The degree of active systolic shortening of the ischemic myocardium can be increased either by a decrease in the after-load [6] or by an increase in the pre-load [12]. However, in the present experiments the after-load was unchanged because the compounds tested did not lower BP. Meanwhile malate and NAD significantly increased IC, which is virtually independent of changes in the pre-load [2]. Accordingly the participation of a primary hemodynamic mechanism in the realization of the positive effect of the compounds on function of the ischemic myocardium can be ruled out. Improvement of contractility of the boundary and intact zones of myocardium was evidently connected with the beneficial effect of malate and NAD on metabolism of the affected heart [4, 7] and also with their ability to increase the blood supply to the ischemic focus in the myocardium [1]. The weak effect of the compounds on myocardial function in the central ischemic zone can be explained either by their inadequate penetration into this zone or by the fact that tissue changes there were already irreversible.

Malate and NAD can thus restore the contractile function of the myocardium in the boundary zone, and this can be interpreted as a manifestation of their antiischemic action.

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LIPOSOME TRANSPORT TO TARGET ANTIGENS AS A POSSIBLE WAY OF STANDARDIZING TARGETED DRUG TRANSPORT

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Targeted drug transport, a field of intensive research in biomedicine, is currently regarded as a promising method of prevention and treatment of several cardiovascular diseases caused by disturbance of integrity of the layer of endothelial cells of the blood vessels [9]. Exposure of the antigenic structures of the intima as a result of this process means that a container containing a drug can be targeted there by antibodies against the corresponding specific structures of the damaged vessel. On the basis of these considerations systems of targeted drug transport to damaged regions of the vascular bed, using liposomes [5], erythrocytes [11], and anticollagen antibodies immobilized on their surface, have been teted both in vitro and in vivo.

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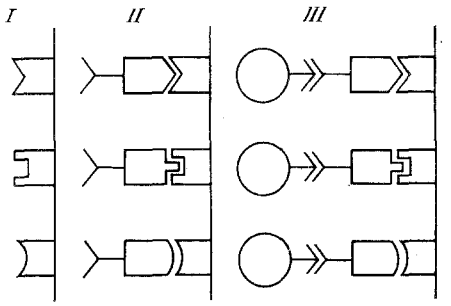

Fig. 1. Diagram of targeted transport of standardized containers to a set of target antigens. I) Antigens exposed on target surface; II) preliminary treatment with mediator molecules; III) specific binding of standardized containers.

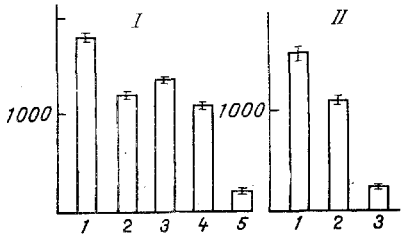

Fig. 2

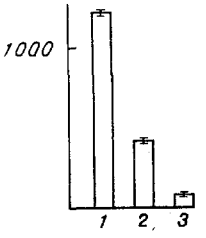

Fig. 3

Fig. 2. Binding of ^{14}C-liposomes with mixtures of antigens A and B adsorbed on a panel. Ordinate, binding (in cpm). I) Mixture A: 1) a mixture of all three biotinylated antibodies was added to the mixture of antigens; 2) biotinylated antibodies against fibronectin; 3) biotinylated antibodies against fibrinogen; 4) biotinylated antibodies against LDL; 5) mixture of three native antibodies; II) mixture B: 1) mixture of three biotinylated antibodies; 2) biotinylated antibodies against fibronectin; 3) mixture of three native antibodies. Mean values of three independent measurements are shown.

Fig. 3. Binding of ^{14}C-liposomes with mixture A of antigens with reduced content of one component (LDL). 1) Mixture of three biotinylated antibodies; 2) biotinylated antibodies against LDL; 3) mixture of three native antibodies. Ordinate, the same as to Fig. 2.

However, many workers have observed that targeting of a container simultaneously against several (more than one) antigens of the target organ, with the use of a mixture of antibodies as vectors, can significantly increase the effectiveness of targeted transport [4, 5, 14].

In this paper we describe a new approach to the concept of targeted transport, by means of which a universal container can be dispatched to several antigens of the target organ at once, and it can also be realized in a model system. Instead of vector molecules, immobilized on the container surface, bifunctional mediator molecules are used, with two types of affinity: for the affected organ and for the standardized container. As molecules of this kind, natural polyvalent macromolecules or artificially synthesized conjugates of several molecules, possessing two different types of affinity, can be used. It is suggested that

the mediators be introduced into the blood stream directly before use of the microcontainers carrying the drug. In this way the containers can be bound with any type of vector (Fig. 1).

In this investigation the glycoprotein avidin was used as the mediator molecule, biotin-modified antibodies as the vectors, and liposomes carrying biotinyl residues on the surface of their membrane as containers. The avid–biotin system was used previously to create the traditional systems of targeted transport of liposomes [12] and erythrocytes [10].

EXPERIMENTAL METHOD

Liposomes labeled with ^{14}C-cholesterol oleate, containing lecithin, biotinylphosphatidylethanolamine, and cholesterol in the molar ratio of 6:1:3 were used in the targeted transport experiments. Liposomes were obtained by sonication on an ultrasonic generator. Biotinylphosphatidylethanolamine was synthesized by the method in [3]. Two mixtures of antigens, each with three proteins, adsorbed on the surface of wells in a titration panel (Titertek, USA) were used as the targets. Mixture A included human fibronectin, fibrinogen, and low-density lipoproteins (LDL), isolated from normal donated blood [2, 8, 13]. Mixture B contained human fibronectin, laminin, and type I collagen. Human laminin and antibodies to it were provided by Dr. J. Martin (National Institutes of Health, USA), the human type I collagen and antibodies to it by S. P. Dogomatskii (All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR). The remaining antibodies were isolated from serum of immunized rabbits on a column with the correspondinng immunosorbent [1]. The surface concentration of each component of the antigen mixture was verified by immunoenzyme assay, based on the avidin–biotin system [7].

Immunoglobulins for biotinylation were modified with the N-hydroxysuccinimide ester of D-biotin by the method in [6]. Into a well in the panel covered with a mixtured of antigen proteins was added 100 µl of a solution of biotinylated antibodies in buffered physiological saline, containing bovine serum albumin in a concentration of 2 mg/ml (BPS-BSA) and the mixture was incubated for 1 h at room temperature. The antibody concentration in the targeted transport experiments was 0.01 mg/ml (total concentration for a mixture of antibodies). Antibodies of each type, adsorbed on the panel with antigens in this concentration, gave an identical signal in the immunoenzyme analysis system. After the panel had been washed, a solution of avidin (10 µg/ml) was added, the preparation was incubated for 10 min, and after repeated washing 100 µl of a suspension of ^{14}C-labeled liposomes was added. In this case incubation was accompanied by mixing in an orbital incubator for 30 min. After the final washing the panel was cut into pieces and firmly bound radioactivity was determined in each well with the aid of a scintillation spectrometer. All washings were done five times, and 200 µl of BPS-BSA was used each time.

EXPERIMENTAL RESULTS

Choice of the antigens in mixture A was determined entirely by accessibility of fewer proteins and antibodies to them, and also by accumulated experience of determination of each component by immunoenzyme analysis. Protein components of the extracellular matrix of the subendothelial layer of the vascular bed, potential targets for targeted drug transport during treatment of the damaged vessel wall, were chosen as components of mixture B.

The results of the experiments to study targeted transport of ^{14}C-liposomes to mixtures A and B of antigens are given in Fig. 2. These results shows that the use of a mixture of vector molecules increases the effectiveness of targeted transport by 30-50% compared with binding due to only one type of antibodies. Values of maximal binding of liposomes with the antigen coated surface were 0.4-0.5 µg of lipid to the surface of one well, which is close to complete covering of its surface with liposomes [5].

Information on the antigenic structure of affected organs can be obtained from the results of immunomorphologic investigations. Very often, however, the relative fraction of accessible antigenic determinants of each type remains unknown, and this information is absolutely essential for success in targeted transport. In this case, the use of the largest possible number of vectors increases the reliability of targeted transport significantly. As an illustration of this statement, we obtained a surface with antigens of mixture A adsorbed on it, and one of the antigens (LDL) was titrated in the immunoenzyme analysis system and found to be about six times less than the total of all three antigens. As a result, tar-

geted transport of liposomes to this surface was three times more effective when all three biotinylated antibodies were used than when antibodies to LDL only were used (FIg. 3).

It was suggested in some publications [4, 5, 14] that containers with affinity for a set of antigens be obtained by direct immobilization of a mixture of immunoglobulins on the container surface. In that case, however, it is quite difficult to verify the degree of binding of the antibodies of a given type with the container. In addition, the presence of antibodies to different antigens on the microcontainer surface may lead to the appearance of steric hindrances during binding of the container with antigenic structures of the affected region.

The system of targeted transport suggested in the present paper is free from the disadvantages of the approach examined above and, in addition, it has the following advantages: 1) the reliability of the system is increased. If one of the antigens of the target organ is inaccessible for the container, the effectiveness of the method, based on immobilization of antibodies of a single type, will be reduced to zero, whereas a container targeted on a number of antigens with the aid of a set of mediator molecules will reach its goal; 2) in the process of preparation of the components of the system the need for the laborious stage of immobilization of immunoglobulins on the container surface is eliminated. The main technical difficulty is reduced to preparation of bifunctional mediators, and in turn, this can be reduced to modification of the protein molecule by a low-molecular-weight modifier; 3) liposomes without immunoglobulins immobilized on their surface are used in this method and, consequently, they can circulate for a longer time in the blood stream [14]; this system of targeted transport allows one of its components to be replaced, while leaving another unchanged, so that effective transport of standardized containers with a particular drug on unique sets of the patient's antigens is possible or, on the other hand, several drugs, enclosed in different containers, can be delivered to particular targets. All components of the set – vectors, mediator molecules, and containers with various drugs – can be made and kept separately, and combined in order to achieve the maximal therapeutic effect.

Any containers (liposomes, erythrocyte ghosts, polymer microcapsules, and so on) and also conjugates based on widely different affinity macromolecules (i.e, on the basis of carbohydrate–lectin, hapten–antibody, interactions, and so on) can be used in the suggested system. The use of such a system in vivo requires additional research in order to discover affinity pairs whose interaction would not be inhibited by blood components.

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