neous incorporation of labeled thymidine by circulating lymphocytes has been demonstrated in chronic lymphatic leukemia [5]. The writers previously studied the dynamics of the rise in spontaneous incorporation of labeled thymidine during physiologically normal pregnancy [1]. These findings agree with those obtained by other workers [6]. The origin of the lymphocyte subpopulation spontaneously incorporating the thymidine label has been associated in different studies with stem cells, precursors of immunocompetent cells, and sensitized lymphoid cells [2-5].

It can be concluded from analysis of the results that the peripheral blood of normal adults contains a definite level of proliferating lymphocytes, on the basis of which a criterion of normal can be deduced for a given age group allowing for the technical conditions under which the test is performed. Reduction of the time of culture and manipulations *in vitro* to the minimum ensures that the test is more physiological than others.

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INTERACTION BETWEEN SYNTHETIC PEPTIDES AND INDIVIDUAL COMPONENTS OF THE BLOOD CLOTTING SYSTEM

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KEY WORDS: heparin; peptides; tuftsin; fibrinogen.

Intensive research is currently in progress into the effect of various peptides on the vascular system. These substances appear in large quantities in the blood stream as a result of the action of blood hydrolytic enzymes — trypsin, thrombin, and plasmin. Peptides formed during plasmin hydrolysis of fibrinogen and fibrin possess vasoconstrictor activity and increase vascular permeability [6]. Investigation of these substances and of their synthetic analogs has shown that the biological activity of these compounds depends on the presence of three amino-acid residues in them: proline, arginine, and lysine [6, 7].

Investigations have demonstrated the effect of peptides on blood coagulation. For instance, placental peptides lengthen the prothrombin clotting time [12]. Peptides obtained during fibrinogenolysis exhibit marked anticoagulant properties [11]. They lengthen the prothrombin and thromboplastin times of blood plasma, reversibly inhibit factors VIII, IX, XI, and XII of the blood clotting system, and control thromboplastogenesis. In the investigations which have been undertaken not only have natural peptides been studied, but compounds with high anticlotting potential have also been synthesized. Stereoisomeric analogs of the α(A)-chain site of fibrinogen in the region of the Arg-Gly bond [2] have been created, which exhibit anticoagulant activity by removing thrombin from the fibrinogen molecule. Synthetic peptides, constituting N-terminal regions of the fibrin α-chain can bind fibrinogen and frag-

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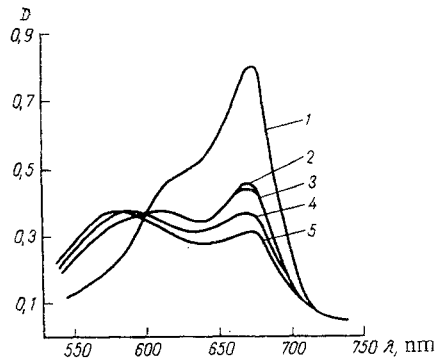

Fig. 1

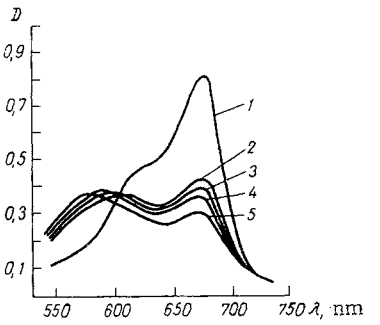

Fig. 2

Fig. 1. Absorption spectra: 1) of methylene blue (MB); 2) MB + heparin + peptide VI; 3) MB + heparin + peptide I or peptide III; 4) MB + heparin + peptide V; 5) MB + heparin. Molar ratio heparin—peptide 1 : 1000.

Fig. 2. Absorption spectra: 1) MB; 2-4) MB + heparin + peptide I; 5) MB + heparin. Molar ratio heparin—peptide I 1 : 2000 (2), 1 : 750 (3), and 1 : 500 (4).

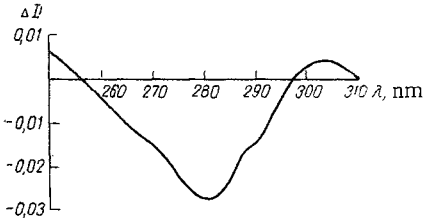

Fig. 3. Differential spectrum of fibrinogen—peptide complex relative to fibrinogen. Molar ratio fibrinogen—peptide 1 : 1000.

ment D, can inhibit polymerization of fibrin-monomer molecules, and can lengthen the clotting time [8, 9]. Peptides (copied) from N-terminal regions of the fibrin β-chain, able to interact with fibrinogen, have no effect on coagulation processes [9].

In the investigation described below, which is part of a complex study of the effect of synthetic peptides of different classes on blood coagulation, the aim was to examine the molecule of tuftsin, the tetrapeptide Thr-Lys-Pro-Arg, contained in the heavy chain of immunoglobulins and stimulating immunogenesis. Its blood concentration is 250-300 μg/liter [5]. The aims were to study the possible effect of tuftsin and also its degradation products — dipeptides Thr-Lys and Pro-Arg — on the various components of the blood clotting system.

EXPERIMENTAL METHOD

Bovine fibrinogen and thrombin were obtained from the Kaunas Microbiological and Bacterial Preparations Factory, and additionally purified on Sephadex G-200 and G-50 respectively; heparin was obtained from Spofa (Czechoslovakia).

The following peptides were synthesized by known methods [1, 8].

I	Pro-Arg,
II	Boc-Pro-Arg,
III	Pro-Arg-OMe,
IV	Boc-Pro-Arg-OMe,
V	Thr-Lys,
VI	Thr-Lys-Pro-Arg,

where Boc [$(CH_3)_3COCO$] protects the N-end and the methyl group Me blocks the C-end of the peptides.

TABLE 1. Effect of Peptides on Characteristic Viscosity of Fibrin-Polymer Solutions in 2.2 M Urea (M ± m)

Peptide	η, dl/g	Peptide	η, dl/g
Monomer	0,51±0,05	Polymer peptide IV	5,9±0,5
Polymer peptide I	4,6±0,4	Polymer peptide V	5,8±0,5
Polymer peptide II	5,5±0,5	Polymer peptide VI	5,6±0,5
Polymer peptide III	5,6±0,5		4,1±0,4

Fibrin monomer was prepared by our modification of Belitser's method [4]. Experiments to determine the characteristic viscosity of the solutions were carried out on Ostwald viscometers in a medium of 2.2 M urea, containing protein in a concentration of 1 mg/ml.

Spectral investigations were done on an SP-8000 UV spectrophotometer (from Unicam, England).

EXPERIMENTAL RESULTS

Interaction of heparin with the peptides was studied by recording absorption spectra in the visible region by the use of a dye (methylene blue). Heparin is known to form a salt-like complex with this dye and to induce a metachromatic shift in its spectrum (Fig. 1: 1, 5). At physiological pH values and in a medium of weak ionic strength (0.01) peptides I, III, V, and VI partially restored spectrum of the dye (Fig. 1: 2-4). Peptides II and IV did not affect the spectrum of the heparin—methylene blue complex. Titration of this complex with increasing concentrations of peptide I restored the spectrum of the dye only a very little (Fig. 2). Similar curves were obtained also for peptides III, V, and VI. These results are evidence that peptides I, III, V, and VI have the property of displacing methylene blue from its complex with heparin and binding with the heparin. Reactivity of this type is not present in peptides II and IV, whose N-end is blocked, and this means that it is the free amino groups of the peptides which take part in the formation of their complexes with heparin. The fact that only partial recovery of the spectrum of the dye takes place even if the peptides are present to excess proves that interaction between molecules in the heparin—peptide complex is of a local electrostatic character.

Investigations in the near UV region using differential spectroscopy revealed a difference in the spectra of native fibrinogen and of the protein bound with peptides I, II, V, and VI (Fig. 3). Peptides III and IV caused no change in the fibrinogen spectrum in the UV region. The shift observed in the spectrum of the protein was due to structural disturbances of organization of the fibrinogen molecules. This blue denaturation shift (Fig. 3) is explained as a rule on the grounds that residues of aromatic amino acids pass from the hydrophobic nucleus of the fibrinogen molecule into a region accessible to the solvent. It must be pointed out that in this case fibrinogen molecules interact with carboxyl groups of the C-end of the peptides, for blocking of these groups in peptides III and IV leads to inactivation of these substances with respect to protein. Evidently complex compounds of low-molecular-weight peptides with thrombogenic proteins and, in particular, with fibrinogen are formed by weak noncovalent forces, and for that reason the presence of these complexes could not be recorded by a method such as chromatography. This method of preparation is unsuitable for compounds with a high degree of dissociation, and in our opinion complexes of heparin and fibrinogen with the peptides studied in these experiments fall into that class.

The viscosimetric studies showed (Table 1) that some decrease in characteristic viscosity, by 22 and 25% respectively, caused by a decrease in the mean numerical degree of polymerization and of branching of the fibrin chains, was observed only in the presence of peptides I and IV. Consequently, certain peptides shift the equilibrium of the monomer—polymer system toward the monomer to a certain degree, although only very slightly. This weak inhibition of the self-assembly of fibrin-monomer molecules is due, in our opinion, to the electrostatic nature of the monomer—peptide complexes. The energy of interaction between the molecules in such a complex is probably much less than the energy of interaction between fibrin-monomer molecules, where a system of noncovalent bonds, including hydrogen bonds, is involved

[3, 10]. It must be pointed out that the antipolymerization activity of tuftsin is due mainly to the Pro-Arg sequence at the C-end.

The experiments thus showed that synthetic low-molecular-weight peptides can interact with different components of the blood clotting system. Heparin binding takes place invariably with the participation of free peptide amino groups, whereas an essential condition for peptide—fibrinogen interaction is the presence of free carboxyl groups. In both cases the dominant role is played by electrostatic forces, which is why the effects observed are weak.

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EFFECT OF PARACHLOROPHENYLALANINE AND 5-HYDROXYTRYPTOPHAN ON THE SERUM TRYPTOPHAN LEVEL AND ITS ABILITY TO STIMULATE TRYPTOPHAN UPTAKE BY CELLS

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KEY WORDS: tryptophan; blood serum; cell uptake.

According to data in the literature one of the factors determining the intensity of tryptophan metabolism in the brain and, in particular, along the pathway of serotonin synthesis, is the serum tryptophan level [3, 6, 10].

On the basis of indirect evidence it can be postulated that feedback (probably negative) exists between the intensity of serotonin biosynthesis in the brain tissue and the blood tryptophan level [4]. If such feedback exists, changes in the intensity of intracerebral serotonin metabolism induced experimentally ought to lead to a corresponding change in the blood tryptophan concentration. The investigation described below was undertaken in order to test this hypothesis experimentally.

As substances modifying the intensity of intracerebral serotonin metabolism, in this investigation it was decided to use parachlorophenylalanine (PCPA), an inhibitor of serotonin synthesis, and the immediate precursor of serotonin — 5-hydroxytryptophan (5-HTP).

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