IMMUNOLOGIC CHANGES IN THE BLOOD SERUM AFTER PROLONGED INJECTION OF TRYPSIN

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Reports have recently been published that the serum of man and animals may have a cytotoxic action in vitro on the cells of their own tissues if these have first been treated with trypsin [7, 8]. It is assumed that trypsin, by breaking down the mucopolysaccharides in the cell membranes, makes the cell accessible to the action of normal tissue antibodies present in the serum.

One of the present authors has demonstrated an increase in the cytotoxic action of immune sera in vitro on tumor cells treated initially with trypsin.

It has been shown experimentally that the injection of another proteolytic enzyme—papain—into rabbits causes an increase in the level of proteins circulating in the blood stream [2, 6] and the serum acquires the ability to react with antigens from the autologous and homologous kidney [5].

The object of the present investigation was to study the immunologic changes in the blood serum of animals receiving injections of trypsin.

EXPERIMENTAL METHOD

Experiments were carried out on 28 male chinchilla rabbits weighing 2.5-3 kg. Before the experiment began the sera of all the animals were tested by the complement fixation reaction (CFR) and by Ourchterlony's gel-precipitation reaction (PR) with antigens from liver, kidney, heart, and brain tissues from hologous animals, and the total protein content and the relative proportions of the protein fractions were determined in their blood sera. The rabbits were then divided into three groups. The animals of group 1 (8) received injections of 5 ml of 2% trypsin solution on alternate days for two weeks. The animals of group 2 (16) received injections of the same dose of trypsin for 45 days. The animals of group 3 (4, control) received injections of physiological saline intravenously in identical conditions. Two weeks after the experiment began, blood was taken from all the animals of group 1, from 7 rabbits of group 2, and from all the control animals for performance of the CFR and PR, and determinations of the total serum protein and protein fractions. Blood was again taken from all the rabbits of experimental group 2 and the control group 45 days from the beginning of the experiment for repetition of the investigations listed above.

Since normal antibodies are known to be more thermolabile than immune antibodies and are destroyed at 65° [1, 3, 4], parallel determinations were made of the CFR with sera heated to 56 and 65°. The antigens used were saline extracts (1:10) of homologous rabbit organs—kidney, liver, heart, and brain. When the CFR was performed with sera obtained from the rabbits of group 2 after 23 injections of trypsin, in 8 cases the antigens used were extracts from autologous organs, but when the results are described, the data for the reaction with homologous and autologous antigens are aggregated.

The antigen for the PR was prepared by centrifuging saline homogenates (1:3) of homologous organs at 5000 rpm for 30 min.

The total protein content was determined by the RPU-1 refractometer. Fractionation of the serum was carried out by electrophoresis on paper in a type AFA-1 apparatus for 18 h with a potential gradient of 2.5 V/cm. Staining was with bromphenol blue. Measurements were made on the FED-56 photoelectric colorimeter.

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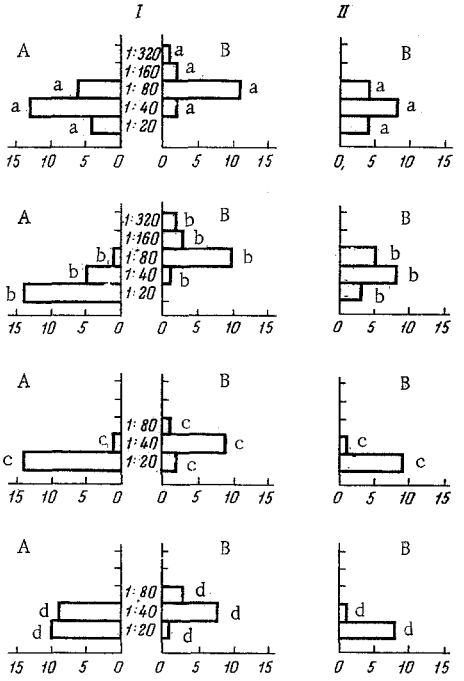

Results of CFR with sera heated at 56° (I) and 65° (II). A) Sera obtained before injection of trypsin; B) sera obtained after 23 injections of trypsin; ordinate-maximal titer of investigated sera; abscissa—number of sera giving positive reaction: a) antigens from kidneys; b) from liver; c) from heart; d) from brain.

Number of Sera (in %) Giving Positive CFR with Antigens from Homologous and Autologous Organs following Prolonged Administration of Trypsin

Conditions of obtaining serum	Sera giving positive reactions with antigens from			
	Liver	Kidney	Heart	Brain
Before injection of trypsin	71,4 / 0	82,1 / 0	53,5 / 0	67,8 / 0
After 7 inections of trypsin	100 / 53,3	93,3 / 53,3	60 / 6,6	66,6 / 6,6
After 23 injections of trypsin	100 / 100	100 / 100	75 / 62,5	75 / 56,2
After injection of physiological saline (control) 14 days	75 / 0	75 / 0	25 / 0	50 / 0
» 45 »	75 / 0	75 / 0	25 / 0	50 / 0

Note. Results obtained with sera preliminarily heated from 30 min at 56° are given in the numerator; results obtained with sera heated for 30 min at 65° are given in the denominator.

EXPERIMENTAL RESULTS

The investigations showed that following prolonged administration of trypsin the number of sera giving a positive reaction to antigens from homologous and autologous organs increased. This increase, moreover, was particularly marked in the case of the sera heated to 65°, in which the proportion of positive reactions increased from 0-100% (see table).

The prolonged administration of trypsin also cause a marked increase in the titer of the investigated sera (see figure). This increase in the serologic activity of the sera was observed in relation to antigens from kidney and liver tissue, and to a rather lesser degree when the CFR was performed with antigens from the heart and brain tissue .

Prolonged administration of trypsin led to the appearance of serologic activity toward these antigens in the sera heated at 65° (see figure); before injection of trypsin no serologic activity was found in any serum.

The PR showed that the sera of normal rabbits formed very weak precipitation lines with antigens from the tissues of rabbits' organs, and when the sera were heated to 65° these lines disappeared. The sera of rabbits receiving trypsin injections for 45 days formed clear precipitation bands with antigens from the tissues of the kidney, liver, heart, and brain. After heating to 65° the sera continued to give precipitation lines, although they were weaker.

Investigation of the effect of repeated injection of trypsin and the total serum protein content and on the relative proportion of the protein fractions showed no marked abnormality. For example, the mean total serum protein concentration of 16 rabbits before the beginning of the experiment was 6.42%, falling to 6.20% after 23 injections of trypsin. Electrophoresis of the serum protein fractions of these same rabbits revealed a decrease in the albumin content from 59-53.7% and in the α-globulin content from 15.6-14.6%, with an increase in the β-globulin level from 13.1-15.7% and in the γ-globulin level from 12.1-15.9%.

Calculation of the albumin-globulin ratio showed a decrease following prolonged administration of trypsin from 1.44-1.32 after 7 injections and to 1.18 after 23 injections.

It may be concluded from all these results that prolonged administration of trypsin gives the serum the property of reacting in a higher proportion of cases and in a higher titer in the CFR and PR with antigens from organs of homologous animals. This increase discovered in the serologic activity of the sera under the influence of trypsin injections is evidently associated with the appearance of antibodies resembling immune antibodies in their thermostability in the serum. This hypothesis is confirmed by the decrease in the albumin-globulin ration.

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