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ENTEROSORPTION IN PROLONGING OLD ANIMAL LIFESPAN

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Abstract—The effect of repeated courses of enterosorption upon the mean and maximal lifespan and some functional and metabolic indices was determined in 28-month-old Wistar rats. Significant increase of mean and maximal lifespan of old rats was noted at certain regimens of enterosorption. The experimental animals demonstrated less marked age-related structural and ultrastructural changes in the liver, kidneys, myocardium, intestines, pancreas, as compared with control animals. Enterosorption leads to a reduction of pentobarbital-induced sleep, decrease of content of cytochrome P-450, blood cholesterol and triglycerides, cardiac and cerebral tissue cholesterol, total lipids, liver cholesterol and triglycerides. Enterosorption was found to increase the RNA and protein biosynthesis in the liver, kidneys and adrenals of old animals.

INTRODUCTION

THE SPECULATION regarding the possible role of toxic metabolites in the genesis of aging, put forward by I.I. Mechnikov as early as at the beginning of this century, justifies the use of modern sorption methods for influencing the course of aging, for prolonging the lifespan. While considering this problem, a note should be taken of the age-related rise of organism's sensitivity to some toxic agents (Frolkis, 1970). Most of the known experimental methods for lifespan prolongation proved to be effective when used well before the onset of aging. If an assumption is made that the toxic metabolites are essential in the genesis of aging, then the sorption methods should, obviously, be used in late ontogenesis, when these waste products alter quantitatively.

At present, sorption methods are widely used in clinical practice. Quite widespread is the use of enterosorption confined to regular daily intake of 20–80 g of microspheric activated carbons of synthetic origin (Bonatskaya & Zinevich, 1982; Nikolaev *et al.*, 1982; Shcherbitskaya *et al.*, 1982). The main mechanism of enterosorption's therapeutic effect lies in the purification of 6–9 litres of digestive juices, secreted in man daily. In other words, enterosorption may be considered as a version of hemosorption, since the sorption of substances from digestive juices also influences the blood composition. Enterosorption is known to modify the lipid and aminoacid spectrum of the intestinal content, and to

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eliminate toxic and some biologically active substances. Lipid- and cholesterol-eliminating effect of orally taken activated carbons, initially demonstrated in patients with chronic renal insufficiency, established the basis for the use of enterosorption in hypercholesterolemia-related atherosclerosis (Nikolaev & Strelko, 1979). In this context, enterosorption as a possible means of effect upon an aging organism can be recommended.

METHODS

Experiments were performed on 120 male Wistar rats aged 28 months. The hemosorption was performed on carbon sorbent SKN-1k and enterosorption on SKN sorbent (non-coated nitrogen-containing carbon, fraction composition—0.3–1.0 mm, pore volume measured by benzol—0.6–0.7 ml/cm^3). Animals were fed sorbent-containing diet (1 ml/100 g b.w.) starting from the 28th month of age. The animals were divided in 4 groups according to enterosorption regimen: Group 1–control animals (fed standard diet); Group 2–sorbent administered by 10-day courses at 1-month intervals; Group 3–sorbent administered by 1-month courses at 10-day intervals; Group 4–sorbent administered by one 20-day course. The mean lifespan at 50%, 80% and 100% mortality, as well as the maximal lifespan, were determined. A number of functional, morphological and metabolic indices were estimated in some animals of each group after 5 courses of enterosorption (34–35 months of age) and in animals of Group 4. The myocardium, liver, kidney, lung, brain, pancreas, adrenals, spleen and intestines of 10 animals from each group were used for light microscopical study. All material was fixed in 10% formaline, paraffin embedded sections were stained with hematoxylin-eosin, pycrofuxin. In addition, fragments of each organ were fixed in 3% glutaraldehyde in phosphate buffer (pH 7.4), post-fixed in 1% OsO_4 in phosphate buffer (pH 7.4) and embedded in Epon 812. Thin sections were made by LKB III ultramicrotome and stained with uranil acetate, lead citrate and examined in JEM-100B electron microscope.

The indices of the state of lipid metabolism were studied in the blood serum, liver, heart and brain. Extraction of lipids from tissues was made by chloroform-methanol mixture (Folch *et al.*, 1957). Total lipids were determined according to Bragdon (1951), triglycerides according to Carlson (1963), total cholesterol according to Ilka (1962). α-cholesterol was determined after the sedimentation of low and very low density lipoproteins from the serum by $MnCl_2$ in the presence of heparin (Ilka, 1962). The concentrations of the studied substrates in blood serum were expressed in g/l for total lipids and mg%—for the rest. In tissues, the concentrations was expressed in mg/g tissue for all indices.

The content of liver cytochromes P-450 and B_5 were determined in post-mitochondrial supernatant (9,000 g) according to Omura and Sato (1964).

The duration of sodium pentobarbital-induced narcotic sleep (25 mg/kg) was estimated from the moment of "side-position" till the recovery of locomotor reactions.

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 blood serum of the same animal at 37°C and constant bubbling with mixture of O_2 and CO_2 (95:5). After about one-hour preincubation, the labeled precursors of RNA (^{14}C-orotate) and protein (^{3}H-leucine) of 10^{2}MBq/ml 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. Sample radioactivity was measured on scintillation counter "Mark-III."

RESULTS AND DISCUSSION

Hemosorption is known to be an effective means of removing the toxic agents from the blood. In the first series of experiments the study was undertaken on the effect of hemosorption upon the old animal organism. It was found that after 4–6 weeks following the course of hemosorption the old rats developed arterial hypertension. Thus, the initial level of arterial blood pressure in 32-month-old rats made 84.6 ± 6.1 mm Hg, as compared to 118.0 ± 5.4 mm Hg, observed 4–6 weeks following hemosorption. Without conducting special experiments, it is difficult to interpret the mechanism of post-hemosorption hypertension in old rats. Noteworthy are the two facts. First, arterial hypertension develops not instantaneously, but in 4–6 weeks after hemosorption; second, the evolved hypertension is of a sustained character and lasts for several months. It can be assumed, therefore, that the development of arterial hypertension is linked not merely with the sorption of some physiologically active substance, but rather with the slowly evolving regulatory renal and hypothalamic rearrangements.

All these consequences of hemosorption and its possible complications (Lopukhin & Molodenkov, 1978) have determined our interest in a milder method of body detoxication, that is enterosorption. We also hoped that quite effective could be a long-term sorption of toxic agents, rather than a short-term course, usually practiced in hemosorption.

Another series of experiments (Groups 2 and 3) were made to study the effect of the two enterosorption regimens upon the lifespan of old rats (aged 28 months). In Group 2 enterosorption resulted in the prolongation of both mean and maximal lifespans. In control group the mean lifespan at 50% mortality was 936.6 days, at 80% 972.1 days, at 100% 992.8 days, and at maximal lifespan 1117.5 days, as compared with 977.4, 1022.8, 1054.7, and 1209.5 days, respectively, registered in experimental animals.

The experimental procedure we have chosen required the estimation of changes in the lifespan since the moment of sorbent intake, that is from 28 months of age. Thus, enterosorption resulted in the increase of mean lifespan by 47.3%, 41.4% and 43.7% at 50%, 80% and 100% mortality, respectively; the maximal lifespan increased by 34.4%. In other words, the data obtained indicate that the enterosorption is an effective means of prolonging old animal life. It is noteworthy that the positive effect can be attained only at certain regimens of enterosorption. Thus, the mean lifespan in Group 3 (one-month enterosorption course at 10-day interval) made only 97.9%, 107.3% and 110.3% at 50%, 80% and 100% mortality; the maximal lifespan made 118.9%, as compared with control.

Our special experiments showed that the enterosorption not only alters the animal lifespan, but also influences the rate of onset of age-related structural and metabolic changes in the organism, and the biological age of animals. We have compared the structural and ultrastructural enterosorption-induced changes in Group 2 with those in control animals. In Group 2 we found less marked age-related structural rearrangements and lack of rough pathological changes. Thus, in the heart of experimental animals the diffuse myofibrosis was less marked, and there were no focal sclerotic changes. In contrast to control, the experimental animals showed no contractural and metabolic lesions in the heart. In only one case there was a small focus of fibrosis, located around the blood vessel, while in the coronary vessels and aortal wall no pathological processes have been found.

In kidneys of experimental animals we failed to find sclerosed renal glomeruli, which was the case in control animals. The basal membranes of the glomerular capillaries were not thickened. In the capsules of Shumliansky-Bowman, the sclerotic processes were not marked. The tubuli appeared to be normal, though in one case there was a widening of renal tubuli and filling of their lumen with protein.

In liver, the architectonics of the lobes was normal. Hepatocytes had predominantly light cytoplasm and large nucleus with the fine network of chromatin and large nucleoli. Their cytoplasm contained much less lipid drops, as compared with control. A significant number of bi-nuclear hepatocytes could be seen. The number of Kupfer cells was increased, their cytoplasm had a somewhat basophilic shape, nuclei were distinct, as compared with control. In pancreas, the sclerotic changes were not marked. Both small and large Langerhans islets could be seen. No pathological changes were found in small and large intestines, in lungs and other organs. Thus, enterosorption prevented the development of many age-related structural changes.

Electronmicroscopic examination of the cardiocytes of experimental and control animals revealed moderate swelling of mitochondria with the preservation of the integrity and clarity of the inner membrane and some clarification of mitochondrial matrix (Figure 1). Some mitochondria had destructive changes such as injury of the crystae and avail-

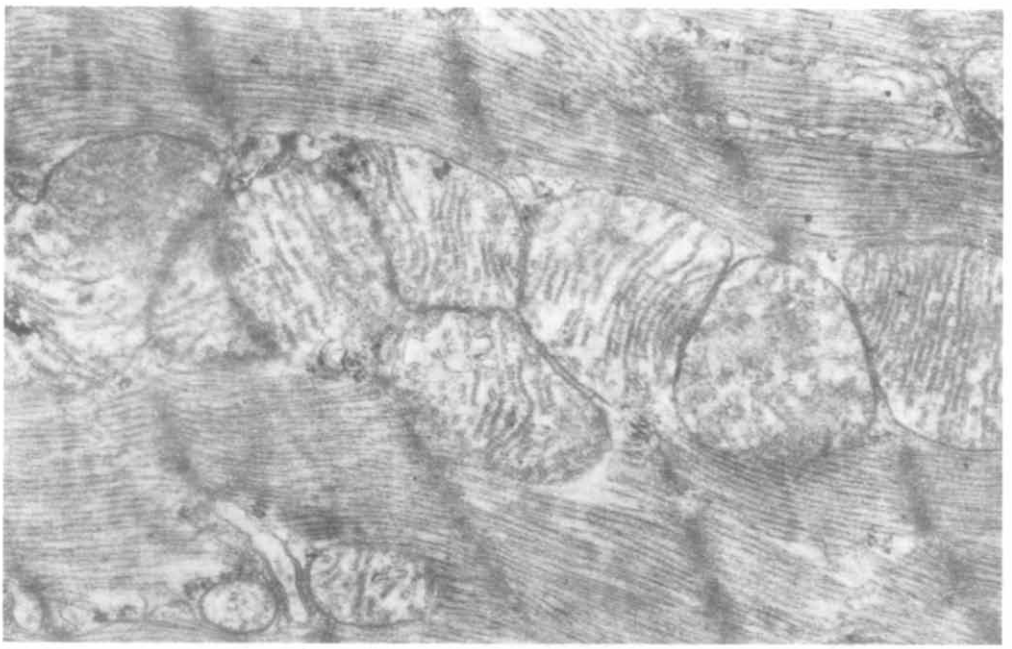

FIG. 1. Electron micrograph of cardiomyocytes of an old rat (Group II) showing insignificant changes in mitochondria (m) and myofibrils (mf) × 22800

ability of the myelin-like structures. However, the destructive changes of the cardiocytes in Groups 2 and 3 were less marked, as compared with control. The activity of the fibroblast cells was found in the myocardium, that is the swollen cisternae of the rough endoplasmatic reticulum and numerous ribosomes on the surface of the cisternae. Lipofuscin granules were found in the cytoplasm of the connective cells (Figure 2).

Very few lipid granules could be seen in the liver cells in Groups 2 and 3, as compared with control. In addition, the irregular-shaped structures of the lipid origin encircled by osmophilic membrane were noted in the cytoplasm of these cells. The rough endoplasmatic reticulum of the liver cells was well developed and the nuclei contained the diffusive chromatin with large nucleoli (Figure 3).

Thus, light and electronmicroscopic studies showed the marked structural differences between Groups 2 and 3 and control in the liver, kidney and myocardium. The diffusion fibrosis of the organs and tissues was less marked after the course of enterosorption. The injury and destruction of the parenchyma cells were not found in the kidney and myocardium of animals of any experimental group.

The results of the light microscopic study showed no structural changes in the organs and tissues in Group 4 (20-day course of enterosorption).

The duration of narcotic sleep is an accepted index for detoxicating function of the liver. Our experiments revealed that the duration of pentobarbital-induced sleep in control group was 78.75 ± 10.47 min as compared with 49.80 ± 8.94 min in experimental group, that is by 37% less.

The enterosorption produced significant decrease of cytochrome P-450 (by 54.9%), that is 4.81 ± 1.1 nmol/g tissue in control group versus 2.17 ± 0.25 nmol/g tissue after a

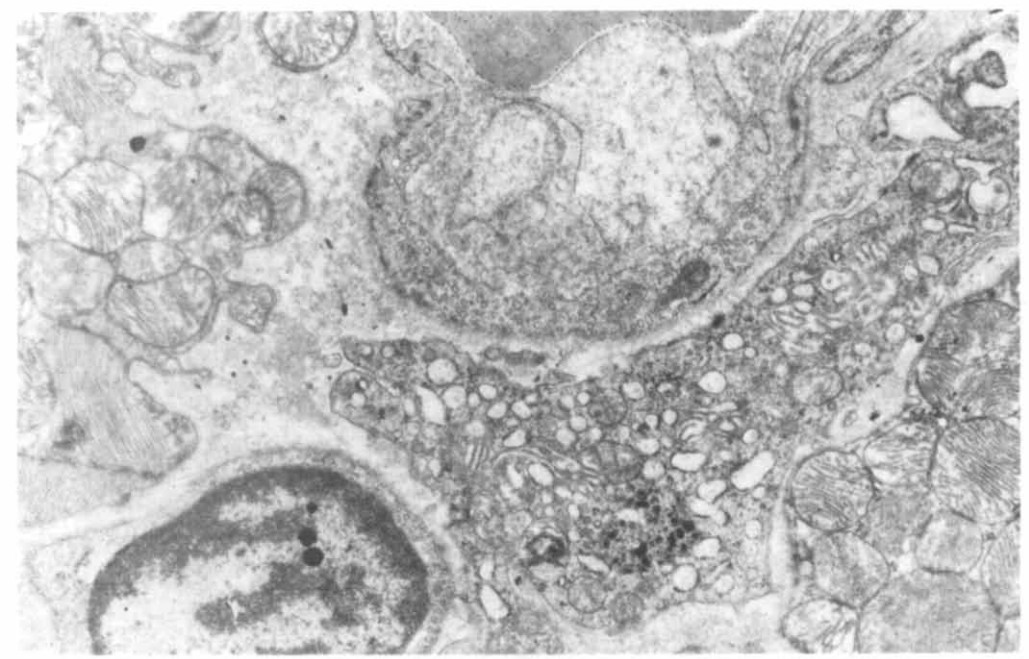

FIG. 2. Ultrastructure of myocardial fibroblast of an old rat (Group II): extended tubules of rough endoplasmatic reticulum (ER) and lipofuscin granules (arrow) in the cytoplasm × 12200

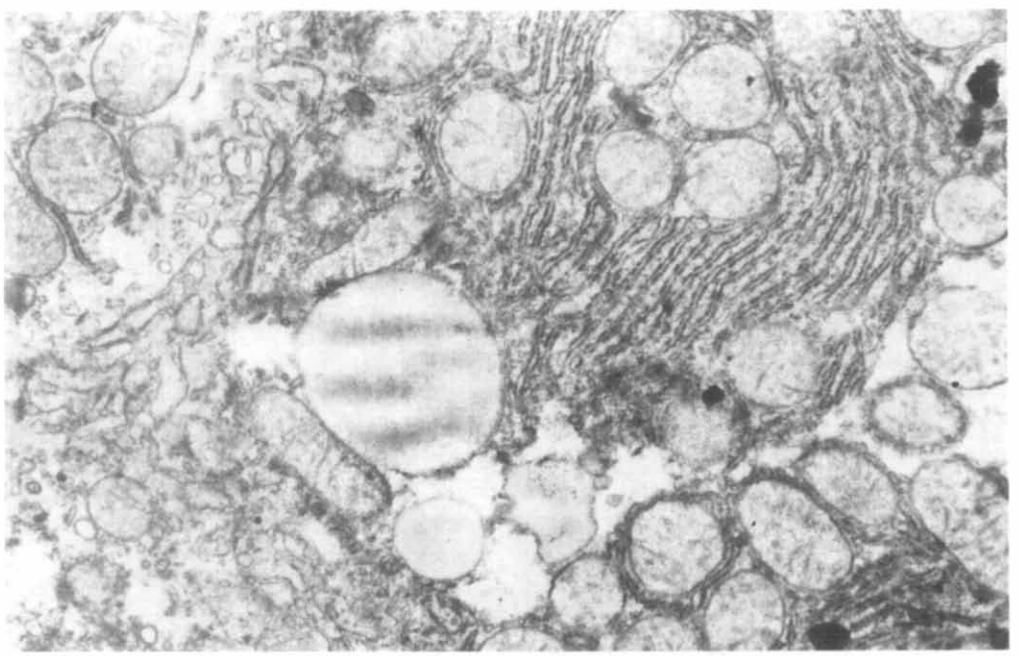

FIG. 3. Electron micrograph of hepatocyte of an old rat (Group II) showing well developed rough endoplasmatic reticulum (ER), normal mitochondria (m) and lipid granules (arrow) × 16000

20-day course. The content of cytochrome B_5 remained almost the same (11.40 ± 3.26 nmol/g tissue versus 12.23 ± 2.34 nmol/g tissue, $p > 0.2$, respectively). Thus, enterosorption shortened the period of narcotic sleep and decreased the content of cytochrome P-450, a key enzyme of the system of microsomal oxidation in the liver. Usually, the reduction of intensity of microsomal oxidation is accompanied by the prolongation of the narcotic sleep, since in these conditions the narcotic substance has a prolonged effect upon the corresponding nervous centers. In our experiments, the decrease of cytochrome P-450 was, apparently, related with the enterosorption-induced decrease of the concentration of toxic agents, circulating in the blood, which were the substrates of the enzymes of microsomal oxidation. During aging, the sensitivity of the central nervous system to some pharmacological substances, including narcotic ones, increases (Frolkis, 1970). It can be assumed that by influencing the rate of aging the enterosorption decelerates the age-related rise of brain sensitivity to narcotics, thus leading to shortening of pentobarbital-induced sleep in experimental animals, as compared with control.

Another important criterion of the enterosorption efficacy is the state of lipid metabolism. This is conditioned by the role it plays in the processes of aging, in the development of age-related pathology. As is known, during the "enterohepatic circulation" cycle the cholesterol and the main products of its degradation-bile acids-are excreted with bile by the liver and then a large share of them is reabsorbed. And here, in physiological conditions in rats and, possibly, in man (Polyakova, 1981) the cholesterol synthesis and formation of bile acids are coordinated by the negative feed-back mechanism. Therefore, the level of reabsorption of cholesterol and bile acids is essential in maintaining the cholesterol homeostasis in the organism. That is why the enterosorption may be used in an attempt to influence the lipid and cholesterol metabolism in old age.

For this purpose, we have estimated the concentration of total lipids, cholesterol and triglycerides in the blood serum, liver, heart and brain, as well as α-cholesterol in blood serum. Also estimated was the cholesterol atherogeneity coefficient (**C**) (2), which presents the relation of the sum of cholesterol of low density and very low density lipoproteins to cholesterol of high density lipoproteins (α-cholesterol):

$$\mathbf{C} = \frac{\textbf{total cholesterol} - \alpha\textbf{-cholesterol}}{\alpha\textbf{-cholestrol}}$$

We found that the enterosorption resulted in changes in the lipid content in serum and organs. The most significant changes have been found in the liver, where the concentration of total lipids, triglycerides and cholesterol fell by 31.8%, 48% and 28.6%, respectively (Table 1). This effect was marked in continuous enterosorption as well, that is the liver cholesterol and triglycerides fell to 1.78 ± 0.07 mg/g and 12.4 ± 1.36 mg/g, respectively or by 48.6% and 29.2% less than in control group.

Liver plays an important role in the processes of formation of lipids and, primarily, of cholesterol both for own membranes, and for export within very low and low density lipoproteins, as well as in the excretion of cholesterol with bile and its oxidation to bile acids. In view of the above, the decrease of total lipids, triglycerides and cholesterol in the liver may be decisive in changes of lipid metabolism in the whole organism and in the prevention of risk factors for the development of atherosclerosis during aging. In fact, in blood serum we registered significant decrease of total lipids (from 2.99 ± 0.13 to 2.41 ± 0.11 g/l, $p < 0.01$ or by 19.4%) and of concentration of total cholesterol (from

TABLE 1. EFFECT OF ENTEROSORPTION ON INDICES OF LIPID METABOLISM IN ORGANS OF 28-MONTH-OLD RATS (MG/G)

	Liver		*Heart*		*Brain*	
Indices	*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 ± 0.03	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*

*$p < 0.05$

95.0 ± 5.0 to 79.6 ± 2.8 mg%, $p < 0.02$ or by 16.3%). In 28-month-old experimental animals these indices approximated those of 20-month-old intact rats (2.41 ± 1.0 g/l and 82.0 ± 6.0 mg%, respectively).

Rats are known not to develop spontaneous atherosclerosis (Klimov, 1981), and experimental atherosclerosis is very difficult or even impossible to induce. In rats, a large share of cholesterol is within high density lipoproteins (α-cholesterol). We found that in both control and experimental animals the main amount of cholesterol is within high density lipoprotein fractions (61.0 and 70.3%, respectively). Still, enterosorption resulted not only in the changes of total cholesterol, but also in the increase of α-cholesterol. The cholesterol atherogeneity coefficient decreased 1.5-fold (from 0.64 ± 0.07 in control to 0.42 ± 0.06 in experimental animals). The concentration of triglycerides in the blood remained unaffected, while in the heart and brain it fell significantly ($p < 0.05$) by (40.7% and 17.3%, respectively).

The data presented suggest that the enterosorption-induced changes in lipid content in blood serum and in the studied organs, especially, in liver, are related with changes in enterohepatic circulation. Obviously, the sorbent adsorbs part of cholesterol and bile acids in the intestines, thus eliminating them from the organism. This results in a decreased reabsorption from the intestines. This leads to the enhanced oxidation of cholesterol to bile acids in liver, and both directly and indirectly promotes the decrease of its concentration in blood serum. Such a mechanism of hypolipidemic effect is postulated for cholesteramine, beta-cytosterine and surgery for partial resection of intestinum ilium, that is for effects aimed at the decrease of cholesterol.

The enterosorption-induced decrease of triglyceride content may account for the disturbance of absorption from the intestines of hydrolysis products-lipids and carbohydrates-which determine the rate of lipogenesis in the liver and control the level of blood triglycerides.

The life-prolonging effect, observed against the background of enterosorption may be greatly conditioned by the shifts in plastic provision. That is why the next series of experiments was designed to study the differences in the intensity of protein and total RNA biosynthesis in sections of some organs of the control and experimental animals (Table 2). Based on specific radioactivity of acid-soluble material, we found no significant changes in the uptake of labeled precursors in any of the studied organs, except for the myocardium and skeletal muscle, in which this index slightly decreased. At the same time, the intensity of protein and RNA biosynthesis, estimated by the relative specific radioactivity, increased markedly in the liver, adrenals and less markedly in the kidneys and remained unchanged in the hypothalamus, pituitary, skeletal muscle and myocardium. The relative

TABLE 2. EFFECT OF ENTEROSORPTION UPON THE INTENSITY OF UPTAKE (SPECIFIC RADIOACTIVITY, SR) AND INCORPORATION IN MACROMOLECULES (RELATIVE SPECIFIC RADIOACTIVITY, RSR) OF LABELED PRECURSORS OF RNA AND PROTEIN IN VARIOUS ORGANS OF RATS

	RNA				*Protein*			
	Control		*Enterosorption*		*Control*		*Enterosorption*	
Organ	*SR* • 10^3	*RSR* • 10^3	*SR* • 10^{-3}	*RSR*	*SR* • 10^{-3}	*RSR*	*SR* • 10^{-3}	*RSR*
Hypothalamus	38 ± 5	1.81 ± 0.33	38 ± 2	1.39 ± 0.11	417 ± 47	0.70 ± 0.06	389 ± 28	0.87 ± 0.14
Pituitary	49 ± 4	15.9 ± 2.3	56 ± 7	15.5 ± 2.5	382 ± 25	11.1 ± 1.5	412 ± 38	10.3 ± 1.5
Adrenals	60 ± 2	1.79 ± 0.16	60 ± 4	2.44 ± 0.31	350 ± 11	2.13 ± 0.24	352 ± 22	4.18 ± 0.44*
Myocardium	78 ± 2	3.05 ± 0.15	70 ± 4	3.17 ± 0.30	406 ± 12	2.78 ± 0.23	370 ± 17	3.09 ± 0.32
Kidneys	74 ± 4	3.54 ± 0.17	70 ± 2	4.25 ± 0.39	368 ± 21	2.56 ± 0.32	346 ± 11	3.00 ± 0.28
Liver	62 ± 5	6.41 ± 1.15	64 ± 5	10.05 ± 1.53	304 ± 15	7.12 ± 1.33	306 ± 22	11.25 ± 1.75

*Significant shifts as compared with control ($p < 0.01$)

specific radioactivity of total RNA and protein in sections of kidneys of experimental animals increased by 17 and 20% as compared with control. In the adrenals and liver, these indices made 59% and 37% and 58% and 57%, respectively. Apparently, the increase of intensity of protein and RNA biosynthesis, which improves the plastic provision of such essential organs as liver, kidneys and adrenals, results in widening the range of some defensive systems of an organism, which, in its turn, leads to lifespan prolongation.

Thus, the data presented suggest that the enterosorption has a potent effect upon an aging organism. This notion can be supported by the enterosorption-induced changes in lifespan prolongation, structural and ultrastructural changes, shifts in protein and RNA biosynthesis and in lipid metabolism, in the content of microsomal enzymes, and so forth. This work substantiates the need for further search for regimens of enterosorption, and combination of it with other effects, aimed at lifespan prolongation. Currently, the question remains whether the described enterosorption effects are related with the sorption of the known or supposed metabolites, or with the changes in the content of some physiologically active substances and subsequent regulatory transformations, or with both. The main thing is that the experiments with enterosorption persuade that altering the humoral environment of an organism may effect the rate of aging, the biological age of animals, and thus their lifespan.

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REFERENCES

BONATSKAYA, L.V. and ZINEVICH, A.K. (1982) In: *Sorption Methods of Detoxication and Immune Correction in Medicine,* p. 4, Kharkov (in Russian).

BRAGDON, G.H. (1951) *J. Biol. Chem.* **190,** 513–517.

CARLSON, L.A. (1963) *J. Atheroscler. Res.* **3,** 334–336.

FOLCH, J., LEES, M. and SLOANE, S.G. (1957) *J. Biol. Chem.* **226,** 497–509.

FROLKIS, V.V. (1970) *Regulation, Adaptation and Aging.* Nauka, Leningrad (in Russian).

ILKA, V.S. (1962) *Ztschr. f. d. Ges. inn. Med.* **17,** 83.

KLIMOV, A.N. (1981) In: *Biochemistry of Lipids and Their Role in Metabolism,* pp. 45–75, Nauka, Moscow (in Russian).

LOPUKHIN, YU.M. and MOLODENKOV, M.N. (1978) *Hemosorption.* Meditsina, Moscow (in Russian).

NIKOLAEV, V.G. and STRELKO, V.V. (1979) *Hemosorption on activated carbons.* Naukova Dumka, Kiev (in Russian).

NIKOLAEV, V.G., STRELKO, V.V., KOROVIN, YU.F., and others. (1982) In: *Sorption Methods of Detoxication and Immune Correction in Medicine,* pp. 112–114, Kharkov (in Russian).

OMURA, T. and SATO, R. (1964) *J. Biol. Chem.* **239,** 2379–2385.

POLYAKOVA, E.D. (1981) In: *Biochemistry of Lipids and Their Role in Metabolism,* pp. 120–128, Nauka, Moscow (in Russian).

SHCHERBITSKAYA, E.V., SANKOVA, I.P. and TARASENKO, L.V. (1982) In: *Modern Problems of Hemodialysis and Hemosorption in Traumatology,* pp. 100–101, Tashkent (in Russian).