increase in the rate of entry of $NaNO_2$ into the blood and additional endogenous methemoglobin generation.

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CHANGES IN BINDING CAPACITY OF HUMAN BLOOD ALBUMIN AFTER UV-IRRADIATION IN THERAPEUTIC DOSES

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Investigation of the primary mechanisms of the therapeutic action of autologous transfusion with UV-irradiated blood (ATUVB), which is widely used at the present time [2], showed that UV irradiation of blood in therapeutic doses gives rise to structural changes both in the blood cells and in the plasma proteins; of the latter, moreover, the thermolabile proteins are the most sensitive [9, 10]. Functional consequences of structural changes induced by UV irradiation have already been described for some such proteins (complement and IgM) [9]. It is only for albumin — the principal blood protein, a highly important transport system of the blood, a regulator of the colloid-osmotic pressure of the plasma, and a reserve protein of the body — that consequences of this kind have not been studied.

The aim of this investigation was to study the effect of UV irradiation of blood in the Izol'da apparatus, which is used for ATUVB in the clinics of the USSR, on the binding capacity of albumin. It is this which determines the transport of ions, physiologically active substances, and also many drugs (predominantly lipophilic) in the body [6, 12]. Since certain

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TABLE 1. Effect of UV Irradiation in Various Therapeutic Doses (1-50 D) on BCA (in % of that for unirradiated plasma) of Blood Plasma from Healthy Adult Donors and Children with HDN (M ± m)

Sample tested	Dose						
	0 (control)	1	2	4	5	10	50
Blood from adult donors (n = 11)	91±7	93±8 (102)	96±6 (104)	98±5 (107)	—	—	—
Blood from children with HDN (n = 13)	44±3	50±4 (115)*	50±3 (115)*	57±3 (136)*	—	—	—
Blood plasma from children with HDN (n = 3)	47±2	—	—	—	58 (123)	56 (120)	55 (117)

Legend. Changes in percent of BCA of unirradiated plasma (control) given in parentheses. *$p < 0.05$; n) number of samples.

pathological states (conjugated hyperbilirubinemia, hemolytic disease of the newborn – HDN, virus hepatitis, etc.) are characterized by reduction of the binding capacity of albumin (BCA), and since their treatment aims at restoring the transport potential of the albumin [1, 4, 11], it was decided to undertake such an investigation not only on blood from healthy donors, but also on blood from patients with one of the diseases mentioned above.

EXPERIMENTAL METHOD

Blood from adult donors and from children with HDN, stabilized with Glyugitsir solution, was irradiated with UV light (254 nm) in the Izol'da apparatus in a standard therapeutic dose (1 D) and also in higher doses (2-4 D). In three experiments blood plasma without platelets was irradiated. Since in the absence of platelets the dose of UV light falling on the plasma was increased almost a hundredfold, the inbuilt filter of the apparatus, reducing the energy tenfold, was used [3]. Under these circumstances, the dose received by the plasma was 5, 10, and 50 D, respectively. Plasma was separated from blood cells by consecutive centrifugation (twice each at 100 and 900 g for 20 min). The binding capacity was investigated by the standard micromethod [14], by staining albumin with the azo-dye 2,4-hydroxyazobenzene-benzoic acid, and the quantity of bound dye was expressed as a percentage of that for a standard 4% solution of albumin. It is generally accepted that BCA can be estimated in this way principally relative to bilirubin [14, 15].

EXPERIMENTAL RESULTS

BCA of the blood from different donors varied from 60 to 127%, with a mean value of 91% (Table 1). After UV irradiation in doses of 1, 2, and 4 D it showed a tendency to rise, to a maximum at 4 D, but this effect was found in not more than 60% of blood samples, and as a whole it is not statistically significant. Analysis of the data revealed correlation between the initial level of BCA and the character of its change after UV irradiation: an increase in BCA was observed more often in blood samples with a low initial level (group 1), and no effect or a decrease in BCA was found in samples with an initially high BCA (group 2). The coefficient of correlation for all samples irradiated in doses of 1, 2, and 4 D was 0.880, 0.755, and 0.779, respectively. After UV irradiation in a dose of 4 D, BCA in the samples of group 1 increased to 119% of the initial value, whereas in the samples of group 2 it fell to 95%.

BCA of the blood from children with HDN varied from 30 to 60, with a mean value of 43.9 for the 13 samples tested, or only half as high as that of the blood donors (Table 1). The effect of UV irradiation in this case was appreciably greater, and became significant (115-136% of the initial value) with all doses tested. Just as in the adult donors, the greatest increase in BCA was characteristic of blood samples with a low initial level (group 1), and a less marked rise or even a fall of this parameter was observed in samples with a relatively high initial BCA (group 2). This relationship is reflected in the high coefficient of correlation: 0.920, 0.846, and 0.681 in a series with UV irradiation in doses of 1, 2, and 4 D, respectively. In these doses UV irradiation led to an increase in BCA in the blood samples of group 1 from 29 to 110, 131, and 172% of the initial level, and in the samples of group 2 to 106, 103, and 108%, respectively.

UV irradiation of three samples of platelet-free plasma in doses of 5, 10, and 50 D led to an increase in BCA, a direct consequence of the action of UV rays on plasma proteins. Increasing the dose to 10-50 D did not lead to any further increase in BCA (Table 1).

Thus UV irradiation of blood in a standard therapeutic dose, and also in doses 2-4 times higher, induces an increase in BCA. This effect is maximal when the initial values of BCA are low. This relationship, on the one hand, affords further confirmation of the opinion expressed by the writers previously on the regulatory action of UV irradiation on the cellular and plasma components of blood [8, 10], and on the other hand, it indicates that the use of UV irradiation of blood may be worthwhile in pathological states characterized by lowering of BCA.

Although the great conformational flexibility of albumin molecules and their high lability on exposure to various factors are well known [12], and structural changes in this protein under the influence of UV irradiation were described a few decades ago [13], nevertheless it is only very recently that the increased UV-sensitivity of albumin compared with other blood plasma proteins was found [5, 7]. Comparison of the results of the present investigation with data obtained previously [9] suggests that the UV-sensitivity of albumin is higher than that of other thermolabile plasma proteins, and in particular, than that of complement and IgM. In our opinion, this may be because aromatic acids are present in the most important regions of the albumin molecule (the first two of the three domains), and also because of complex formation between albumin circulating in the blood and tryptophan [6, 12]. Since these amino acids absorb well in the UV region of the spectrum (250-300 nm), they may play the role of chromophores of UV light. The important fact is that it is those regions of the albumin molecule that contain chromophores that possess the highest specificity for binding bilirubin, tryptophan, certain hormones, and various aromatic anions, which includes many drugs. Hence it follows that binding of these compounds by the plasma albumin will be modified by the greatest degree as a result of UV irradiation of the blood.

Essentially the rise of BCA after UV irradiation is evidently not due to displacement of transported bilirubin from its molecule. This conclusion is supported by the fact that this effect of UV irradiation is found in blood samples not only of patients with HDN, but also of healthy adult donors in whose blood bilirubin is present only in traces. Moreover, even if it is accepted as a fact that the binding of albumin with bilirubin is disturbed by UV irradiation, during the time from the end of the procedure until determination of BCA (usually from 12 to 18 h) the albumin would have bound again with bilirubin. It can therefore be considered that UV irradiation does not increase the amount of bilirubin not bound with albumin.

Discussion of this question is important because certain drugs interact with albumin in precisely this way, and as a result the risk of a toxic action of bilirubin on the CNS is increased. Since there is no danger of an increase in the amount of bilirubin not bound with albumin, UV irradiation of the blood can be tested in the treatment of various forms of HDN.

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EFFECT OF CORTISOL, ALONE AND IMMOBILIZED ON POLYVINYLPYRROLIDONE, AND OF ADENYLATE CYCLASE ACTIVATORS ON CYCLIC AMP LEVELS IN RAT THYMOCYTES

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There is as yet no general agreement regarding the mechanism of the effect of steroid hormones on the adenylate cyclase system of target cells. Some workers have observed a decrease in phosphodiesterase activity of the soluble cell fraction in the presence of steroids [7]. This kind of action is exhibited if the hormones are present in a concentration of about 10^{-6}-10^{-4} M. There is evidence in the literature that steroids may interact with adenylate cyclase at the level of the cytoplasmic membrane of hormone-sensitive cells [2]. By using cortisol immobilized on polyvinylpyrrolidone (PVP-HC), and thus not penetrating into the cell, we have demonstrated in our laboratory that high-affinity glucocorticoid binding sites are present on the cytoplasmic membrane of the thymocytes of adrenalectomized rats [5]. The question of the biological role of the specific binding sites thus revealed needs an answer. The possibility cannot be ruled out that they participate in interaction between glucocorticoids and the adenylate cyclase system of the cells. Thymocytes are a convenient object with which to study the effect of glucocorticoid hormones and adenylate cyclase activators, for on the one hand they are target cells for glucocorticoids, and on the other hand, receptors of biologically active compounds, which exert their action on thymus lymphocytes through activation of adenylate cyclase [9], are located on the plasma membrane of thymocytes.

The aim of this investigation was to study the action of cortisol, PVP-HC, and progesterone on the cyclic AMP (cAMP) concentration in the thymocytes of adrenalectomized rats and to compare it with that of known adenylate cyclase activators (adenosine, isoproterenol, sodium fluoride).

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 120-150 g were used. On the 4th-5th day after bilateral adrenalectomy the animals were killed under superficial anesthesia. Thymocytes were isolated by the method described previously [3]. The viability of the cells was estimated by their ability to stain with trypan blue. In all experiments the viability of the thymocytes after isolation was not less than 95%. After preincubation of the cell suspension for

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