PROTEOLYTIC ACTIVITY IN DERMATOSES: STUDIES OF BLOOD OF PATIENTS WITH PRURITUS*

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In a previous study, the role of proteolytic enzymes in the production of itching was investigated (1). Localized itching was produced regularly by the intradermal injection of streptokinase, profibrinolysin, fibrinolysin, and epidermal protease. Itching could be produced also by endopeptidases found in plants and animals, for example, trypsin, ficin, and papain. In some normal individuals, the intradermal injection of streptokinase, human fibrinolysin, or bovine crystalline trypsin was followed by scattered itching lasting from one to eight hours. These observations prompted measurements of proteolytic activity of blood in normal controls and in patients with severe itching. Additional observations were made before and after the intradermal injection of bovine crystalline trypsin.

METHODS

Protease activity of plasma or serum was estimated by measuring the hydrolysis of a synthetic substrate: toluylsulfonyl-arginine-methyl ester (TAME). On hydrolysis, the carboxylic acid became free, displaced CO_2 from the bicarbonate buffer and CO_2 was measured manometrically in a Warburg apparatus.

As a routine the enzyme containing globulin fraction was precipitated out and the precipitate dissolved in the original volume of saline. Two-tenths (0.2) milliliters of globulin was incubated for 60 minutes at 37° Centigrade with 20 micromoles of substrate. Activity of the enzyme was expressed in terms of micromoles of enzyme hydrolyzed per hour by 1 milliliter of plasma (spontaneous plasmin activity).

Antifibrinolytic activity was measured by adding small concentrations of serum: 0.01 to 0.05 milliliters to 2 milligrams of bovine fibrinolysin and measuring the decrease of activity of the enzyme. Results were expressed as the amount of enzyme activity supressed by 1 milliliter of serum (as found by extrapolation).

Activator content was measured by adding 0.2 milliliters of plasma globulin to 0.05 milliliters of a standard inactive and activator-free human globulin. Activity above the added spontaneous actions of the two components was considered due to the activator present in the plasma sample and expressed as above.

Solutions of crystalline trypsin* were freshly prepared, the solvent being a specially prepared buffered saline[1]. Five-hundreths (0.05) to 0.1 milliliter of a 1:1000 solution was then injected intradermally.

RESULTS

Normal subjects

Plasma fibrinolysin (protease) and serum fibrinolysin inhibitor were measured in sixteen subjects and plasma fibrinolysin activator in eight of these. The range of fibrinolysin in units/milliliters of plasma varied from 1.4 to 10.9 with an average of 5.76. The corresponding serum inhibitor levels varied from 0.6 to 7.6 with an average of 3.22. The range of fibrinolysin activator was 0 to 7.8 with an average of 4.1.

Patients with pruritus

Thirteen patients with various types of severe pruritus were studied. The results are shown in Table I.

In this group, fibrinolysin activator levels varied from 0 to 7.8 units/milliliter with an average of 2.6.

The plasma fibrinolysin levels varied from 5.1 to 8.7 with an average of 6.9 units/milliliter.

Serum fibrinolysin inhibitor levels varied from 0.5 to 5.4 units/milliliter with an average of 2.7.

Levels of plasma fibrinolysin activator, plasma fibrinolysin and serum fibrinolysin inhibitor were obtained before and two hours after the intradermal injection of trypsin in eight subjects. Five of these were normal controls, while the other three were patients with severe scattered itching of undetermined etiology. In three of the five normal subjects, the injection of trypsin was followed by scattered itching beginning some twenty minutes after injection and lasting from two to four hours. This phenomenon was not

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* Obtained from the Worthington Biochemical Company, Freehold, New Jersey.

TABLE I

Proteolytic Activity in Blood of Patients with Severe Pruritus

Diagnosis	Activator Plasma	Plasma Fibrinolysin	Serum Inhibitor
	units/ml.	*units/ml.*	*units/ml.*
1. diffuse neurodermatitis	—	7.8	4.9
2. stasis eczema and neurodermatitis	2.2	8.2	1.2
3. scattered pruritus, psychogenic (?)	1.3	6.5	3.2
4. psychogenic itching	2.1	4.3	0.4
5. chronic pruritus, osteoporosis, arthritis	0	5.6	1.1
6. severe itching with gall bladder disease	3.3	5.1	1.5
7. diabetes, cirrhosis with jaundice, pruritus	2.4	6.5	1.9
8. erythroderma, lymphoblastoma (?)	3.3	8.35	3.1
9. erythroderma, lymphoblastoma (?)	0.45	6.7	3.4
10. severe itching, lymphoblastoma (?)	0.2	8.7	5.4
11. Hodgkin's disease	5.2	7.6	3.1
12. Hodgkin's disease	0	2.4	0.8
13. pruritus, cancer (?)	7.8	5.1	0.5

noted in the other five subjects tested. Significant deviations from the levels obtained in normal controls were not found either before or after the intradermal injection of trypsin.

COMMENT

A comparison of levels of plasma protease activator, plasma protease (fibrinolysin), and serum protease inhibitor in normal subjects and in patients with severe, extensive pruritus did not reveal significant differences. These findings suggest that if proteolysis is responsible for extensive pruritus, it may occur primarily in the skin. If the stimulus to protein breakdown and pruritus is transported by the blood, it must be present in a form not currently detectable.

In three subjects in whom scattered itching had been produced by the intradermal injection of trypsin, a comparison of pre- and post-trypsin proteolytic activity in the blood did not show significant variations. The experimental production of scattered itching is apparently not due to the activation of profibrinolysin by trypsin. It is of interest that scattered itching could not be produced by the intradermal injection of trypsin in three patients with chronic intractable generalized pruritus. In these patients, the serum fibrinolysin inhibitor was not elevated, and it is postulated that failure to develop pruritus may be due to an unknown host mechanism possibly residing in the skin.

The possibility that increased proteolytic activity of blood is related to extensive pruritus could be neither confirmed nor disproved by the present study.

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REFERENCE

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