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CHOLESTEROL EXTRACTION FROM RABBIT BLOOD INTO MULTIPLE EMULSIONS

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Components of biological fluids can be extracted from blood with the aid of liquid membranes, in the multiple emulsions version [5, 6]. For instance, experiments with solutions of lipoproteins, isolated by preparative ultracentrifugation, have shown that emulsions containing glycosides in their internal phase selectively extract nonesterified cholesterol (ChS) of atherogenic β- and pre-β-lipoproteins (LP).

The aim of this investigation was to study the possibility of extracting ChS directly from the blood of a rabbit with experimental atherosclerosis, followed by reinjection of the decholesterolized blood into the animal.

EXPERIMENTAL METHOD

To extract the ChS an emulsion of the following composition was prepared: the liquid membrane — mineral oil (39 ml) and sorbitan oleate (surfactant, 1 ml), internal phase — buffered isotonic solution, pH 7.4 (90 ml), and digitonin (500 mg, 4.06 mM in the internal phase).

The freshly prepared emulsion obtained by dispersion of the internal phase in the membrane by mixing on a propeller mixer, was exposed for 30 min in a graduated funnel with the isotonic buffer solution. This last operation prevents hemolysis (Fig. 1) due to the fact that during preparation of the emulsion a certain amount of digitonin (1-3%) may be adsorbed on the outer surface [3, 6]. No digitonin is subsequently lost into the extracted phase (blood) from an emulsion prepared in this way, and the high stability of the emulsion thus prepared (the half-separation time into layers exceeds 24 h) makes it possible for ChS to be extracted from blood without preliminary separation of the erythrocytes.

Under intraperitoneal pentobarbital anesthesia (40 mg/kg) the rabbit's femoral vein was exposed and catheterized, for removal and reinjection of the blood. To prevent the blood from clotting heparin was injected intravenously in a dose of 500 U/kg. Portions of rabbit blood (20 ml) were exposed with the digitonin emulsion in the separating funnel at room temperature and with moderate mixing. The ratio of the phases of blood and emulsion was 1:1 by volume. Under these circumstances the emulsion was distributed among the volume of blood in the form of globules measuring 2-3 mm. After extraction for 40 min the phases were separated and the blood returned into the rabbit's femoral vein. For subsequent exposure blood was again taken from the femoral vein. The process of blood extraction was repeated five times, and 24 h later it was repeated a further four times.

In the course of 1 day of the experiment, i.e. from the 1st through the 5th extraction on the 1st day and from the 1st through the 4th extraction on the 2nd day, the concentration of ChS in blood samples taken from the femoral vein did not change significantly. Accordingly, total initial concentrations were used for the calculations: 11.90 mM (1st day) and 12.10 mM (2nd day). The ChS concentration in the blood stream after the end of each day of the experiment was determined 3.5-4 h after the blood sample had been returned into the vein.

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In the course of the experiment the total ChS concentration (by Ikles' method) and the degree of hemolysis were monitored in blood samples taken from the blood stream and after extraction of the emulsions.

EXPERIMENTAL RESULTS

The results are given in Table 1. The average level of ChS extraction during the procedure was 23.4%. The total quantity of ChS extracted reached $0.5 \cdot 10^{-3}$ mole from 180 ml of rabbit blood, equivalent to 1 mg ChS per gram of emulsion or 0.28 μmole of ChS per gram of emulsion. Incidentally, on average 90-95% of the extracted ChS was accounted for by free ChS, and only 5-10% by ChS esters. The small increase in the ChS concentration in the blood sample taken from the blood stream before the beginning of the 2nd day of the experiment can be explained by its release from the tissue depots.

Emulsions containing glycosides in their internal phase extract nonesterified ChS from solutions of lipoproteins in the form of an equimolecular complex:

$$C_{27}H_{45}OH + C_{56}H_{92}O_{29} \longrightarrow C_{27}H_{45}OH \cdot C_{56}H_{92}O_{29}\downarrow \quad (1)$$

With the aid of Eq. (1) the use of digitonin can be evaluated. The 180 g of emulsion used for extraction contained about 640 mg, or $0.52 \cdot 10^{-3}$ mole, of digitonin, and about $0.5 \cdot 10^{-3}$ mole of ChS was extracted. Consequently, the degree of complex formation of digitonin with ChS under homogeneous conditions was achieved (about 91%) [4]. For each type of emulsion, when the fraction of the internal phase b has been determined, and knowing the degree of complex formation of the glycoside used with ChS (f), and having determined by known methods [1, 2] the concentration of nonesterified ChS of atherogenic β- and pre-β-lipoproteins (LP) c_{ChS}, and having assigned the phase ratio, the required concentration of glycoside in the internal phase of the emulsion c_{glyc} can be calculated:

$$c_{glyc} = \frac{c_{ChS} V_{plasma}}{f \cdot V_{int}} = \frac{c_{ChS} \cdot V_{plasma}}{f \cdot b \cdot V_{emul}} \approx \frac{c_{ChS}}{2 \cdot f \cdot b} \cdot \frac{V_{plasma}}{V_{emul}}, \quad (2)$$

where c_{ChS} denotes the concentration of nonesterified ChS of atherogenic lipoproteins in the blood; c_{glyc} the concentration of glycoside in the internal phase of the emulsion; f the degree of complex formation under homogeneous conditions; V_{plasma} the volume of plasma in contact with the emulsion; V_{blood} the volume of blood, and as a rule, $V_{blood} \approx 2V_{plasma}$; V_{int} the volume of the internal phase of the emulsion; V_{emul} — the volume of emulsion in contact with blood; b the fraction of the internal phase; $V_{int} = b \cdot V_{emul}$.

Formula (2) assumes complete extraction of the nonesterified ChS of β- and pre-β-LP, but in reality the degree of extraction of ChS from low- and very low-density lipoproteins from

TABLE 1. Extraction of ChS from Rabbit Blood (M ± m, n = 3)

Days	Samples	ChS concentration, mM	Degree of extraction, %	Degree of hemolysis, %
1st	Original rabbit's blood	11,90 ± 0,05	—	0,0 ± 0,5
	After 1st extraction	9,50	20,1 ± 0,8	1,0
	After 2nd extraction	9,15	23,1	1,0
	After 3rd extraction	8,50	26,9	1,0
	After 4th extraction	9,55	19,8	1,5
	After 5th extraction	9,30	21,8	1,0
	From blood stream after end of 1st day of experiment	11,10	6,7	0,0
2nd	Original rabbit's blood	12,10	—	1,5
	After 1st extraction	9,60	20,7	2,5
	After 2nd extraction	9,35	22,7	2,5
	After 3rd extraction	8,90	26,5	3,0
	After 4th extraction	8,55	29,4	2,5
	From blood stream after end of 2nd day of experiment	11,00	9,1	2,0

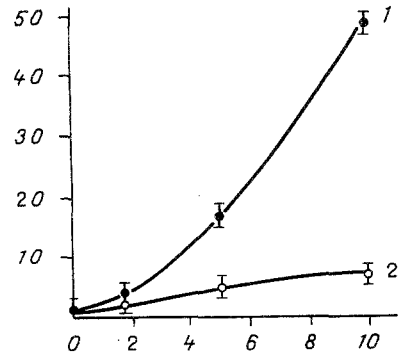

Fig. 1. Dependence of degree of hemolysis on digitonin concentration in internal phase of emulsion. Abscissa, digitonin concentration in internal phase of emulsion (in mM); ordinate, degree of hemolysis (in %). 1) Freshly prepared emulsion; 2) emulsion treated with isotonic buffer solution.

the blood of rabbits with alimentary hypercholesterolemia is 80-85%. By the calculation given above it is possible to avoid any unintentional increase in the concentration of glycosides in the internal phase of the emulsion, leading to the risk (as in the case of digitonin) of increasing the degree of hemolysis. Although glycosides such as tomatonine and solanine are characterized by a lower degree of complex formation than digitonin, their investigation with a view to their use in the internal phase of emulsions would seem to be worthwhile because of their lower hemolytic activity.

The present investigation has thus demonstrated that emulsions containing digitonin in the internal phase may be used to extract ChS from rabbit's blood, followed by return of the decholesterolized blood to the animal. The method of preparation, composition of the membrane, and preliminary treatment of the emulsion with buffered isotonic solution allow extraction to be carried out without any significant increase in the degree of hemolysis in the blood. A method of calculating the concentration of glycosides in the internal phase of the emulsion for ChS extraction is suggested.

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