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EFFECT OF ENTEROSORPTION ON ANIMAL LIFESPAN

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Experiments were performed on Wistar male rats, starting from the 28th month of age. The effect of dietary sorbent (non coated nitrogen-containing carbon administered as 10 day courses at 1 month intervals in dosage of 10 ml/kg) on lifespan and a number of biological indices were studied. Enterosorption resulted in the increase of mean and maximal lifespan by 43 and 34% respectively. Analysis of the effect of enterosorption on activity of microsomal enzymes, intensity of total RNA and protein biosynthesis, lipid metabolism, formation of free radicals etc. showed that it produced a positive influence on the functional state of the studied systems and increased the organism's adaptive capacities. Enterosorption was found to delay the rate of onset of age-related structural changes in the organs and tissues.

INTRODUCTION

An increasing chemization of the environment and an expansion of the arsenal of synthetic medicamentous drugs have highlighted the role that xenobiotics may play in the formation of age-related abnormalities and in the development of age pathology. Apart from xenobiotics, the autointoxicating factors play a definite role in the complex of stochastic and genetically determined damaging agents.

Current conceptions about their role in the genesis of aging have dated to the classical investigations by I.I. Metchnikov. The effect of toxic substances depends not only on their amounts and activity of detoxicating systems, but also on tissue sensitivity. According to our data, in rats the basal level of one of the most essential detoxication processes - microsomal oxidation - decreases in young age and remains mainly unchanged till old age, while the sensitivity to the products of protein metabolism increases (1,2).

All this has determined our interest in an experimental search for the means of body detoxication aimed at prolonging the lifespan and delaying the onset of age-related changes. Enterosorption, which appeared to be an effective means in the cases of poisoning, immunological diseases, liver ailments, atherosclerosis, etc., is a successful method of body detoxication (3).

MATERIALS AND METHODS

Experiments were performed on 120 male Wistar rats. The experimental animals were administered non-coated nitrogen-containing SKN carbon (10 ml/kg b.w.; fraction composition - 0.3-1.0 mm, pore volume measured by benzol - 0.6-0.7 ml/cm^3) starting from the 28th month of age. The control animals were rats of the same age and of the adult age (6-8 months) fed a standard diet. The biochemical and morphological studies were performed in the animals administered sorbent by a 20-day course. The shifts of lifespan were estimated both by the mean and maximal lifespan, and by the corresponding coefficients of age-related kinetics of mortality in Gompertz' coordinates (4). The myocardium, liver, kidney, lung, brain, pancreas, adrenals, spleen and intestines were used for light microscopical study. All material was fixed in 10% formalin, sections were stained with hematoxyllin-eosin, pycrofuxin. In addition, Schiff's test as well as lipid and lipofuscin tests were performed. For electron microscopic analysis, the fragments of myocardium and liver were fixed in 3% glutaraldehyde in phosphate buffer (pH 7.4), post-fixed in 1% O_sO_4 and embedded in Epon 812. Thin sections were made by IKB ultramicrotome and examined in a JEM-100B electron microscope.

The indices of the state of lipid metabolism were studied in the blood serum, heart and brain. Extraction of lipids from tissues was made by chloroform-methanol mixture (5). Total lipids were determined as described (6), triglycerides and total cholesterol were measured as described (7,8), respectively. Cholesterol was determined after the sedimentation of low and very low density lipoproteins from the serum by $Macl_2$ in the presence of heparin (8). The content of liver

cytochromes P-450 and B_5 were determined in post-mitochondrial supernatant (9000 g) according to Omura and Sato (9). Hydroxylase activity of the supernatant was determined in vitro by addition of aminopurine (10) and aniline (11) substrates. Phenobarbital ("Merk" Firm, FRG) was administered in the dose of 80 mg/kg for three days. The intensity of in vitro biosynthesis of total RNA and protein was determined in the frontal cortex of large hemispheres, hypothalamus, pituitary, skeletal muscle, left ventricle of the myocardium, adrenals, kidney and liver by the incubation of their sections in the serum of the same animal at 37° and constant bubbling with a mixture of O_2 (95:5). After about one-hour of preincubation, the labeled precursors of RNA (^{14}C-orotate) and protein (^{3}H-leucine) of 100 and 10 MBq/ml (final concentration), respectively, were added. After one hour the sections were homogenized in 10% trichloroacetic acid and acid-insoluble material was separated from acid-soluble one by filtration through nitrocellulose membrane filters (mesh 0.22 nm, Millipore, USA). Sample radioactivity was measured on scintillation counter "Mark-III" (USA). The intensity of electron magentic resonance (EMR) signals from cylindric liver tissue samples was registerd on spectrometer E-109 ("Verian", USA) at temperature 77° (12). Data were analyzed by means of several statistical methods (13).

RESULTS

Enterosorption resulted in the increase of mean and maximal lifespan of rats by 43.4% and 34.4%, respectively (Fig. 1). Age-related dynamics of mortality for control (1) and experimental (2) animals expressed in Gompertz' equation coordinates aredescribed as follows:

$$\ln R_t = (-14.26 \pm 1.75) + (0,0097 \pm 0,0018)t;\ r=0.94 \quad (1)$$

$$\ln R_t = (-13.08 \pm 1.70) + (0,0078 \pm 0,0016)t;\ r=0.94 \quad (2)$$

where R_t - mortality at time t (days $^{-1}$)

t - age (days)

r - the coefficient of correlation between $\ln R_t$ and t (mean values and standard deviations of the coefficients of regression equations are given in brackets).

The content of liver cytochrome P-450 and the activity of aminopurine demethylase (2.17 nmol/g and 8.67 nmol/g/min, respectively) were twice as lower in experimental old rats compared to intact animals (4.81 nmol/g and 18.87 nmol/g/min). While the content ofliver cytochrome B_5 (12.23 nmol/g and 11.40 nmol/g in control and experimental rats, respectively) and the activity of aniline hydroxylase (2.2 nmol/g/min in control and 2.67 nmol/g/min in

experimental animals) were almost the same. The duration of phenobarbital-induced narcotic sleep in experimental group (50 min) was 37% shorter as compared with intact animals (79 min). On administering phenobarbital the inductive synthesis of cytochrome P-450 and the activity of aniline hydroxylase and aminopurine demethylase were much lower in old versus adult rats. After enterosorption the inductive effect of phenobarbital in old animals did not differ statistically significantly from that of adult rats (Fig. 2).

The study by means of EMR of liver enzyme systems, catalyzing reduction-oxidation reactions, showed that in adult animals enterosorption produced a significant weakening of the intensity of signals from cytochrome P-450 (g=2.25), iron-sulphur proteins of the mitochondrial respiratory chain (g=1.94), mitochondrial flavoproteins in free-radical form (g=2.00), Mn^{2+} containing proteins of the endoplasmatic reticulum (g=2.16 and g=2.10), nitrosyl complexes on non-heme iron and Cu^{2+} containing proteins (g=2.035), while the intensity of signals from mitochondrial sulphitoxidase (g=1.97) remained unchanged. In old rats there was a significant decrease in the intensity of signals from cytochrome P-450 and a sharp increase in that from sulphitoxidase (Table I).

Based on the shifts of relative specific radioctivity of total RNA, we found that the intensity of transcription decreases in all studied brain structures and increases in the remaining tissues. Enterosorption-induced changes in the intensity of protein biosynthesis were in many aspects analogous to the shifts of RNA synthesis, i.e. it was decreased in brain structures and increased in other organs (Fig. 3).

Lipid contents underwent organ-specific changes due to enterosorption (Table II). The most marked changes occurred in the liver where the concentration of total lipids, triglycerides and cholesterol decreased by 32%, 48% and 29%, respectively. There were approximately the same shifts in the myocardium and the brain. Brain serum total lipids decreased from 3.0 ± 0.1 to 2.4 ± 0.1 g/l. Addition of sorbent to the diet resulted in the redistribution of lipids in plasma membrane. For instance, in hepatocyte membranes there was an increase in lysophosphatidyl choline from 3% to 4.2% ($p < 0.05$) and phosphatidyl choline from 38 to 45% ($p < 0.05$), and in myocardiocyte membranes there was only a slight decrease in lysophosphatidyl choline - from 2.5 to 2.0% ($p < 0.05$).

The morphological investigations also revealed the retardation of the rate of aging due to enterosorption. In the experimental old rats, using the light microscopic examination, we found a decrease in myofibrosis and myocardiodystrophy occurring against the background of

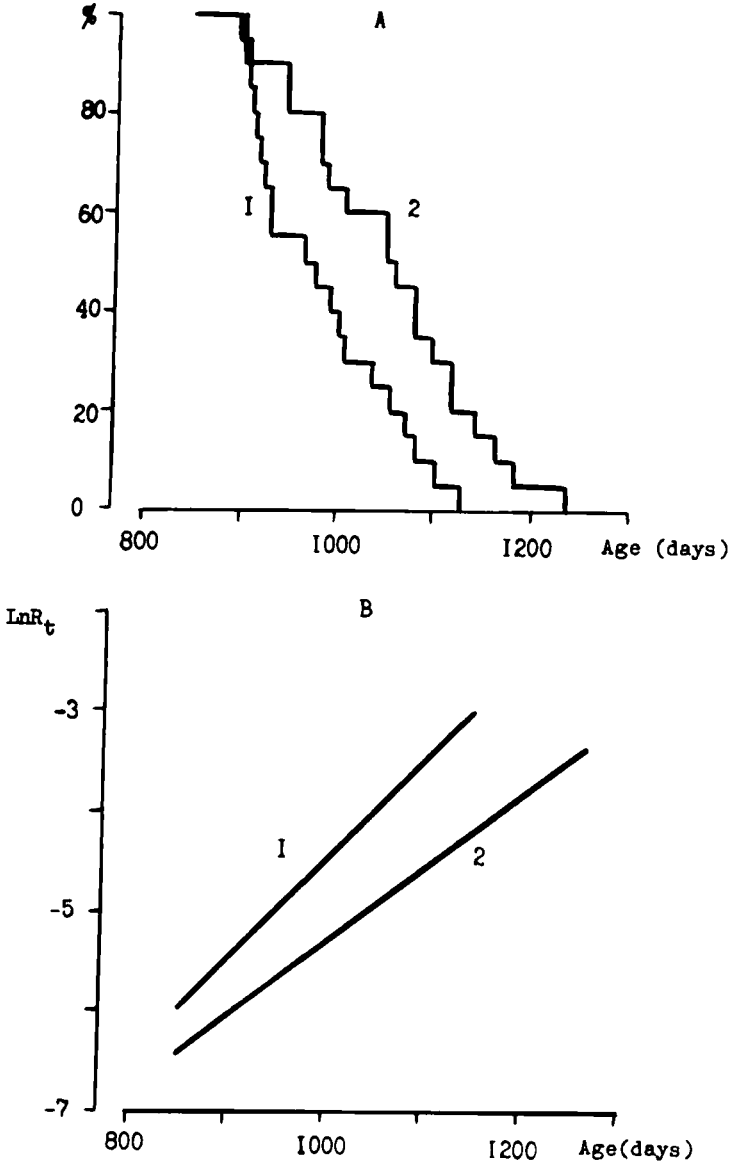

Figure 1: The effect of enterosorption on the dynamics of survivorship (A) and mortality in Gompertz' coordinates (B) in rats.
1- control 2- enterosorption

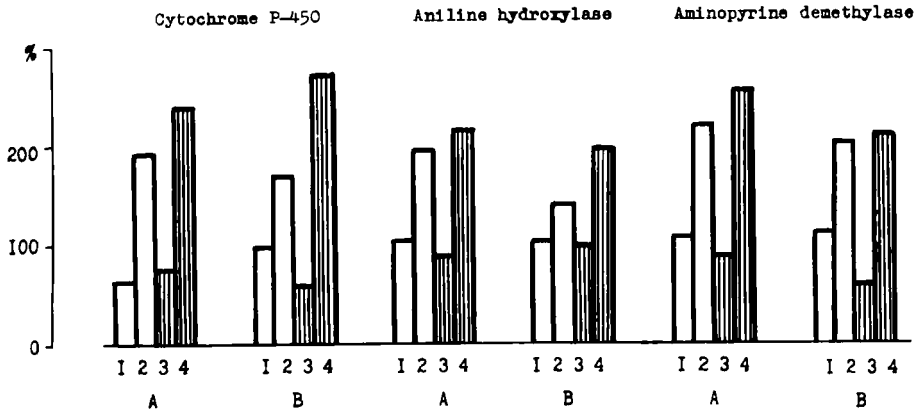

Figure 2: The effect of enterosorption on the contents of cytochrome P-450, aniline hydroxylase and aminopurine demethylase of the liver microsomes following an administration of phenobarbital in the adult (A) and old (B) rats.
1- control, 2- phenobarbital, 3- enterosorption, 4- enterosorption + phenobarbital

TABLE I

Effect of enterosorption on the intensity of EPR signals from the liver in rats of various ages ($\bar{x} \pm m$)

EPR signal (g factor)	Intensity (nominal units)			
	Adult		Old	
	Control	Experiment	Control	Experiment
1.94	0.89±0.02	0.72±0.06*	0.77±0.10	0.80±0.08
2.00	0.52±0.04	0.42±0.03*	0.43±0.04	0.40±0.07
2.25	1.10±0.20	0.90±0.10*	0.70±0.10	0.60±0.10*
2.035	0.12±0.04	0.06±0.01*	0.05±0.01	0.05±0.02
2.16	0.20±0.01	0.15±0.02*	0.14±0.04	0.13±0.03
2.10	0.10±0.01	0.08±0.01*	0.07±0.01	0.07±0.02
1.97	0.38±0.04	0.40±0.04	0.30±0.09	0.49±0.04*

* - $p < 0.05$

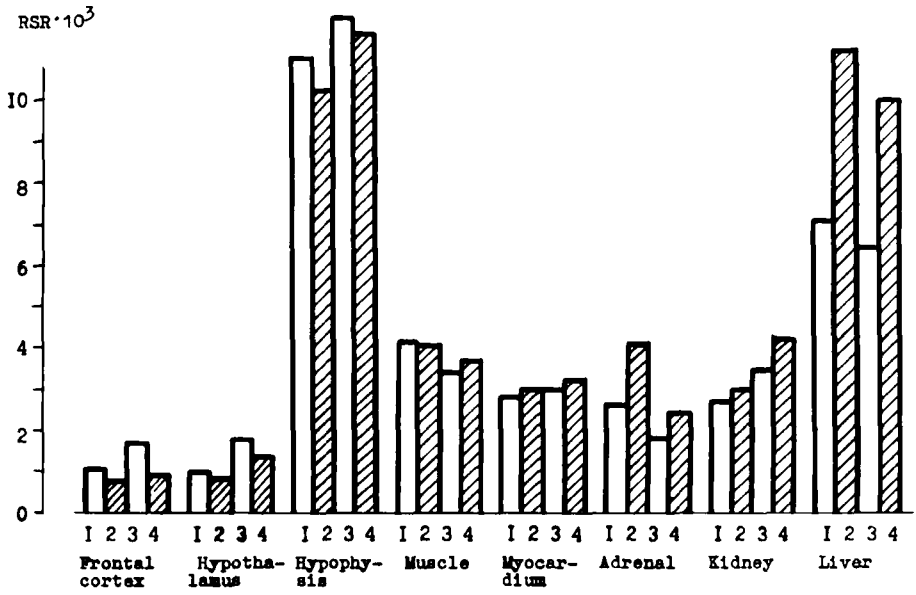

Figure 3: The effect of enterosorption on relative specific radioactivity (RSR) of total RNA and protein in different organs of the rats.
1,3 - protein and RNA RSR in control rats;
2,4 - protein and RNA RSR in experimental rats.

TABLE II

Effect of enterosorption on indices of lipid metabolism in organs of old rats (mg/g)

Indices	Liver		Heart		Brain	
	Control	Experiment	Control	Experiment	Control	Experiment
Total lipids	24.9±2.7	17.0±1.0*	10.8±0.5	9.9±0.5	34.4±1.3	33.9±1.3
Total cholesterol	3.5±0.1	2.5±0.2*	1.4±0.07	1.2±0.03	21.9±0.7	24.0±0.7
Triglycerides	17.5±2.9	9.1±0.7*	4.3±0.4	2.5±0.3*	9.3±0.8	7.7±0.3*

* $p < 0.05$

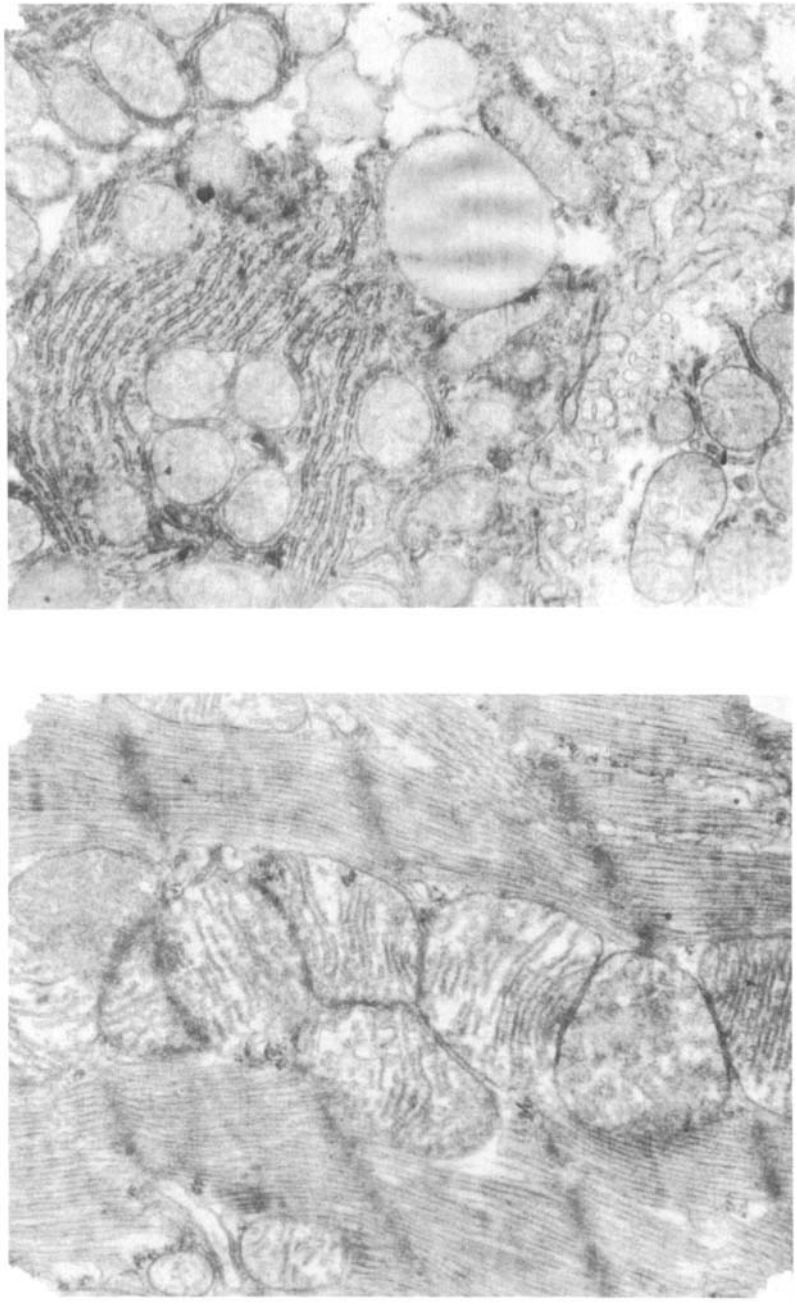

Figure 4: The ultrastructure of hepatoctye (a) and cardiomyocyte (b) in the old rat (35 mo) after enterosorption.

a- well developed granular endoplasmic reticulum whose cysterns contact mitochondria, x 14 200;

b- fine structure of the myofibrils is preserved, mitochondrial matrix is slightly clarified, x 19 600

less marked focal perivascular changes. The animals showed no contractural or metabolic lesions of the heart. In the brain there was a lesser degree of atrophic changes in neurons. In kidneys of the experimental animals we failed to find sclerosed renal glomeruli. Hepatocytes had predominantly light cytoplasm and large nucleus with the fine network of chromatin and nucleoli. Electron microscopic examination (Figure 4) revealed a decrease in the destructive changes of organelles and mitochondria of the myocardium, as well as disturbances of the contractile apparatus, myofibrils. Hepatocytes contained less lipid granules and secondary lysosomes and more channels of the granular endoplasmic reticulum and free polyribosomes. Mitochondria had a preserved integrity of the ultrastructure of the inner membrane forming the crystae.

DISCUSSION

As is seen from Figure 1, enterosorption results in a noticeable increase of the lifespan and a decrease of the age related dynamics of mortality. Comparison of the constants of equations (1) and (2) has shown that in experimental animals the intersect is somewhat increased but the regression coefficient is decreased. According to the existing conceptions (4), such shifts of the constants are rather a consequence of the retardation of aging processes than an improvement of the maintenance conditions. Retardation of the rate of aging in sorbent treated animals can be accounted for by the "additional" detoxication of the organism. Since in conditions of a whole organism this function is mainly performed by microsomal oxidases of the mixed liver function, the decrease, due to sorbent leads to the decreased content and activity of microsomal enzymes. In old animals the enterosorption increases an adaptive capacity of microsomal detoxication system. That is the inductive synthesis of cytochrome P-450 and the hydroxylase activity of microsomes in old rats against the background of phenobarbital approaches the level of adult animals. Shortening of the narcotic sleep, an integral index of the activity of microsomal monooxygenases may suggest that by influencing the rate of aging the enterosorption decelerates the age related rise of brain sensitivity to barbituates.

The decrease of cytochrome P-450 was also confirmed by the decreased intensity of its signals as revealed by the EMR method. This decrease is probably related to the enterosorption-induced elimination of toxic metabolites, circulating in the blood, which are the substrates and endogenous inductors of cytochrome P-450. It is noteworthy that the occasional malfunctioning of enzymes, catalyzing the reduction oxidation reactions in a cell, including iron-sulfur

proteins, flavorproteids and cytochrome P-450, might lead to the one electron oxygen reduction and the formation of free radicals. The enterosorption induced decrease in the activity of electron transport enzyme systems results in the decrease of the concentration of the metabolites of the free radical nature, which, apparently, along with the decrease of other toxic agents, underlies the geroprotective action of the enterosorption.

The mechanism of enterosorption action in an old organism is linked with its altering effect on the contents of total lipids, cholesterol and triglycerides in organs and tissues. The age related changes of lipid metabolism are known to create predispositions for the development of the atherosclerotic process. Therefore the positive effect of enterosorption on the age related changes of lipid metabolism can be used in the prevention of the development of atherosclerosis.

The data on retardation of the rate of onset of the age realted structural changes observed in the myocardial cells hepatocytes and neurons are an objective evidence for the effect that enterosorption may produce on the aging process.

In conclusion, the data presented suggest that enterosorption is an effective means of lifespan prolongation. Based on the findings of the complex of biochemical, morphological and physiological analyses, the increase of lifespan is accompanied with the delay of onset of age related changes and the enhancement of functional possibilities of a number of systems. In real fact, on entering the body the sorbent performs the role of an additional "organ" whose function lies in body detoxication, hemeostasis stabilization and prevention of free radical generation. While considering the attractiveness of the use of enterosorption with the aim of prolonging the life, a note should be made of its harmlessness that has been sufficiently checked in clinical practice at various pathological conditions. Moreover, the obvious advantage of this method is its effectiveness in late ontogenesis, when most geroprotectors become less effective. This work substantiates large perspectives for the use of enterosorption as the means of prolonging the human life.

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