netic flux density in the pulse of 9.5 mT, permeability of the structures of the cornea and their metabolic activity were increased, and this was accompanied by increased penetration of the radioactive substance into the eye. This effect, and also the immediate effect of MF on the structures tested, can explain the greater accumulation of radioactive lebel in the lens, vitreous body, retina, and vascular membrane of the eye. The decrease in the concentration of radioactive substance in the cornea and aqueous humor taking place immediately afterward was evidently due to the effect of MF on the drainage system of the eye and on the blood-eye barrier. Evidence in support of this conclusion is given by results showing a marked increase in concentration of the radioactive substance in the peripheral blood of animals whose eyes were exposed to MF, and also by the results of investigations by other workers [2].

It must be emphasized that the pulsed MF, under the conditions of exposure which were used, ruling out any possibility of injury to the cornea, can be used in clinical practice in order to increase the concentration of thereaputic substances in the tissues of the eye and to enhance their therapeutic action.

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SOME PRINCIPLES GOVERNING MONOMERIC ^{239}Pu ELIMINATION FROM THE SKELETON AND LIVER BY LIPOSOME-ENCAPUSLATED PENTACIN

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KEY WORDS: liposome; pentacin; ^{239}Pu.

The problem of removal of ^{239}Pu, deposited in the organs, still remains unsolved. We know that the complexone calcium-trisodium salt of diethylene-triaminepenta-acetic acid (DTPA), or pentacin, which is used to eliminate some radionuclides and toxic metals from the body, passes with difficulty through cell membranes and is ineffective in eliminating ^{239}Pu from cells [1, 9]. Meanwhile, when the complexone is used encapsulated in liposomes, it can enter the cells of organs in which the radionuclide is deposited much more readily, and the effectiveness of the compound as a means of eliminating ^{239}Pu from the liver and skeleton is enhanced [2-4, 7, 8]. To discover the principles governing the action of the compound and its rational usage, it is necessary to know how the action of the lysosomal form of pentacin (LP) depends on dose. This problem has not been studied in detail as regards the liposomal form both of complexones and of other thereapeutic substances.

The aim of this investigation was to study the effectiveness of LP as a means of eliminating monomeric ^{239}Pu from the liver and skeleton and to determine how the effect depends on dose and the concentration of the complexone in the liposomes.

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TABLE 1. Content of ^{236}Pu in Rat Organs (in % of injected radioactivity on 7th day after complexone treatment)

Preparation	Dose, μmoles/kg	Skeleton	Liver
Control		81,1±5,4	17,5±1,2
FP	200	69,9±3,8	5,8±0,6
LP*	200	56,8±3,8	2,5±0,2
LP**	200	59,0±3,2	2,8±0,3
LP*+FP	100+100	52,8±2,9	3,8±0,3

Legend. Preparations injected 24 h after radionuclide. *) Liposomes of phosphatidylcholine and cholesterol in molar ratio of (1:1), **) liposomes of phosphatidylcholine, cholesterol, and stearic acid, in minor ratio of (7:7:1).

EXPERIMENTAL METHOD

Liposomes were formed from an equimolar mixture of chromatographically pure egg phosphatidylcholine and cholesterol by the method described previously [5]. Before removal of pentacin from the aqueous phase outside the liposomes, the suspension was pressed through a nuclear Dacron filter with pore diameter of 0.3 μ [6]. The dose of pentacin introduced into the liposomes varied. In one case liposomes were formed with phosphatidylcholine in a concentration of 54 mM in a 75 mM solution of pentacin, and equal volumes of the liposomal preparation (but different numbers of liposomes) were injected into the animals. The doses of phosphatidylcholine under these circumstances were 50, 100, 200, and 400 μmoles/kg and the corresponding doses of pentacin were 50, 100, 200, and 400 μmoles/kg. In the other case liposomes were formed with phosphatidylcholine in a concentration of 54 mM in pentacin solutions with concentrations of 18.5, 37, 75, or 150 mM; the resulting preparations were injected in a dose of 1 ml each.

The content of complexone in the liposomes was determined with the aid of ^{14}C-pentacin. The complexone which was outside the liposomes was removed by sedimenting the liposomes by centrifugation (100,000g, 60 min), followed by their resuspension in glucose solutions isoosmotic with the contents of the liposomes.

Experiments were carried out on 100 noninbred male rats weighing 160-180 g. A citrate complex of monomeric ^{239}Pu was injected intravenously into the rats in a dose of 180 kBg/kg. Liposomal preparations and free pentacin were injected by the same method 24 h after injection of the radionuclide. The rats were decapitated under ether anesthesia 6 days after treatment. The ^{239}Pu content in the liver and femur was determined by means of a Gamma-Track scintillation spectrometer (USA) with respect to characteristic x-ray emission (16.8 keV). The radioactivity of the skeleton was calculated as the radioactivity of the femur multiplied by 20.

At each experimental point five or six animals were used. The results are presented in the form of mean values and standard errors of the means.

EXPERIMENTAL RESULTS

LP removed 1.5-2.5 times more monomeric ^{239}Pu from the liver and skeleton than nonencapsulated, free pentacin (FP) (Table 1, Fig. 1). The radionuclide was eliminated by a lesser degree from the skeleton than from the liver, and the difference between the actions of the two forms of pentacin was less marked.

The effectiveness of action of LP with a negative surface charge (with stearic acid) was the same as in neutral liposomes (Table 1). The absence of effect of surface charge of the liposomal membranes on elmination of ^{239}Pu also was demonstrated previously [8]. The effectiveness of elimination of ^{239}Pu from the liver by a preparation consisting of 50% of FP and 50% of LP (100 μmoles/kg of each) was only two-thirds of that when LP was used, but was 1.5 times greater than when FP was used. This preparation removed not less of the radionuclide from the skeleton than did LP in a dose of 200 μmoles/kg.

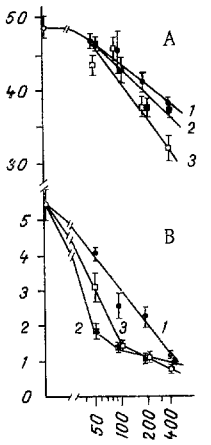

Fig. 1. Content of monomeric ^{239}Pu in skeleton (A) and liver (B) of rats after treatment with complexones. Abscissa, dose of pentacin (in μmoles/kg); ordinate, percentage of injected radioactivity of ^{239}Pu. 1) FP; 2) LP, dose of which was varied by concentration of pentacin in liposomes; 3) LP, dose of which was varied by number of injected liposomes, with constant concentration of pentacin in them. Preparations injected 24 h after ^{239}Pu (results of determination on 7th day).

As Fig. 1 shows, elimination of ^{239}Pu from the liver and skeleton increased with an increase in dose of the preparations, and the dose–effect curve was logarithmic in character. The difference between LP and FP as regards effectiveness of elimination of the radionuclide from the liver was greatest when the complexone was given in a dose of 50 μmoles/kg. The effect of injection of that dose in a large number of liposomes (200 μmoles/kg) was 1.8 times greater than when it was injected in a smaller number of liposomes (50 μmoles/kg) (Fig. 1B, 2) with a higher concentration of the complexones (Fig. 1B, 3). LP was 2.3 and 1.3 times more effective respectively than FP. With an increase in the dose of LP up to 100 μmoles/kg by an increase in the number of liposomes injected, elimination of ^{239}Pu from the liver was increased by 1.2 times (Fig. 1B, 3), whereas, with an increase in the concentration of the complexone in the liposomes, this parameter was increased by only a very little (Fig. 1B, 2). Within the dose range from 100 to 400 μmoles/kg, any additional increase in ^{239}Pu elimination was not significant and the two liposomal preparations were virtually equally effective, and in large doses their action exceeded that of FP by only a little.

This character of the action of LP and FP was due, in our opinion, to the fact that pentacin can remove only monomeric, minimally hydrolyzed ^{239}Pu sufficiently effectively from cells [2, 4], and to do so, a dose of the complexone in the liposomes of 50-100 μmoles/kg, or a dose of FP of 400 μmoles/kg is sufficient. Probably the radionuclide remaining in the organ, because of hydrolysis and polymerization, has become inaccessible for the complexone, and a further increase in the dose of the preparations was not accompanied by any marked increase in its elimination from the liver cells. Dependence of the effectiveness of action of LP on the number of liposomes injected is evidence that to remove monomeric ^{239}Pu from the liver it is advantageous to introduce the complexone into the largest number of "sites" in the cells, thereby increasing the probability of contact between pentacin and the deposited radionuclide, rather than to create a high local concentration of pentacin.

Differences between LP and FP as regards elimination of ^{239}Pu from the skeleton were most marked when the complexone was used in doses above 100 μmoles/kg. To remove the radionuclide from bones it is more effective to use liposomes with concentrated solutions of pentacin. An increase in the concentration of encapsulated complexone leads to a marked increase in the effectiveness of LP (Fig. 1A, 3). It can be tentatively suggested that as a result of osmotic shock some pentacin is released from the liposomes, which contain a solution of the complexone that is hypertonic relative to blood, into the blood, and the combined action of LP and FP is observed on ^{239}Pu deposited in the skeleton. As the data in Table 1 show, simultaneous injection of LP and FP gives a quite high effect of radionuclide elimination from the skeleton.

To remove monomeric ^{239}Pu from the liver and also, probably, from other parenchymatous organs, it is worth while using pentacin in a dose not exceeding 50 μmoles/kg, injected in a large number of liposomes (200 μmoles/kg), whereas to remove the radionuclide from the skeleton

it is better to use liposomes with concentrated solutions of the complexone, or to inject LP and FP simultaneouly. However, this latter procedure requires further experimental study.

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LIPID PEROXIDATION IN OUTER AND INNER MITOCHRONDRIAL MEMBRANES DURING ANOXIA

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534.121.2+612.273

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It has been shown recently that products of lipid peroxidation (LPO) participate in the mechanism of injury to membranes during hypoxia [3]. Analysis of experimental results obtained during a study of LPO in various organelles during anoxia *in vitro* and *in vivo* shows that mitochondria are particularly sensitive to anoxia and are liable to undergo oxidative destruction [2]. We know that the outer and inner mitochondrial membranes differ not only in their chemical composition, but also in the functional properties of their own ultrastructures [6].

The aim of the present invetigation was to study the character of development of LPO in the inner and outer mitochondrial membranes during anoxia, and also to examine whether labilization of membranes of other organelles by LPO products accumulating in the mitochondria is possible.

EXPERIMENTAL METHOD

Experiments were carried out on 250 Wistar albino rats. Mitochondria were isolated from the liver of the rats by the method in [7]. The inner and outer membranes of the mitochondria were isolated by the method in [8]. Tissue anoxia *in vivo* was induced by ligation

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