evidently similar in all cases and consists of disturbance of adhesive interactions of keratinocytes, either among themselves or with the basal tissue membrane.

The high sensitivity of the chosen parameters to the destructive changes investigated suggests that the study of adhesive interactions between keratinocytes may prove to be useful both when studying the mechanisms causing these changes and when assessing the efficacy of therapeutic and preventive measures.

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QUANTITATIVE CHARACTERISTICS OF INTERACTION BETWEEN SERUM LIPOPROTEINS AND STEROID HORMONES

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The role of the blood lipoproteins (LP) in the transport of lipids [10], fat-soluble vitamins [11], xenobiotics [17], and thyroxine [4, 8] has recently been conclusively proved. Our previous results indicate that blood plasma LP can bind with and transport steroid hormones [1, 5, 6]. New, previously unknown properties of LP, determining their involvement in the regulation of intracellular metabolism, have been discovered. It has been shown that LP can penetrate into cells, take part in metabolism, and exert a regulatory influence [3]. Apoproteins are known to have coenzyme functions [9]. However, the role of the protein component of LP (apo-LP) in the body has been studied extremely inadequately. For instance, in order to understand the mechanisms of oriented transport and how certain hormones realize their effect, it is very important to study the possibility of their interaction with apo-LP.

In the present investigation, the glucocorticoid-binding capacity of the blood serum LP and of their protein components (apo-LP) was analyzed quantitatively by methods of fluorescent spectroscopy and equilibrium dialysis.

EXPERIMENTAL METHOD

Preparative isolation of LP from blood plasma was carried out by ultracentrifugation in KBr solution [12] on a "Beckman L-75" centrifuge with 75 Ti rotor. The very low density LP (VLDL, density under 1.006 g/cm^3), low density LP (LDL, density 1.006-1.063 g/cm^3), and high density LP (HDL, density 1.063-1.21 g/cm^3) thus obtained were dialyzed for 24 h against 0.15 M NaCl, pH 7.4, containing 0.005 M EGTA-Na_2 at 4°C. The LP were delipidized with a cold chloroform–methanol (2:1) mixture and then washed with ether. ApoA-1 was obtained by gel-filtration

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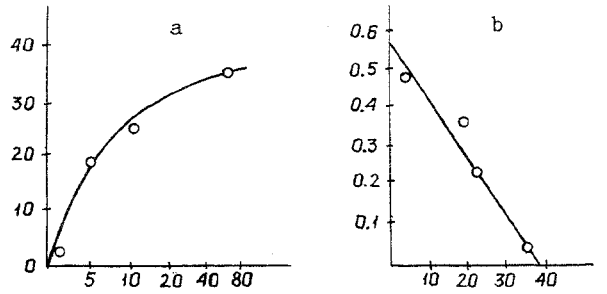

Fig. 1

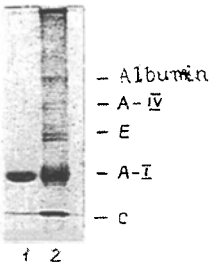

Fig. 2

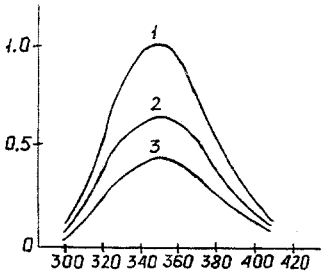

Fig. 3

Fig. 1. Binding of rat blood serum LDL with ^{3}H-corticosterone: a) saturation curve. Abscissa, corticosterone added (in $M \cdot 10^{-6}$); ordinate, bound corticosterone (in $M \cdot 10^{-7}$); b) Scatchard plot. Abscissa, bound corticosterone (in $M \cdot 10^{-7}$); ordinate, ratio of bound to free corticosterone.

Fig. 2. PAG (12.5%) electrophoresis by Laemmli's method: 1) apoA-1 after chromatographic purification; 2) total apo-HDL. Protein bands visualized by 0.1% Coomassie G-250 in a mixture of ethanol and 10% acetic acid (1:1).

Fig. 3. Fluorescence spectra of apoA-1 in 0.05 M Tris-acetate buffer (pH 7.4). 1) ApoA-1 without addition of corticosterone; 2) apoA-1 + corticosterone (30 μl) 5 min after beginning of experiment; 3) the same, 60 min after beginning of experiment. Abscissa, wavelength (in nm); ordinate, fluorescence (in relative units).

on sepharose 4B ("Pharmacia," Sweden) in 0.01 M Tris-HCl buffer containing 6 M urea, pH 8.6. Peak II, corresponding to apoA-1, was repurified by ion-exchange chromatography, using the strong anion-exchange resin "DEAE-Toyopearl 650M" ("TSK," Japan), equilibrated with 0.01 M Tris-HCl with 6 M urea, pH 8.6. Elution was carried out with the original buffer with a linear NaCl gradient from 0 to 0.5 M. The purity of the resulting fraction was tested by PAG-SDS electrophoresis [12]. For equilibrium dialysis, 0.5 ml of a solution of LP (0.1-0.3 mg protein/ml) was introduced into 8/32 dialysis bags ("Serva," Germany). Dialysis was carried out for 48-72 h at 4°C against ^{3}H-corticosterone or ^{3}H-cortisol (10^{-8}-10^{-7} M) in 0.05 M Tris-acetate buffer, pH 7.4. Under these circumstances the concentration of the unlabeled steroid in the outer buffer solution increased relative to the labeled up to a thousandfold excess. Nonspecific sorption on the dialysis membrane was measured during a control experiment. It amounted to 11% of the total quantity of added steroid. Radioactivity was measured on a "Mark III" instrument (USA). The binding constants were calculated on Scatchard plots [16]. Protein tryptophan fluorescence was recorded on an MPF-4 fluorescent spectrophotometer ("Hitachi," Japan) at 4°C and 20°C between wavelengths of 300 and 430 nm. Excitation and emission wavelengths were 285 and 335 nm respectively. The protein concentration in 0.05 M Tris-acetate buffer, pH 7.4, was measured spectrophotometrically by Lowry's method [15]. Solutions of cortisol ($5.5 \cdot 10^{-3}$ M) and corticosterone ($5.8 \cdot 10^{-3}$ M) in 96% ethanol were used. Titration was carried out by addition of aliquots of steroid hormones (1 μl) to 2 ml of a solution of LP, total apo-LP, and apoA-1. The alcohol concentration at the end of the experiment did not exceed 1.5%. Binding constants and the number of binding sites were calculated

TABLE 1. Binding Constants of Corticosterone and Cortisol with Blood Serum Lipoproteins on Scatchard Plots

Class, LP	Corticosterone,	Cortisol, $\cdot 10^6$ M^{-1}
HDL	9,0±0,2	56,0±0,1
LDL	0,1±0,04	4,0±0,3
VLDL	8,0±0,1	8,0±0,9

Legend. Rat blood serum LP were used in the experiments with corticosterone, human blood serum LP in experiments with cortisol. Mean values for three parallel measurements are given.

TABLE 2. Binding Constants of Corticosterone and Cortisol with Total Apo-Protein Fractions by LP Classes Obtained by the Method of Attallah and Lata

Apo-LP	Corticosterone,	Cortisol, $\cdot 10^6$ M^{-1}
Apo-HDL	0,8±0,24	0,2±0,05
Apo-LDL	0,6±0,12	0,2±0,01
Apo-VLDL	0,8±0,18	0,66±0,15

Legend. Mean values for five parallel experiments are given.

by the method of Attallah and Lata [7]. The molecular weights of HDL, LDL, and VLDL were taken to be $2 \cdot 10^2$ kD, $1 \cdot 10^3$ kD, and $5 \cdot 10^3$ kD respectively [2].

EXPERIMENTAL RESULTS

The method of equilibrium dialysis was used to compare interaction of the different LP classes with steroid hormones. This method is considered to be the most reliable for the study of complex formation in a state of thermodynamic equilibrium [14]. By way of example, a saturation curve of rat blood plasma LDL with corticosterone, reflecting dependence of complex formation on the concentration of an excess of unlabeled hormone, added to the outer buffer solution, is shown in Fig. 1. The curve is saturable in character, and indicates enlargement of the LP–corticosterone complex during binding. Scatchard plots were drawn on the basis of these data, and used to calculate binding constants of corticosterone and cortisol with three types of rat and human blood serum LP. The results of the investigation are summarized in Table 1. They show that affinity of blood LP for glucocorticoids is sufficiently high. The highest association constants were obtained for BDL and LDL.

Next the binding constants for corticosterone and cortisol were calculated relative to the protein component of the lipoproteins – apo-HDL, apo-LDL, and apo-VLDL respectively, obtained after delipidization, and isolated by ultracentrifugation of the LP fractions. The method of quenching of fluorescence during titration of the protein with ligand was used for this purpose. The results are given in Table 2. They were closely similar to those obtained by equilibrium dialysis. The small decrease in the value of the binding constant was probably due to removal of lipids necessary to maintain conformation relations typical for intact LP by apoproteins.

Maximal binding of glucocorticoids was demonstrated for HDL, in which the main protein is apoA-1. Separation of the total apo-HDL fraction by PAG electrophoresis and also the position of apoA-1 after chromatographic purification are shown in Fig. 2. ApoA-1 migrated as a single band in the gel as a homogeneous protein with mol. wt. of 28 kD.

Investigations in Tris-acetate buffer, pH 7.4, showed that addition of corticosterone to a solution of apoA-1 was not reflected in the shape of the spectrum and caused virtually no change in its half-width (Fig. 3). Weakening of fluorescence when the hormone concentration was 300 μg/mg protein amounted to 40% of its initial value.

Binding constants and number of binding sites for corticosterone were calculated from the fluorescence quenching curves. Their values were $0.35 \pm 0.08 \cdot 10^6$ M^{-1} and 11 per apoA-1 molecule respectively.

We thus showed that blood lipoproteins bind glucocorticoids with sufficiently high affinity and can play the role of active transport form of these substances in the body. HDL and VLDL have the greatest binding capacity. Apoprotein A-1, 1 mole of which combined 11 moles of the hormone, is one of the main proteins interacting with steroid hormones.

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