The Protease and Antiprotease of Plasmas of Patients with Cancer and Other Diseases*

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MacFarlane and Biggs (5) recently reviewed the literature dealing with the plasminogen-plasmin, an active proteolytic enzyme and an antiprotease which are present in plasmas in health and disease. Satisfactory quantitative data for the plasminogen-plasmin relationship and for the spontaneously active proteolytic enzyme in disease are limited. Although the antiprotease has been studied rather extensively, its relationship to the proteolytic enzymes is not well established.

The active proteolytic enzyme observed in the plasmas of diseased individuals has been designated chiefly as fibrinolysin and "plasmin." The characterization of this enzyme is inadequately defined. With a few exceptions (5), the results obtained for the activities of this proteolytic enzyme in the plasmas of patients cannot be considered as quantitative.

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Von Dungern (2) first attempted to determine the trypsin inhibitor quantitatively in the plasmas of patients with osteomyelitis. Subsequently, many observations have been made on antiprotease with a variety of procedures. It is generally agreed that this inhibitor is increased in a large variety of diseases (3, 4). Christensen (1) studied the kinetics of the inhibitor-cryotryptic trypsin reaction and was able to develop a well standardized procedure for determining inhibitor activity.

This investigation is concerned with the plasmin, spontaneously active proteolytic enzyme and antiprotease activities of the plasmas of patients (a) shortly after admission and usually before therapy is instituted, and (b) at intervals after operation or during therapy.

EXPERIMENTAL

Plasma was separated by centrifuging the oxalated blood of fasting subjects. The plasmas of 3 groups were analyzed: (a) healthy male medical students; (b) patients with about 20 different types of cancer; (c) patients with acute bacterial and virus diseases and a variety of chronic diseases. The diagnosis in each cancer case was confirmed by histologic examination.

The procedure described by Ratnoff (6) for determining chloroform activated plasmin was used except for the initial buffering of the casein substrate. Casein was dissolved in 0.85 per cent saline buffered with M/20 phosphate at pH 7.4.

The spontaneously active proteolytic enzyme is designated as proteolysin in this paper. It is present in the euglobulin precipitate which is obtained by diluting plasma 20 times with distilled water, adjusting the pH to 5.2 with acetic acid and centrifuging. The precipitate representing 2 ml. plasma is dissolved in the buffered saline-phosphate and brought to a 5 ml. volume. The activity of a 2 ml. aliquot is determined after a 1 hour incubation with an equal volume of the buffered casein substrate.

The trypsin inhibitor (antiprotease) is found in the euglobulin supernatant. The details of the procedure for determining this inhibitor are outlined since they represent modifications of a number of methods, particularly those of Christensen. The supernatant is diluted serially with buffered saline at pH 7.4, and 1 ml. aliquots are incubated for 10 minutes with 1 ml. of a standard solution containing 0.02 mg. crystalline trypsin. At the end of this time 2 ml. of 0.5 per cent buffered casein are added and incubated at 37°C for 15 minutes. Digestion is stopped by adding HCl and sulfosalicylic acid to 1 ml. of the inhibitor digestion mixture according to Ratnoff (6). The turbidity is read in a Klett-Summerson colorimeter. The values for a minimum of 6 different dilutions of a plasma are plotted and a sigmoid curve is obtained. The inhibition unit represents the amount of a plasma which causes a 50 per cent inhibition in the digestion of 0.3 per cent buffered casein by 0.02 mg. crystalline trypsin.

RESULTS

Measurements of the plasmin, proteolysin, and trypsin inhibitor in cancer and miscellaneous dis-
cases are shown graphically in Figure 1. These patients were studied shortly after admission to the hospital. The control ranges represent the limits of variation in 16 normal subjects. The chloroform activated plasmin values of one half of the cancer cases and 37 per cent of the other cases are elevated. The proteolysin values for 30 of the 33 patients with cancer are above normal; the 3 values within the control range are obtained in patients with (a) chronic myelogenous leukemia, (b) multiple myeloma, and (c) a small squamous cell carcinoma of the lip. In the miscellaneous diseases, 26 of the 31 values for proteolysin are elevated; the cases in the normal range represent non-inflammatory disorders. The increased proteolysin activities may be roughly correlated with evidence of tumor necrosis or with inflammatory processes. The trypsin inhibitor values are elevated in 76 per cent of the 39 cancer cases and 90 per cent of the 45 cases of miscellaneous diseases. The 4 subnormal values of the cancer group are seen in 2 cases of multiple myeloma, an early case of carcinoma of the breast and an early untreated case of Hodgkin's disease. A third patient with multiple myeloma has a normal inhibitor value. All elevated inhibitor values are observed in patients showing evidence of tissue destruction or inflammation.

All elevated proteolysin activities are plotted against their respective inhibitor values in Figure 2. In all cases in which both the proteolysin and inhibitor values are elevated, well established evidence of tissue breakdown is present. The normal or subnormal inhibition values which are observed in patients with elevated proteolysin activities are difficult to interpret.

The proteolysin and trypsin inhibitor values were determined at various intervals after operation or after instituting therapy in 30 selected patients. The cases are divided into the following 4 groups: (a) partial surgical removal of cancerous tissue; (b) complete surgical removal of the tumor; (c) pneumonia under penicillin therapy; and (d) various chronic diseases. Typical changes observed in each group are shown in Figures 3 and 4.

Partial removal of tumor tissue does not affect the proteolysin activity during the first 3 weeks following surgery. The trypsin inhibitor values increase in these cases (Fig. 3).

The proteolysin activity of the plasma dropped to zero within one week after removal of the urinary bladder in 2 cases of carcinoma of the bladder and after excision of an epidermoid carcinoma of the buccal mucosa in a third patient (Fig. 3). A similar pattern was observed after pneumonectomy for bronchiogenic carcinoma. The values for the 2 cases with initially elevated inhibitor values are not affected, while an initially low value is increased after operation.

The changes in proteolysin and inhibitor in 3 patients with pneumococcal pneumonia treated
with penicillin are shown in Figure 4. It is seen that the proteolysin disappears during the afebrile stage while the inhibitor concentration increases during this period. One patient recovering from pneumonia developed a chill and temperature of 108° F. on the ninth afebrile day. A high proteolysin activity of the plasma appeared within 10 hours following the chill and disappeared 24 hours later when the temperature was normal; the trypsin inhibitor concentration was not affected.

The data for 3 patients with chronic diseases are shown in Figure 4. (a) The edema disappeared and a slight trace of proteinuria was present 2 weeks after tonsillectomy in Miss D who was admitted with glomerulonephritis; at the time of improvement, the proteolysin disappeared, but the inhibitor concentration remained high. (b) A young woman (C) with rheumatic fever (joints) showed no clinical improvement during salicylate administration; both the proteolysin and the inhibitor concentrations remained elevated. (c) A 35 year old man (R) with early miliary tuberculosis was treated with streptomycin and gradually improved during the 4 week period of observation; the proteolysin activity gradually decreased and was no longer present at the end of 3 weeks, but the inhibitor concentration remained elevated.

**DISCUSSION**

Chloroform activated plasmin is not a reliable measure for the plasma protease content (1, 6). Attempts to correlate the plasmin concentrations with the plasma protease (proteolysin) or with the trypsin inhibitor are unsuccessful.

The proteolysin and trypsin inhibitor values are elevated in practically all patients with malignancy and in the febrile stage of disease. During the course of frequent studies in individual patients, it becomes apparent that the proteolysin is a comparatively sensitive indicator of the effectiveness of treatment or the progress of disease. The protease content of the plasma disappears after successful therapy and remains elevated if the patient does not respond to surgery or drugs. The determination of this enzyme may be a useful guide for determining the progress of a patient's recovery.

The rapid increase in proteolysin concentration probably reflects the presence of a tissue product which is a stimulant to enzyme formation, or perhaps represents an in vivo activation of plasminogen. A fairly good correlation between the proteolysin and elevated temperature may be obtained. The elevated values observed in cancer cases are probably associated with tumour necrosis. The removal of the abnormal stimulus by surgery or therapy is accompanied by a rapid disappearance of the proteolysin.

The trypsin inhibitor concentration increases following acute infections and surgery, but the rise is not as rapid as that seen for proteolysin. The inhibitor returns to the normal range slowly. In 2 patients, one recovering from bronchopneumonia and another recovering from a subphrenic abscess following appendectomy, the inhibitor values returned to the control range 3 weeks after their temperatures were normal. It appears that this inhibitor is associated with the destruction and resolution of tissue.

**SUMMARY**

The activities of chloroform activated plasmin, spontaneously active protease (proteolysin) and
trypsin inhibitor of the plasma of healthy young men and of patients with cancer and with a variety of acute and chronic diseases were determined.

Observations made on a group of patients shortly after admission to the hospital showed elevated values for the proteolysin and trypsin inhibitor in most cases. No relationship between plasmin, proteolysin, and inhibitor concentrations could be established.

Periodic analyses of the proteolysin and trypsin inhibitor were made on patients (a) after incomplete removal of cancer, (b) after complete removal of cancer, (c) during recovery from pneumonia, and (d) with a variety of chronic diseases. The proteolysin concentration dropped to zero soon after complete surgical removal of a cancer, and in other cases after successful therapy or surgery. The trypsin inhibitor remained elevated for an appreciable period.

The appearance of proteolysin in plasma is associated with necrosis and with inflammatory processes accompanied by elevated temperatures. The trypsin inhibitor concentration remains elevated in patients who have been operated on and in those whose tissues are undergoing readjustment to the normal state.

The significance of the changes noted in the plasmas of diseased individuals is discussed.

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