# IMMUNOCHEMICAL ANALYSIS OF THE SERUM PROTEIN PROFILE IN PSORIASIS PATIENTS AFTER HEMOPERFUSION

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The introduction of the technique of extracorporeal hemoperfusion [1] into clinical practice and its successful use, for example, in psoriasis [2], requires a fuller interpretation of the mechanisms of the beneficial effect of this method. It is natural to suppose that the clinical effect of hemoperfusion is attributable to the removal of particular substances related to the pathogenesis of psoriasis from the blood.

The object of this investigation was to analyze changes in the serum protein profile of psoriasis patients after hemoperfusion.

## EXPERIMENTAL METHOD

Altogether 14 patients with disseminated psoriasis in the exacerbation stage, all treated by a single session of hemoperfusion on IGI charcoal, were studied. The technique of hemoperfusion was described previously [1, 2]. Individual serum proteins were determined by double immunodiffusion in agar by means of monospecific standard test systems [4] and also by radial immunodiffusion [5]. Standard antisera (from "Behringwerke," West Germany) and also monospecific antisera against various proteins, prepared in the writers' laboratory by immunizing rabbits with preparations of the corresponding antigens, and by adequate exhaustion of the resulting antisera by different protein fractions, and checked by immunodiffusion analysis, were used in the work. The schemes of immunization of the rabbits and also the methods of determining the physicochemical parameters of particular identified antigens were described previously [3].

To determine proteins adsorbed on charcoal in the course of hemoperfusion, they were eluted from charcoal, previously washed with physiological saline to remove blood, with a 0.5 M solution of disodium hydrogen phosphate.

## EXPERIMENTAL RESULTS

Data on the dynamics of the plasma levels of various individual proteins in patients with psoriasis after hemoperfusion are given in Table 1. Immediately after hemoperfusion a tendency was observed for the levels of lipoproteins, fibrinogen, the three classes of immunoglobulins, and also the $C_3$- and $C_4$-components of complement to fall, but this decrease was small and not statistically significant. No significant changes likewise were found in the serum concentration of these proteins during the 3 weeks after hemoperfusion. These results agree with those of the determinations of proteins adsorbed on charcoal in the course of hemoperfusion, for analysis of the eluates showed that the relative capacity of IGI charcoal for these proteins is of the order of 1 mg/g of adsorbent.

Because of the low relative capacity of charcoal for the principal normal plasma proteins, whose concentrations in the serum is high, it seems unlikely that the beneficial clinical effect of hemoperfusion in psoriasis is due to adsorption of these proteins. The more likely explanation would seem to be that the clinical value of hemoperfusion in diseases with

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TABLE 1. Immunodiffusion Analysis of Changes in the Serum Protein Profile of 14 Patients with Disseminated Psoriasis after Hemoperfusion (M ± m)

| Test proteins | Protein concentration in blood plasma, mg% | | | | |
|---|---|---|---|---|---|
| | before hemoperfusion | immediately after hemoperfusion | 1 week later | 2 weeks later | 3 weeks later |
| $\alpha_1$-Lipoprotein | 270±34 | 260±31 | 280±37 | 290±35 | 290±34 |
| $\beta$-Lipoprotein | 530±62 | 490±57 | 490±61 | 470±54 | 520±68 |
| Apolipoprotein H | 14±2 | 12±2 | 10±3 | 18±4 | 18±3 |
| Fibrinogen | 270±33 | 240±29 | 260±31 | 280±35 | 280±33 |
| IgG | 1150±140 | 1080±120 | 1200±140 | 1260±150 | 1120±120 |
| IgA | 230±24 | 200±28 | 190±31 | 190±29 | 180±26 |
| IgM | 120±18 | 110±16 | 120±19 | 120±17 | 120±19 |
| $C_3$-complement | 90±11 | 70±13 | 80±11 | 100±16 | 100±14 |
| $C_4$-complement | 34±5 | 30±4 | 32±6 | 34±6 | 32±7 |
| CRP | 0,9±0,1 | 0,9±0,1 | 0,6±0,1 | 0,2±0,1 | — |
| PZP | 0,7±0,1 | 0,7±0,1 | 0,6±0,1 | 0,8±0,1 | 0,9±0,1 |
| LTAG | 0,6±0,1 | 0,2±0,1 | 0,4±0,1 | 0,2±0,1 | 0,2±0,1 |
| AMGP | 0,8±0,1 | 0,3±0,1 | 0,4±0,1 | 0,3±0,1 | 0,2±0,1 |

Legend. $P < 0.05$ compared with protein level before hemoperfusion. CRP) C-reactive protein, PZP) pregnancy zone protein, LTAG) lymphocytic thermostable α-globulin, AMGP) $\alpha_2$-macroglycoprotein.

an autoimmune component, of which psoriasis is one, may be due to adsorption of certain biologically active immunoregulatory molecules present in the patients' blood in comparatively low concentrations, but playing the role of a special kind of trigger. Very probably if the course of psoriasis is severe, a stable pathological equilibrium, or vicious circle, becomes established in the patients' immune system, and often this cannot be broken by conservative treatment. After removal of certain molecules which play a key role in this pathological equilibrium by means of hemoperfusion, the vicious circle is broken and this is followed by clinical remission.

On the basis of these views attention was concentrated on two particular "pathological" antigens, identified in the course of this investigation. These antigens are not found in the blood sera of healthy blood donors (or are found only in trace amounts), they are leukocytic in nature (they are found in lysed leukocytes both of patients and of healthy persons), and they are present in high titers (up to 500 mg%) in pus of varied origin. One of these antigens (with mol. wt. 380,000 daltons), on the basis of the physicochemical parameters studied, was called $\alpha_2$-macroglycoprotein (AMGP), the other (with mol. wt. 90,000 daltons) was called lymphocytic thermostable α-globulin (LTAG).

The function of AMGP and LTAG is not yet known, but the following two hypotheses can be put forward on this account. First, these proteins may play the role of immunoregulators (lymphokines, lymphocytic chalones, or their complexes with carrier proteins). Second, LTAG and AMGP may be structural elements of leukocytes secreted into the surrounding medium when leukocytes are destroyed, such as during autoimmune attack, for which they may serve as evidence.

Immediately after hemoperfusion on the patients with psoriasis there was a statistically significant fall in the LTAG and AMGP levels in their blood serum, on average by two-thirds (Table 1); the concentration of these proteins remained low, moreover, for 3 weeks after hemoperfusion. An important property of LTAG and AMGP is their high affinity for charcoal, reflected in the highest coefficients of selectivity of adsorption (the ratio of the specific capacity of the adsorbent and serum concentration), which are tens or hundreds of times higher than these coefficients for the principal normal blood proteins. An essential feature distinguishing IGI charcoal, which must be taken into account, is its ability to adsorb LTAG and AMGP on contact with a suspension of washed leukocytes (either leukocytes react to contact with charcoal by releasing LTAG and AMGP into the external medium, or partial leukocytolysis takes place). Accordingly, the quantity of LTAG and AMGP removed from a patient with psoriasis as a result of hemoperfusion may be even greater than that estimated from the difference between the serum levels before and after hemoperfusion. The possibility that adsorption of LTAG and AMGP may play an important part in the beneficial clinical effect of hemoperfusion in psoriasis is a matter which requires special study.

It will be clear from Table 1 that hemoperfusion does not change the serum levels of C-reactive protein (CRP) or of pregnancy zone protein (PZP) in the blood serum, and these proteins, moreover, likewise were not found in eluates from the charcoal. Nevertheless, the CRP concentration in the blood serum of psoriasis patients fall significantly later, but this evidently only reflects the development of a clinical remission.

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# EFFECT OF PYRIDOXINE, RIBOFLAVINE, AND GLUTAMIC ACID ON RAT LIVER AND SERUM LYSOSOMAL HYDROLASE ACTIVITY DURING TRAUMATIC STRESS


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Stress causes considerable disturbances of cell metabolism [1, 3, 4]. In particular, glutamic acid metabolism is suddenly interrupted on the path of formation of inhibitory intermediates and of succinate, accompanied by a marked deficiency of pyridoxine and riboflavine [2, 14, 15]. Disturbances of relations between excitation and inhibition arising under these circumstances in the CNS lead to qualitative changes in neuroendocrine regulation of metabolism [8]. In turn, changes in the hormonal status of the body affect different aspects of its physiological activity, including the functional state of the lysosomal apparatus of cells in various organs and tissues [7, 9]. This influence is manifested as destabilization of the lysosomal membranes and the outflow of large quantities of hydrolases into the cytoplasm, followed by their appearance in the systemic circulation.

The object of this investigation was to study the effect of pyridoxine, riboflavine, and glutamic acid on the functional state of the lysosomal apparatus of the liver cells and hydrolase activity in the blood in traumatic stress evoked by crushing of the soft tisses of the hind limbs in rats.

## EXPERIMENTAL METHOD

Experiments were carried out on 128 male Wistar rats weighing 250-280 g. The rats, divided into eight series with nine animals in each series, were given glutamic acid (0.25% of the diet) and a fourfold excess of pyridoxine and riboflavine (0.008 mg) *per os* through a special tube daily for 14 days [7]. On the 15th day, after starvation for 12 h, the soft tissues of the rats' thighs were crushed. The control consisted of 56 animals, divided into eight series with seven rats in each series. Trauma was applied in both experimental and control rats, by crushing the soft tissues of the hind limbs with special forceps, designed to a model suggested in the Department of Pathological Physiology, S. M. Kirov Military Medical Academy [4]. To evaluate the effect of a combination of pyridoxine, riboflavine, and glutamic acid on the lysosomal system of the rat liver cells more completely, a modified model of long-term crushing of the soft tissues was adopted: the forceps were not removed until the end of the experiment, so that the main mass of crushed thigh muscles was isolated from


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