide	650	males	17	22
		females	12	27
Meclophenoxate	120	males	7	15
		females	39	23
Ionol	0.001%	males	30	–
		females	16	–
Butylhydroxyanisole (BHA)	0.01%	males	12	–
		females	5	–

The procedure whereby biologically active substances retarding ageing are selected can be greatly shortened by employing populations of microscopic polycellular nematodes (*Caenorhabditis origgsae*) and rotifers (*Bdelloid rotifer*)[93-98], whose life span is close to 1 month and which can be grown simply and in large amounts without much expense. The presence of α-tocopherylquinone in the culture medium retarded the ageing process of nematodes whose age at the beginning of the experiment was one day, and increased by 30% the average life span. A positive effect was not observed if the age of the animals was 20 days, since irreversible changes associated with ageing had occurred[99]. The influence of inhibitors of the biosynthesis of proteins, antimetabolites, and cytostatic agents on the life span of rotifers has been studied[100]. The following inhibitors of biosynthesis were found to retard ageing: actinomycin D, streptomycin, Acridine Orange, tetracycline, and chromomycin A_3.

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Progress in this field of experimental gerontology depends on scientific advances in the understanding of the principal mechanisms of ageing. The next urgent task is to combine the search for substances "capable of protecting the cells from senile changes in a manner similar to that occurring on restricting the diet"[87] with improved methods for recording the rate of ageing.

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