The results of the experiments with pronase showed that if pronase almost free from nuclease activity was used, the DNA concentration in the samples was unchanged after incubation with the enzyme, but after dialysis it was reduced on average by 50%. These results showed that the DNA, in the form of complexes with serum proteins, was dialyzed and that, consequently, it was of low molecular weight. This was found in the case of both patients and healthy donors. When pronase containing nuclease activity was used in the experiments the DNA concentration fell immediately after incubation with pronase and remained practically unchanged after dialysis (15 and 14.3 μg/ml – the initial values for donor and patient with SLE respectively were: 9.1 and 8.3 μg/ml after hydrolysis and 8.9 and 8 mg/ml after dialysis). Cryoprecipitins isolated from the blood serum of patients with SLE were incubated with pronase subjected to prior autodigestion.

The DNA content in the samples in this case was unchanged both after incubation with the enzyme and after dialysis. Consequently, the DNA contained in immune complexes was nondialyzable.

It can be concluded from these experiments that the DNA which circulates in the blood plasma of healthy persons and patients with SLE is mainly of low molecular weight and exists in the form of complexes with serum proteins. Meanwhile, a DNA of higher molecular weight may also be present in the serum of patients with SLE. This DNA forms complexes with other serum proteins (immunoglobulins).

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SPIN PROBE INVESTIGATION OF THE STATE OF BLOOD PLASMA

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Spin probes and labels have recently found wide application in molecular biophysics for the study both of relatively simple systems (proteins, nucleic acids, bilayer lipid membranes) and of certain complex supramolecular formations, especially biological membranes, by the use of electron paramagnetic resonance (EPR) [2].

The object of this investigation was to develop approaches to the use of the EPR method for the study of a heterogeneous system such as blood plasma, one of the most frequently tested objects in medicine and experimental biology. Rabbits with alimentary atherosclerosis were used as the experimental animals, and a hydrophobic probe of the benzocarboline series, binding of which with albumin, the principal plasma protein, has been investigated in detail previously [3], was used as the spin probe.

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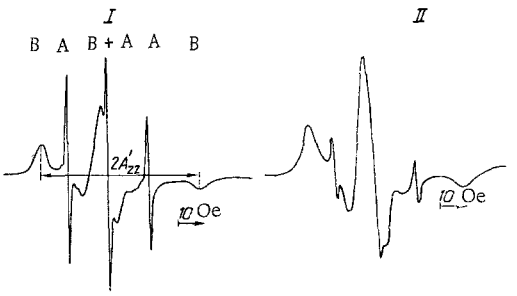

Fig. 1. Examples of EPR spectra of probe-radical ($6 \cdot 10^{-4}$M) in plasma of normal rabbit No. 2 (I) and of rabbit No. 4 with experimental atherosclerosis (II).

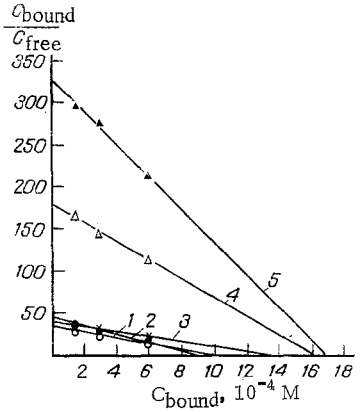

Fig. 2

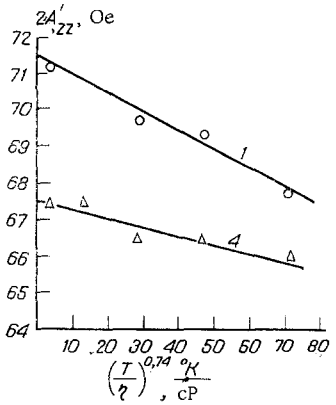

Fig. 3

Fig. 2. Adsorption isotherms of probe-radical in rabbit plasma. Nos. of isotherms correspond to Nos. of rabbits. C_{bound} and C_{free}) Concentrations of bound and free radicals.

Fig. 3. Splitting of A'_{ZZ} as a function of plasma viscosity η for probe-radical adsorbed in plasma of normal rabbit No. 1 and of rabbit No. 4 with experimental atherosclerosis.

EXPERIMENTAL METHOD

Male Chinchilla rabbits were used. Control rabbits Nos. 1 and 2 received a standard diet. For 140 days, rabbits 3, 4, and 5 received additional cholesterol daily by mouth in a dose 200 mg/kg body weight [1]. Plasma was obtained by centrifugation of blood containing EDTA-Na_2 in a concentration of 1 mg/ml, at 800g for 15 min. The plasma cholesterol concentration was determined in the plasma with an accuracy of within 5% by means of the Technicon A-II automatic analyzer.

The spin probe 2,2,4,4-tetramethyl-1,2,3,4-tetrahydro-5,6-benzo-γ-carbolin-3-oxyl [4], dissolved in ethanol, was added to plasma in a concentration of $1.5 \cdot 10^{-4}$, $3 \cdot 10^{-4}$, and $6 \cdot 10^{-4}$M to study adsorption, and in a concentration of $2 \cdot 10^{-4}$M to study its mobility. The ethanol concentration in each series of experiments was kept constant: at 6% to study adsorption of the radical and at 4% to study its spin mobility. In the last series, the viscosity of the solution was changed by the addition of crystalline sucrose to concentrations of 15, 30, 45, and 60%, and was determined from a nomogram [5].

EPR spectra were recorded on the Varian E-4 radiospectrometer with the temperature of the sample in the resonator of 20 ± 1°C, amplitude of modulation 2G, and power 10 mW. The ratio between the concentrations of bound and free probe molecules was determined by double integration of different regions of the spectra. The constant of hyperfine interaction A_{ZZ}, depending on the polarity of the environment of the radical, was determined as $\lim_{\tau \to \infty} A'_{zz}$. The value of τ, characterizing spin mobility of the immobilized radical, was determined by the equation:

$$\tau = 4{,}17 \cdot 10^{-10} \left(1 - \frac{A'_{zz}}{A_{zz}}\right)^{-1{,}5} \cdot \frac{32}{A_{zz}} C.$$

The values of τ were converted for standard conditions corresponding to the viscosity of pure water at 20°C, by obtaining the value of τ^{20}. All technical details concerned with EPR spectroscopy are examined in detail in the monograph [2].

EXPERIMENTAL RESULTS

The morphological and biochemical tests showed that rabbits Nos. 4 and 5 developed marked hypercholesteremia, accompanied by lesions of the whole of the thoracic aorta, whereas only solitary lipid stains were observed in the aorta of rabbit No. 3 and the plasma cholesterol level was close to normal. In control rabbits Nos. 1 and 2 the plasma cholesterol level was normal; no lipid stains were present in their aorta.

The general shape of the spectrum of all samples tested was superposition of spectra of the fast (A) and slow spinning (B) radicals (Fig. 1). The slow spinning spectrum was due to probe molecules adsorbed on high-molecular-weight components of the plasma (proteins or lipoproteins), whereas the fast spinning spectrum was due to molecules of the free probe.

For quantitative investigation of probe adsorption, adsorption isotherms were plotted for the plasma of each rabbit during variation of the probe content in the system (Fig. 2). The parameters of binding (KN – the total binding constant, K – the binding constant of the radical with a single adsorption center, N – the concentration of binding centers in the plasma), calculated from these isotherms with the aid of Langmuir's equation, are given in Table 1. It will be clear from this table that, as the degree of pathological involvement of the aorta increases and the plasma cholesterol level rises, both the number of binding centers in the plasma and the binding constant characterizing them also increases. The greatest relative increase is found in the total binding constants.

For plasma of a rabbit with mild atheromatosis (No. 3) the binding constants correspond to normal, whereas the concentration of binding centers is intermediate in value between that for the control animals and that for rabbits with marked atheromatosis. The values of the binding constants (K and KN), it must be noted, increase with an increase in the plasma cholesterol concentration.

It will be clear from Table 1 that the value of A_{ZZ} is low for the plasma of rabbits with marked atheromatosis and hypercholesteremia. To discover the causes of these changes, plasma of rabbits Nos. 1 and 4 was additionally investigated by measurement of viscosity. The relationships thus obtained between A'_{ZZ} and viscosity η are illustrated in Fig. 3. It will be clear from Fig. 3 that extrapolated values of A_{ZZ} were 35.8 ± 0.2 and 33.8 ± 0.2 Oe for rabbits Nos. 1 and 4, respectively. For the same samples taken without sucrose the values of τ were 32 ± 4 and 119 ± 40 nsec, and of τ^{20}, 21 ± 2 and 65 ± 20 nsec. The decrease in the value of A_{ZZ} for the blood plasma of rabbits with experimental atherosclerosis was thus due to an increase in the degree of hydrophobicity of the environment of the radical fragment and a decrease in the spin mobility of the radical.

Unfortunately, no conclusion regarding the nature of the binding particles in the plasma can be drawn from these results. The most direct answer to this question can be obtained by a further study of binding of the probe with the various components of plasma. Such experiments would not only help to localize the radical used in the plasma, but would also help to select probes binding selectively with the various high-molecular-weight components of the plasma, so that the state of these components could be investigated by examination of whole plasma. Such investigations, conducted with spin-labeled biologically active substances, could help to establish the principles governing their binding with blood plasma and changes in the parameters of this binding in various pathological states.

TABLE 1. Some Parameters of Rabbit Plasma (M ± m)

Rabbit No.	Character of lesions in aorta	Plasma cholesterol concentration, mg%	KN	K, 10^4 M^{-1}	N, 10^{-4} M	A'_{zz}, Oe
1	Control	29	33,5±1,7	3,5±0,4	9,6±1,5	33,7±0,1
2		35	44,0±2,6	4,8±0,7	9,2±1,9	33,7±0,1
3	Solitary lipid stains	49	38,5±0,8	2,9±0,2	13,3±0,9	33,6±0,2
4	Total involvement of thoracic aorta	676	179,5±3,7	11,2±0,1	16,0±0,4	32,4±0,1
5		772	327,0±7,2	19,2±1,8	17,0±2,0	32,6±0,2

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