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CHOLESTEROL EXTRACTION FROM BIOLOGICAL MEMBRANES BY POSITIVELY CHARGED PHOSPHATIDYLCHOLINE MICELLES

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Phospholipids (PL) have the property of extracting cholesterol (CH) from biological membranes [2, 3]. The writers have shown that CH-accepting properties *in vitro* are most marked in positively charged micelles of polyunsaturated phosphatidylcholines (PCH).

This paper describes the results of a study of the possibility of extracting CH from biological membranes *in vivo* in rabbits with experimental atherosclerosis by intravenous injection of positively charged soy PCH micelles.

EXPERIMENTAL METHOD

Experimental atherosclerosis was induced in male rabbits weighing 2.5-4 kg by feeding with CH for 3 months [4]. Half of the animals (experimental group), 2 weeks after stopping the high cholesterol diet, were given PCH intravenously at the rate of 3 or 4 injections per week for 5 weeks. The total dose of PCH given to one animal was 10 g. Animals of the second (control) group were kept under conditions of spontaneous regression of their atherosclerotic lesions. Total CH, PL, triglycerides, and CH and PL of high density lipoproteins (HDL) in the blood serum were determined on an "SMA-12/60 Technicon" automatic analyzer [4]. The methods of isolating ghost erythrocytes, extracting lipids, and determining CH and PL in lipid extracts were described previously [3]. The fatty acid composition of HDL phospholipids was studied on a "Tsvet-106" gas-liquid chromatograph in the Silar 10-ts phase, column temperature 196°C, vaporizer temperature 200°C, detector temperature 200°C. Carrier gas nitrogen, flow rate 40 ml/min. Erythrocyte ATPase activity was determined by the method in [6]. The microviscosity of HDL and the erythrocyte ghosts was investigated by determining the ratio between fluorescence of pyrene excimers and monomers F_{470}/F_{391} [2]. The intensity of fluorescence was measured on a Hitachi-2MPF spectrofluorometer. Platelet aggregation

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TABLE 1. Lipid Composition, Microviscosity, and ATPase Activity of Rabbit Erythrocyte Membranes (M ± m)

Experimental conditions	CH/PL, mole/mole	PE	PCH	SM	PI+PS+ LPCH	PCH/SM	F_{470}/F_{391}	Mg^{++}-ATPase	Na,K-ATPase	Ca^{++}-ATPase
Spontaneous regression	0,82±0,07	30	32	32	6	1,0	1,00	39±4,0	6,8±0,52	87±10
PCH (6)	0,66±0,06	28	40	26	6	1,5	1,54	40±4,2	15±1,10	144±12

Legend. PE) Phosphatidylethanolamine, PI) phosphatidylinositol, PS) phosphatidylserine, LPCH) lysophosphatidylcholine. Activity of ATPases expressed in nanomoles P_i/min/mg PL of erythrocytes. Individual PL fractions separated by unidimensional thin-layer chromatography (TLC) with double distillation in a system of chloroform–methanol–28% ammonia (62:25:5 by volume). Content of individual PL determined as P_i [2] and expressed in relative percentages. Here and in Tables 2-4, number of animals shown in parentheses.

TABLE 2. Lipid Composition of Rabbit Blood Serum at End of CH Feeding (A) and after Administration of PCH for 1 (B), 2 (C), and 5 Weeks (D) (M ±m)

Lipids, mg%	A		B		C		D	
	Control (7)	Experiment (7)	Control (7)	Experiment (6)	Control (7)	Experiment (6)	Control (6)	Experiment (5)
CH_T	670±75	690±76	410±50	320±47	290±25	250±20	139±26	129±17
CH_{HDL}	24±2,4	22±4,1	27±3,7	32±4,4	26±3,3	32±2,6	30±3,0	37±2,5
AI	29±3,8	34±5,5	17±1,0	9±1,0	11±2,2	6±0,5	4±0,8	2±0,2
Triglycerides	160±51	170±44	210±55	100±50	160±47	130±36	115±40	130±45
PL_T	710±67	540±85	400±45	450±57	240±38	300±26	145±40	250±35
PL_{HDL}	35±6,0	27±6,4	34±3,0	41±4,6	31±3,0	40±5,9	36±3,2	42±3,5

TABLE 3. Microviscosity and Lipid Composition of Rabbit HDL

Experimental conditions	F_{470}/F_{391}	Free CH	CH esters	PGP	PE	PCH	SM	LPCH	PCH/SM
Spontaneous regression (7)	0,107	51	49	8	11	44	15	22	2,9
PCH (6)	0,137	38	62	2	7	52	9	30	5.8

Legend. PGP) Polyglycerophosphatides. Content of free CH and CH esters expressed as relative percentages. CH esters were separated from free CH by TLC in a system of hexane–diethyl ether–acetic acid (85:15:1 by volume).

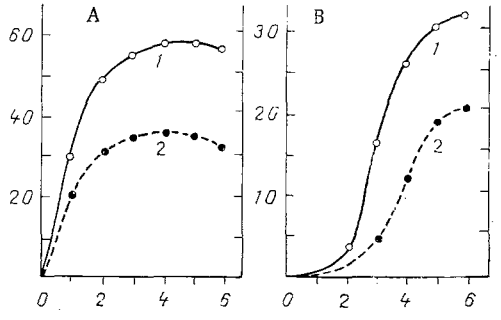

Fig. 1. ADP-Induced (A) and collagen-induced (B) aggregation of rabbit platelets. Abscissa, time of recording (in min); ordinate, degree of aggregation (in % of maximal); 1) spontaneous regression, 2) PCH.

TABLE 4. Fatty Acid Composition of Phospholipids of Rabbit HDL

Experimental conditions	14:0	15:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:3	20:4	20:5
Spontaneous regression(6)	1,77	1,28	26,5	2,48	1,66	13,0	13,7	29,6	1,16	1,17	5,32	2,38
PCH (6)	1,66	1,34	21,7	1,98	1,55	17,1	12,9	31,3	1,82	5,89	1,13	1,63

Legend. Content of fatty acid fractions expressed as relative percentages. Each value is mean of two determinations. PL of pooled extracts of lipids from HDL separated from CH esters and triglycerides by TLC in system of hexane—diethyl ether—acetic acid (85:15:1 by volume).

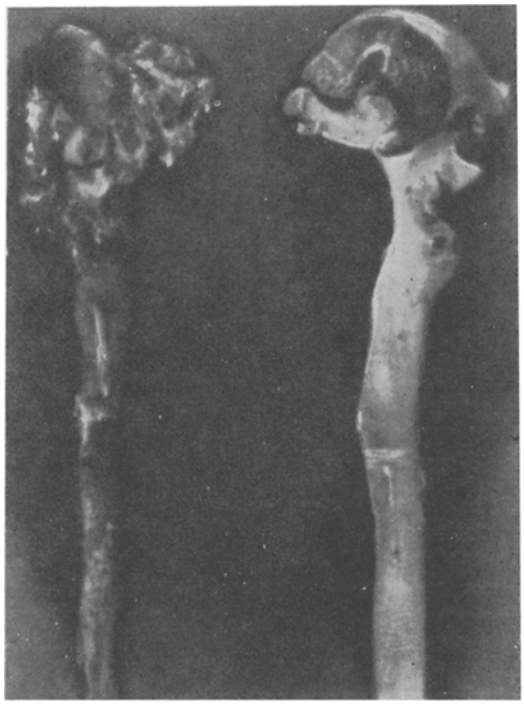

Fig. 2. Aorta of rabbit in stage of spontaneous regression (left) and of rabbit receiving PCH (right). Stained for fat with oil red.

was initiated by the addition of ADP or collagen and investigated on an Elvi-840 aggregometer [5]. The area of the affected parts of the aorta was calculated by the method in [1] after staining for fat with oil red.

EXPERIMENTAL RESULTS

A fall in the CH/PL ratio, an increase in the PCH/sphingomyelin (PCH/SM) ratio, an increase in the F_{470}/F_{391} ratio, and an increase in activity of Na,K-ATPase and Ca^{++}-ATPase activity were found in erythrocyte membranes of animals receiving PCH (Table 1).

The fall in the velocity of ADP- and collagen-induced platelet aggregation in response to administration of PCH can be explained by the decrease in the CH/PL ratio in platelet membranes to 0.92 ± 0.08 in the experimental group (0.99 ± 0.08 in the control) (Fig. 1).

The most marked differences in the lipid composition of the blood lipoprotein of the experimental and control animals consisted of an increase in the PL and CH content in the HDL fraction, a decrease in the atherogenicity index (AI) $(CH_T - CH_{HDL})/CH_{HDL}$, where CH_T and CH_{HDL} denotes total CH and CH in HDL respectively, and a decrease in the triglyceride content in the experimental animals during the first weeks of PCH administration (Table 2). PCH administration was accompanied by a decrease in microviscosity of HDL (Table 3), which

can be explained by an increase in the total PL content, an increase in the PCH/CM ratio, a decrease in the fraction of free CH, and removal of free CH from the surface monolayer of HDL particles, responsible for their CH-accepting properties. The increase in the fractions of CH esters and of lysophosphatidylcholine in HDL on administration of PCH indicates definite activation of lecithin:CH acyltransferase. Investigation of the fatty acid composition of HDL-PL revealed no marked differences between animals of different groups (Table 4).

The area of the aortas occupied by atherosclerotic lesions in animals receiving PCH was only half of that in the control animals: 14 ± 2.5 and 32 ± 4.3% respectively (Fig. 2).

The results of this investigation thus demonstrate that excess CH can be extracted from biological membranes of animals with experimental atherosclerosis by intravenous injection of positively charged PCH micelles.

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ROLE OF BILIRUBIN AS A NATURAL ANTIOXIDANT IN REGULATION OF THE INTENSITY OF LIPID PEROXIDATION IN ACUTE VIRUS HEPATITIS

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Liver damage in acute virus hepatitis (VH) leads to various metabolic disturbances, among which an important place is occupied by disturbances of lipid metabolism, expressed as high serum levels of phospholipids, triglycerides, cholesterol, and nonesterified fatty acids in the patients. Several workers [7, 8, 13] have found high serum levels of lipid peroxidation (LPO) products in patients, correlating with the severity of the disease. Bilirubin, whose level also is regularly raised in accordance with the severity of VH, exhibits the properties of an antioxidant *in vitro* — an inhibitor of radical reactions [9, 11]. Data showing that bilirubin can perform the function of natural antioxidants *in vivo* (especially, in regulation of the intensity of LPO during VH) are not to be found in the literature.

The aim of this investigation was to study the effect of bilirubin on changes in some LPO parameters, namely levels of diene conjugates and antioxidant activity (AOA) of lipids, in the serum of patients with VH of different degrees of severity.

EXPERIMENTAL METHOD

Altogether 63 patients with VH (A and B) of different degrees of severity were studied at the climax of the disease. The degree of severity of the disease was determined on the basis of the usual clinical and clinical-biochemical parameters. Since preliminary investigations revealed no significant differences in the biochemical parameters of patients with VH A and VH B, patients were placed together in the corresponding groups of severity of course of the disease irrespective of the type of VH. The control group consisted of 35 clinically healthy subjects.

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