THE CHANGE OF THE TYPE ANTIGENIC PROPERTIES OF HUMAN ERYTHROCYTES UNDER THE EFFECT OF SOME PROTEOLYTIC ENZYMES

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(Presented by Active Member of the Acad. Med. Sci. USSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 12,
pp. 69-71, December, 1961
Original article submitted February 17, 1961

Many authors have observed the weakening of antigenic properties of erythrocytes under the effect of trypsin [5,6], ribonuclease [3] and enzymes of some microbes [2]. Because trypsin and chemotrypsin act basically on different peptide bonds [1,4], we thought it relevant to compare the effects of these proteases on type antigens of erythrocytes.

METHOD

We have tested the effect of crystalline preparations of trypsin, chemotrypsin and of their mixture, on erythrocytes. The enzyme solutions in isotonic phosphate buffer (pH 7.6) were used in concentrations of 1.5 and 6 mg/ml. One volume of washed erythrocytes was mixed with an equal volume of the enzyme and $1\frac{1}{2}$ volumes of the buffer solution. The mixtures were incubated in test tubes at 37-38°C. Samples of erythrocytes were taken 5, 10, 20, 30, 40 and 60 minutes after the beginning of the experiment. Agglutination reaction, on a flat surface and in tubes after centrifugation at 1500 rpm, and adsorption reaction were tested.

RESULTS

Blood samples from 47 persons with the following type and group antigenic characteristics were tested: 4 persons - OM, 10 - AM, 4 - BM, 2 - ABM, 5 - ON, 2 - AN, 3 - BN, 2 - ABN, 4 - OMN, 8 - AMN and 3 - BMN.

Solutions of a concentration of 1 mg/ml were found to be hardly effective. Their contact with erythrocytes for 40 minutes did not have any effect on the agglutinating properties of the cells. Erythrocytes which were treated with trypsin and chemotrypsin solutions in the concentration of 5 mg/ml, 7 to 10 minutes later began to lose their capacity to be agglutinated by type sera anti-M and anti-N (Table 1). The weakening of agglutinating properties under the effect of proteases increased with the time of exposure. However, the erythrocytes, during the entire period of incubation with enzymes, retained completely their capacity to be agglutinated by group sera, and in no case acquired the property of non-specific agglutination. Changes occurring in erythrocytes under the effect of proteolytic enzymes were also clearly manifested in the adsorption reaction (Table 2). Samples of fresh erythrocytes, not subjected to enzyme action, almost completely adsorbed the antibodies of anti-M and anti-N type sera. Meanwhile, erythrocytes, after incubation with proteases, lost their ability to adsorb type antibodies. Titers of sera after the adsorption by treated erythrocytes were almost unchanged.

In the next series of experiments we compared the ability of both enzymes (trypsin and chemotrypsin) to act on type antigenic properties of erythrocytes. The effects of these enzymes were studied on the same samples of erythrocytes, using same concentrations and times of exposure of the enzymes.

It will be seen in Table 3 that trypsin is more active than chemotrypsin. However, a mixture of trypsin and chemotrypsin is more effective than trypsin alone. It is possible that a certain summation of the action of the trypsin-chemotrypsin mixture may be explained by a greater activity of each of these enzymes at different stages of proteolysis.

Thus, trypsin and chemotrypsin, when incubated with erythrocytes at 37-38°C, destroy the type antigens of the latter. However, the treatment of erythrocytes with trypsin and chemotrypsin has no effect on their agglutinating and adsorption properties toward group sera. This may be due to the fact that group antigens, unlike type antigens, are of a polysaccharide nature.

TABLE 1. Change of Titer of Type M- and N-Antigens of Erythrocytes after Treatment with Enzymes in Concentration of 5 mg/ml for 1 hour (standard protocol)

No. of blood sample	Treatment	Antigenic characteristic of erythrocytes	Specificity of standard serum	Serum dilutions								Control: erythrocytes in normal saline
				1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
46	Before enzyme treatment	BM	Group A (II)	+++	+++	+++	+++	++	+	+	-	-
46	Trypsin plus chemotrypsin	BM	The same	+++	+++	+++	+++	++	+	+	-	-
46	Before enzyme treatment	BM	Type, anti-M	+++	+++	+++	++	+	-	-	-	-
46	Trypsin plus chemotrypsin	BM	The same	+	+	±	-	-				
47	Before enzyme treatment	AB	Type, anti-N	+++	+++	++	+	+	-	-	-	-
47	Trypsin plus chemotrypsin	AB	The same	+	+	-	-	-				-

Legend: +++ agglutinate is not broken with slight shaking of tube; ++ agglutinate breaks into several large lumps with slight shaking of tube; + agglutinate breaks into numerous small lumps; ± agglutination seen only under a microscope; - no agglutination.

TABLE 2. Change of Adsorption Ability of Erythrocytes after Treatment with a Mixture of Trypsin and Chemotrypsin in a Concentration of 5 mg/ml (standard protocol)

No. of blood sample	Erythrocytes by means of which adsorption was performed	Erythrocytes used in reaction	Serum specificity	Serum dilutions						Control: erythrocytes in normal saline
				1:2	1:4	1:8	1:16	1:32	1:64	
4	Fresh, not treated with enzymes	Fresh erythrocytes of AM blood group	Type, anti-M	+	-	-	-	-	-	-
4	Enzyme-treated for 30 min	The same	The same	++	++	++	+	-	-	-
4	Before adsorption	The same	The same	++	++	++	++	+	-	-
45	Fresh, not treated with enzymes	Fresh erythrocytes of AN blood group	Type, anti-N	±	-	-	-	-	-	-
45	Enzyme-treated for 1 hr	The same	The same	+++	+	+	-	-	-	-
45	Before adsorption	The same	The same	+++	++	+	-	-	-	-

Legend: as in Table 1.

TABLE 3. Effects of Trypsin, Chemotrypsin and of Their Mixture on Erythrocytes (standard protocol)

Erythrocyte antigens	Nature of treatment	Serum dilutions				
		1 : 2	1 : 4	1 : 8	1 : 16	1 : 32
AM	Before enzyme-treatment	+++	+++	++	+	–
AM	40 min exposure to trypsin, 5 mg/ml	++	+	±	–	–
AM	40 min exposure to chemotrypsin, 5 mg/ml	++	++	+	–	–
AM	40 min exposure to a mixture of trypsin and chemotrypsin	+	+	–	–	–

Legend: as in Table 1.

SUMMARY

Erythrocytes subjected to the action of trypsin and a mixture of trypsin with chemotrypsin lost their capacity to be agglutinated by the type specific sera and to adsorb antibodies. Trypsin and a mixture of trypsin with chemotrypsin destroy the type specific antigens, but do not act on the group properties of human erythrocytes.

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