Fibrinolytic Activity of Normal Urine

It has been established that human plasma contains an active fibrinolytic and proteolytic enzyme (plasmin), its inactive precursor (plasminogen), and also an inhibitor (antiplasmin) that normally overneutralizes the enzyme[1,2]. Plasmin and plasminogen are associated with the globulin fraction, antiplasmin with the albumin. Fibrinolytic activity can be induced in plasma by the addition of chloroform, which destroys antiplasmin, or of streptokinase, which activates plasminogen. Activity also occurs spontaneously in human subjects who have suffered surgical operation[3], trauma, fear[4], strenuous exercise, or the injection of adrenalin[5].

The last of these observations revealed the fact that the activity induced by these means disappeared from the circulating blood within a few minutes of the cessation of the stimulus, suggesting that plasmin is rapidly inactivated or eliminated. Among other possibilities its excretion by the kidney was considered, and the consequent examination of the urine by methods already described for plasma fractions[2] has, in fact, demonstrated considerable fibrinolytic activity in all samples examined. This activity existed even when no stimulus had been applied, and though it varied in intensity in the same subject from time to time, and from one subject to another, it was, on the average, four or five times greater than that of active plasmin, complete lysis of fibrin being produced within 24 hours by urine diluted 1/2,000–1/4,000. Though this titre increased considerably after exercise, the amount of urine passed was so much less as compared with control periods of rest that the total active principle excreted was not significantly greater than normal, and it seems improbable that the post-exercise fall in blood-plasmin can be explained by its simple elimination by the kidney.

Nevertheless, in some respects the fibrinolytic agent of urine resembles plasmin. It is not diffusable, being retained by 'Cellophane'. It is precipitated by 50 per cent saturation with ammonium sulphate, full saturation with sodium sulphate, or by bringing the *p*H of the urine to 5·5 after removing all electrolyte by dialysis. It is also precipitated by 50 per cent alcohol or acetone. It will digest fibrinogen, fibrin, albumin and casein, as judged by estimation of acid soluble tyrosine. The *p*H of optimum activity is 7·4, and of maximum heat stability 6·8, these being very close to the corresponding figures for plasmin.

There are, however, points of difference. The urine factor is less heat-labile than plasmin, 5 per cent activity remaining after 30 min. at 80° C., but it is totally destroyed by boiling. The urine factor is inhibited by antiplasmin and soya-bean trypsin inhibitor, and this inhibition is relatively more effective per unit of activity than is the case with plasmin. Plasmin is very strongly adsorbed by fibrin; the urine factor less so.

It is not certain at present, therefore, what relationship the proteolytic enzyme of the plasma bears to the rather similar factor present in the urine. Neither the points of similarity nor of difference are conclusive evidence for or against the two being identical. However, the existence of such an activity of urine is itself of some interest.

Previous observations on the proteolytic activity of urine are few. Loeper and Baumann[6] studied the excretion of 'pepsin' in the urine of normal and pathological subjects, and there is a recent paper on the urinary content of a similar enzyme, with a review of other references, by Farnsworth, Speer and Alt[7]. In view of the clear-cut differences between the properties of pepsin and those of the agent described here, this previous work probably has little bearing on the present observations.

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Use of Trypsin in the Detection of Incomplete Anti-*Rh* Antibodies

In an attempt to define the properties of the enzyme present in the filtrate of a culture of vibrio cholera, which causes cells sensitized with an 'incomplete' anti-*Rh* antibody to agglutinate[1], cultures of other organisms and pure enzyme preparations have been tested. During this investigation trypsin has been found to cause agglutination of cells sensitized with an 'incomplete' antibody, and also to enhance the specific agglutination of other hæmagglutinins in the absence of any detectable antibody of the 'incomplete' type.

If trypsin is added to the serum or to the cell antigen antibody mixture, its action may be inhibited by the natural trypsin inhibitor present in all sera[2]. This can be overcome by the addition of an excess of the enzyme or removal of the serum inhibitor, or by washing cells already sensitized before exposing them to the trypsin; if this latter method is used, a single washing is sufficient to remove the inhibitor. Test cells may also be incubated with trypsin and, on incubation with sera, show enhancement of agglutination with *iso*agglutinins and immune agglutinins, and agglutination with sera of the 'incomplete' type; and after such treatment are not susceptible to the normal serum inhibitor or to soya bean trypsin inhibitor. With fresh undiluted sera, however, there is also enhancement of rouleau formation, which can be abolished by the addition of saline.

In this investigation the trypsin preparations used have been crystalline trypsin (Plaut Research Laboratory, New Jersey) and *liquor trypsini co.* (Allen and Hanbury). The stock solution of crystalline trypsin has been a 1 per cent solution in *N*/20 hydrochloric acid, and has remained stable for three months. Before use the stock solution is diluted with phosphate buffer (*p*H 7·2) to a concentration of 1 : 400 to 1 : 10,000; and the *liquor trypsini co.* is diluted with buffer to give a final concentration of 1 : 5 of the original solution. With a 1 : 400 solution of crystalline trypsin, complete agglutination of fully sensitized cells occurs within 20 min. With increasing dilution of the enzyme the time of agglutination is lengthened.

As with the enzyme present in the filtrate of vibrio cholera, trypsin appears to act on the surface of the red cell but does not affect any of the known hæmagglutinogen loci. Neither removal of the normal