ization of their membrane through a microelectrode inserted into the cell. The investigations were carried out separately on the outer and inner layers of the two bellies of DM. It will be clear from Table 1 that not only the values of RMP, but also CDL and LP (outer and inner layers) and the amplitudes of AP (inner layer) differ in the anterior and posterior bellies. Comparison of the parameters of excitation of the myocytes in the outer and inner layers of the posterior belly of DM revealed a difference only in the amplitude of the AP generated. All these facts taken together indicate that there are unequal quotas of muscle fibers of different types in the anterior and posterior bellies of DM. More excitable fibers with high levels of polarization predominate in the anterior belly, whereas less excitable fibers with low and average levels of polarization predominate in the posterior belly. Incidentally, the results of electrophysiological testing of the types of muscle fibers agree largely with the results of histochemical investigations of the fiber composition of DM [4, 6].

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# EFFECT OF COMPLEMENT COMPONENT C1q ON BLOOD COAGULATION AND FIBRINOLYSIS


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KEY WORDS: components of complement, blood coagulation, fibrinolysis, hemostasis.


Complement plays its primary role in the regulation of and interconnection between the cellular and humoral components of immune reactions and, consequently, in all physiological functions of the body including the hemostasis system, rather than in the efferent system of protection of the organism [2]. Its highly active substances include activation products of the complementary cascade C5a, C3a, C4a, C3b, etc., that under normal conditions are quickly inactivated. Meanwhile physiologically active components may also be found in the form of inert complexes, whose biological effect is induced during their formation and dissociation. Accordingly, the study of the subcomponents of the first component of complement and, in particular, of C1q, is of great interest.

This paper describes a study of the effect of C1q on blood coagulation and fibrinolysis *in vitro*, undertaken for the first time.

## EXPERIMENTAL METHODS

C1q was isolated by affinity chromatography [3] on macroporous glass from the euglobulin fraction of serum. The preparation contained IgG and fibronectin. The investigation was conducted on whole blood and platelet-enriched and platelet-deprived plasma from healthy blood donors, and plasma deficient in factors V, X + VII. To 0.1 ml of plasma an equal quantity of C1q was added in concentrations of 240, 120, 60, 30, 15, 8, 4, and 2 μg/ml, which was followed by incubation for 30 sec and addition of an agent to initiate clotting. In the con-


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TABLE 1. Effect of Clq on Coagulation of Whole Blood (M ± m)

| Parameter, sec | Control | Concentration of Clq added (ratio 1:1), μg/ml | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 240 | 120 | 60 | 30 | 15 | 8 | 4 | 2 |
| Clotting time | 198±10 | 81±3† | 105±6† | 134±8† | 150±7* | 161±9 | 184±9 | 183±9 | 170±9 |
| Cephalin time | 61±2 | 33±1† | 40±2† | 52±1† | 55±2 | 59±2 | 59±3 | 59±2 | 61±2 |
| Kaolin time | 115±6 | 50±3† | 65±5† | 83±6* | 93±5* | 104±6 | 103±5 | 111±4 | 110±4 |
| Prothrombin time | 35±2 | 29±1* | 29±1* | 33±2 | 34±2 | 34±2 | 34±2 | 35±2 | 35±3 |
| Thrombin time | 45±1 | 46±1 | 46±1 | 45±1 | 45±2 | 44±1 | 45±1 | 46±1 | 46±1 |

Legend. *P < 0.05, †P < 0.001.

TABLE 2. Effect of Clq (120 μg/ml) on Plasma Deficient in Blood Clotting Factors V and X + VII (M ± m)

| Parameter, sec | Plasma deficient in factor V | | Plasma deficient in factors X and VII | |
|---|---|---|---|---|
| | control | Clq | control | Clq |
| Cephalin time | 271±6 | 150±1 | 260±3 | 93±2 |
| *P* | <0,001 | | <0,001 | |
| Prothrombin time | 134±2 | 117±2 | 182±5 | 155±3 |
| *P* | <0,001 | | <0,001 | |

trol tests end-dialysis solution was used (Clq was freed from the preservative immediately before the experiment). The clotting time of the test plasma, the kaolin, prothrombin, and thrombin times, and euglobulin fibrinolysis were determined [1]. The numerical results were subjected to statistical analysis by Student's test.

## EXPERIMENTAL RESULTS

Addition to Clq to whole blood in a near-physiological concentration (180 μg/ml) reduced the clotting time of the test plasma and the cephalin and kaolin times about by half ($P < 0.001$). The effect decreased depending on dose and disappeared when the original Clq preparation was diluted 20-fold (Table 1). Significant changes also were found in the prothrombin time when 25-sec thromboplastin was used. The thrombin time showed no significant change. Similar results also were obtained by a study of the effect of Clq on platelet-deprived and platelet enriched plasma.

Contamination with IgG had no effect on coagulation. This is shown by the following data. Addition of extra IgG to the solution of Clq and addition of pure IgG did not shorten the clotting time of the test plasma. The effect of Clq on the blood clotting parameters disappeared completely on inactivation of the thermolabile component of Clq at 56°C for 15 min, whereas heating pure IgG in the same concentration did not change the clotting time of the test plasma. The fraction which we used was not contaminated with thrombin, for incubation of plasma at 37°C with Clq for 6 h did not lead to clot formation.

Addition of Clq to a 0.4% solution of fibrinogen did not change the clotting time in the presence of thrombin. Addition of Clq to plasma deficient in factors V and X + VII reduced the prothrombin and cephalin times (Table 2). Clq evidently accelerates prothrombinase formation but does not affect the conversion of fibrinogen into fibrin.

Investigation of the effect of Clq on fibrinolysis showed no significant changes in the rate of lysis of the euglobulin clot.

The experimental results indicate that the first subcomponent of complement has thromboplastin-like properties and they suggest that it has a role as physiological stimulator of hemostatic reactions. Any external environmental influences that mobilize the protective forces of the organism lead initially to activation of the hemostasis system and mobilization of immunogenesis. The evolutionarily older defensive cells, namely macrophages, when activated, synthesize increased quantities of Clq which, on the one hand, can perform the function of an $F_c$-receptor [4] and, on the other hand, ensure readiness of the hemostasis system to respond to harmful agents, and thus constitute a powerful link which simultaneously

raises the defensive potential of both immune and hemostatic reactions of the organism, as the results of our investigation show.

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# HORMONAL ACTIVITY OF AN $ACTH_{4-10}$ ANALOG — A LONG-ACTING LEARNING STIMULATOR


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KEY WORDS: long action; adrenocorticotrophic hormone; steroidogenic activity; melanocyte-stimulating activity; corticosterone.


The ACTH fragments $ACTH_{4-7}$ and $ACTH_{4-10}$ affect learning processes in animals and man in very small doses (10-100 μg/kg) [3, 6]. An essential defect of these compounds is the short duration of their action, possibly due to rapid degradation by enzymes. To increase the metabolic resistance of these compounds the writers have synthesized a number of $ACTH_{4-10}$ analogs and have found that $ACTH_{4-7}$ Pro—Gly—Pro: methionyl-glutamyl-histidyl-phenylalanyl-prolyl-glycyl-proline (I) which, unlike the natural $ACTH_{4-10}$ fragments, acts for a much longer time in virtually the same dose [4], is the most effective analog. It was interesting to study peptide I for its ACTH-like hormonal activity. Some ACTH fragments are known to possess hormonal activity to a certain degree, and this is undesirable when these compounds are to be used as regulators of higher nervous activity.

In this investigation the steroidogenic and melanocyte-stimulating activity of peptide I was compared with that of $ACTH_{1-24}$ and $ACTH_{5-10}$. Steroidogenic activity was determined *in vitro* as the quantity of corticosterone formed after incubation of isolated fascicular cells of the adrenal cortex with different concentrations of the test substances [5]. $ACTH_{1-24}$, obtained from Ciba-Geigy AG (Switzerland), was used as the standard compound. Compound $ACTH_{5-7}$ (from Serva, West Germany) was found to have the maximal steroidogenic effect in a concentration of $10^{-5}$ M, producing the same increase in the corticosterone concentration

TABLE 1. Steroidogenic Activity of ACTH Fragments on Isolated Rat Adrenal Cells ($M \pm \Delta m$)

| Concentration of $ACTH_{1-24}$, M | Corticosterone, μg per sample | Concentration of peptide, M | Corticosterone, μg per sample of $ACTH_{4-7}$ |
|---|---|---|---|
| 0 | 0,0016±0,003 | $10^{-6}$ | 0,018±0,02 |
| $10^{-11}$ | 0,047±0,006 | | |
| $10^{-10}$ | 0,136±0,008 | $10^{-5}$ | 0,018±0,003 |
| $10^{-9}$ | 0,460±0,007 | | |
| $10^{-8}$ | 0,490±0,003 | $10^{-4}$ | 0,017±0,004 |


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