THE IDENTIFICATION AND QUANTITATION OF THROMBOLYTIC AGENTS[1,2]

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The three fundamental approaches to induce thrombolysis *in vivo* are through the intravenous injection of 1) an activator of the normal fibrinolytic system (streptokinase, urokinase, streptokinase-globulin, etc.), 2) fibrinolysin* (plasmin), or 3) a combination of the two agents. Which of these preparations will prove to be most effective remains to be determined.

We have examined preparations representing four different manufacturers and while each was labeled "fibrinolysin," we were able to distinguish markedly different types of activity. It is particularly important to be able to distinguish and quantitate the different types of activity since the effective dosage levels and safety limits may vary widely with the different types of thrombolytic agents. This report describes a method for identification of each active component in each preparation and a method of quantitating the type of activity exhibited.

All the preparations are capable of lysing plasma clots *in vitro* (formed by recalcification of plasma). Indeed, they also are capable of lysing human blood clots or clots resulting from the addition of thrombin to solutions of human fibrinogen. On the basis of such limited observations one could erron-

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* Fibrinolysin is defined as a proteolytic enzyme formed from an inactive plasma precursor and is capable of hydrolyzing a variety of proteins, among them fibrin and casein, at neutral pH.

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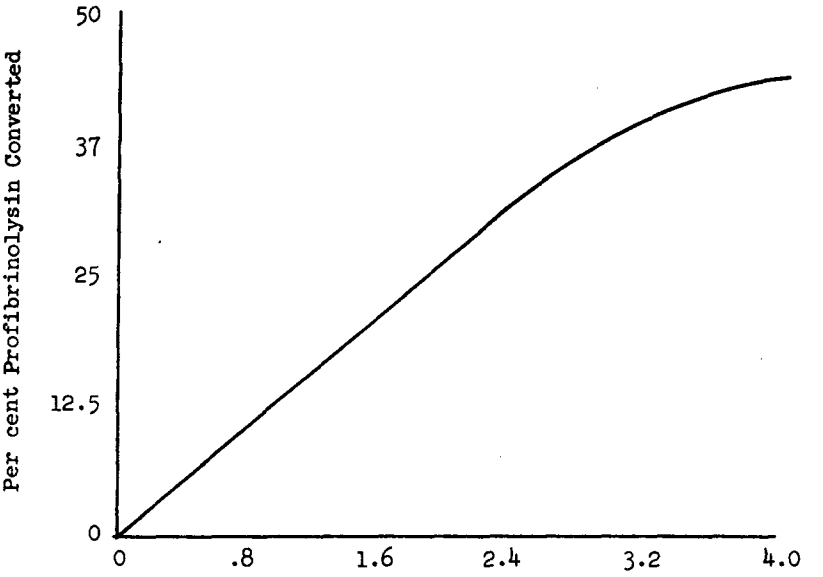
eously conclude that the preparations were similar and were fibrinolysin. However, when these preparations were qualitatively screened by the method of Sgouris, Inman, McCall and Anderson (1961) it was observed that all the preparations exhibited some proteolytic activity against heated human fibrin plates (containing no intrinsic profibrinolysin). Two of the preparations (Sources III and IV) showed much greater activity toward the unheated human fibrin plates as compared to the heated plates. This indicated the presence of activator activity in addition to the direct fibrinolytic activity in these latter preparations (i.e., additional fibrinolysin was formed by converting the profibrinolysin present in the unheated human fibrin plate). The preparations tested thus contained one or both of the following types of activity: 1) direct proteolytic activity (fibrinolysin), and 2) activator activity, i.e., capable of converting human profibrinolysin to fibrinolysin. None of the preparations contained detectable amounts of profibrinolysin (plasminogen).

Direct proteolytic activity (fibrinolysin) was quantitatively determined using a casein substrate as described by Sgouris, Inman, McCall, Hyndman and Anderson (1960). Activator activity was measured essentially in the same way, with the exception that the preparation was preincubated with a human profibrinolysin solution for an activation period of ten minutes. The increase in proteolytic activity was related to fibrinolysin formed by the action of activator on profibrinolysin.

The conversion of profibrinolysin by "activator" is shown in the figure below. When the substrate (profibrinolysin) was in excess, there was a linear relationship between activator concentration and the amount of fibrinolysin formed. The assays were conducted so that this linear portion of the curve was utilized, and preferably in the area representing conversion of 20 per cent or less of the available profibrinolysin. The amount of profibrinolysin substrate chosen was eight caseinolytic units per milliliter, in order to maintain the linear relationship of tyrosine released by the formed fibrinolysin on casein.

Conversion of Profibrinolysin by Activator



This activating medium consisted of 0.5 ml (various dilutions) of the preparation being tested; 0.5 ml of human profibrinolysin (eight caseinolytic units/ml); 1.0 ml of a pH 7.4, 0.1 M sodium phosphate - 0.9 per cent sodium chloride buffer; and 0.3 ml casein (six per cent w/v). One activator unit produces one caseinolytic unit of fibrinolysin upon incubation for ten minutes in the presence of excess profibrinolysin. One caseinolytic unit is defined as the amount of fibrinolysin which liberates 450 μg acid soluble tyrosine from four per cent casein in one hour of incubation, at 37°C.

The total activity in the activator assay necessarily reflects both activator activity and direct proteolytic activity, if the latter is present. Activator activity is calculated as the difference between total and direct proteolytic activity. In these preparations where there was predominantly activator activity, the dilution necessary to test this material properly resulted in no detectable direct activity when measured at this level by the direct method; therefore, no correction was necessary.

The results of our assays of 15 different preparations were as follows:

Source	Preparation	Unitage/bottle Activator Activity	Unitage/bottle Direct Activity
I	A	0	1,165
	B	0	1,620
	C	0	1,690
	D	0	1,430
II	E	0	441
III	F	12,580	33.9
	G	8,160	28.2
	H	13,985	31.6
	I	12,000	34.8
	J	11,900	46.7
	Ave.	11,725	35.0
IV	K	1,915	2.1
	L	928	1.8
	M	606	2.7
	N	2,151	1.9
	O	1,138	1.0
	Ave.	1,348	1.9

It is apparent from the data that although all the preparations were labeled "fibrinolysin," some preparations possessed direct proteolytic activity (fibrinolysin) alone, while the others possessed primarily activator activity with only a trace or a small quantity of direct proteolytic activity.

It is also interesting to note that preparations from Sources III and IV, from two different manufacturers, were all labeled as containing approximately 50,000 fibrinolytic units. However, on the average, Source III contained 8.7 times as much activator as Source IV. These differences in potencies shown by the caseinolytic assay indicated that either different assay systems or different definitions of a fibrinolytic unit were used by the producers involved.

In summary, we have extended the standard caseinolytic assay for fibrinolysin to also measure activator concentration. We have used the caseinolytic assay to identify and quantitate preparations labeled "fibrinolysin" from four different manufacturers. Two were correctly labeled since they contained

fibrinolysin alone. The other two were predominantly activator with very small amounts of direct proteolytic activity.

REFERENCES

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