NEW METHOD OF STUDYING THE RELEASE OF FIBRINOLYSIS ACTIVATORS IN TISSUE CULTURES*

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Tissue culture in the presence of a standard fibrin clot containing plasminogen can be used to detect and estimate quantitatively the release of fibrinolysis activators into the media (by measuring the quantity of fibrin–fibrinogen degradation products). By the method described it is possible to study regulation of the release of fibrinolysis activators by tissues in vitro.

KEY WORDS: fibrinolysis activators (release); tissue cultures.

The endothelium of certain vessels, especially veins, contains an enzyme which activates the conversion of plasminogen into plasmin [4]. This enzyme is continuously being released into the blood and is responsible for its spontaneous fibrinolytic activity [8]. Various factors changing the diameter of the vessels, such as injection of adrenalin or venous stasis, intensify the release of fibrinolysis activators and temporarily increase the fibrinolytic activity of the blood. Factors modifying the tone of the vessel walls are of great importance for the prevention of thrombosis [5], in thrombolysis, and in the recanalization of the vessels [6, 10].

Tissue cultures can be used to study the mechanisms of release of fibrinolysis activators. If fragments of human superficial veins are cultured in vitro the concentration of fibrinolysis activators in them, determined by a histochemical method, remained unchanged for 3 weeks [9]. However, the release of these activators into the culture medium by tissues other than those of the kidney has proved to be very difficult to determine. This is evidently because of continuous denaturation of activators released into the solution at 37° C.

A new method whereby release of enzymes activating fibrinolysis could be determined in a solution used for tissue culture has recently been developed [2]. Fragments of various organs are cultured on sterile absorbent gelatin sponge with hemostatic properties (Spongostan R), placed close to a standard fibrin clot containing plasminogen, but not touching it. Fibrinolysis activators released during growth of the tissue culture into the nutrient medium reach the clot and convert the plasminogen in it into plasmin. This is accompanied by gradual lysis of the clot. The quantity of degradation products of fibrinogen and fibrin (FDP) in the medium rises and the degree of this rise can be used to judge the quantity of activators released. A scheme illustrating the principle of the method is shown in Fig. 1.

Ordinary Leighton's tubes with two pieces of Spongostan in each tube and with three tissue fragments to each piece of Spongostan are used. It is desirable that the nutrient medium be a pure synthetic solution uncontaminated with serum, which could contain fibrinolysis inhibitors. As an example, Parker 199 solution (SBL) may be used as the synthetic nutrient medium, 1 ml being added to each tube. Tissues from human fetuses (abortion material) is convenient for the purpose, for they keep longer when cultured in synthetic nutrient medium than tissues from the adult.

The fibrin clot is obtained by mixing 1 ml of a 1% solution of human fibrinogen (Kabi) in distilled water with 0.04 ml thrombin solution (Topostasin) in the proportion of 75 units to 1 ml 0.15 M NaCl. Small quantities of nutrient medium (0.03 ml) are withdrawn every 24 h, and the content of FDP in them is determined by immunochemical analysis [7]. The sensitivity of the method is 0.5 mg per 100 ml. During culture of very

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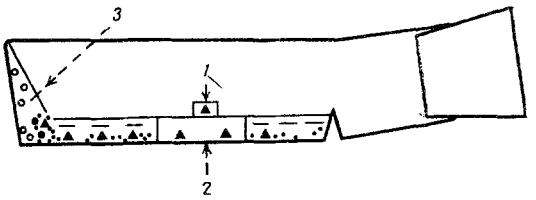

Fig. 1. Principle of the method used to determine release of fibrinolysis activators in tissue culture. 1) Tissue fragment; 2) gelatin film; 3) fibrin clot. Empty circles – plasminogen, filled circles – plasmin, triangles – plasminogen activator, dots – fibrin degradation products.

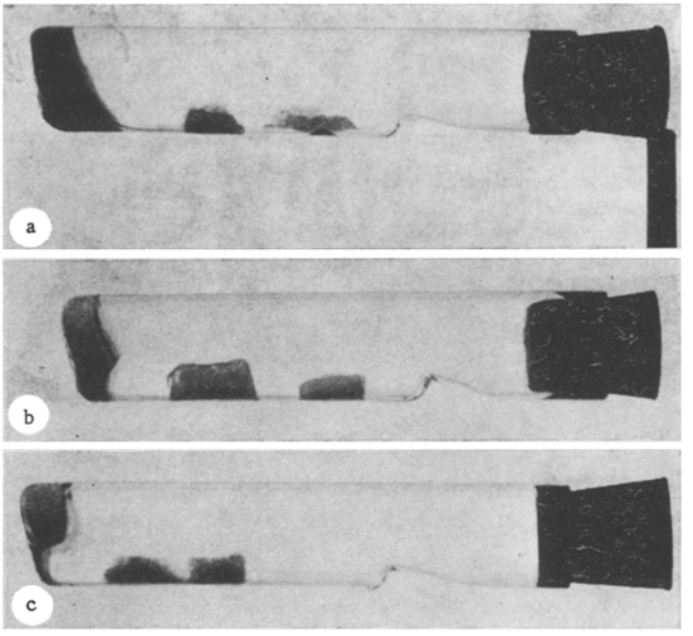

Fig. 2. Different degrees of fibrinolysis in tissue culture: a) macroscopically unaffected standard fibrin clot (low or zero activity); b) macroscopically visible lysis (moderate activity); c) massive fibrinolytic action (high activity). Nutrient medium removed.

active tissues, such as kidney, measurable quantities of FDP accumulated in the medium as early as 12 h after the beginning of the experiment. By the immunochemical method fibrinolysis can be discovered long before complete lysis of the clot.

Lysis of the fibrin clot takes place gradually; where it is in contact with the nutrient medium a defect forms (Fig. 2). After 4 days in tissue culture lysis of most of the clot can be observed. Control tests with the same ingredients but without fragments of tissue are set up at the same time.

All tissues tested except liver and placenta were found to be capable of releasing fibrinolytic activators [1]. Examples of the fibrinolytic activity of various tissues are given in Table 1. The highest activity was found in the experiments with kidney tissue. In these experiments the clot was completely lysed after 2 days. Denaturation of the plasminogen in the clots by heat completely prevented its lysis. Addition of tranexamic acid, an inhibitor of fibrinolysis, delayed lysis of the fibrin clot in experiments with all tissues. These results indicate that the substance released by the tissue fragments is in fact a plasminogen activator, for tranexamic acid, in the concentration used, inhibits mainly the activation of plasminogen.

TABLE 1. Release of Fibrinolytic Activators during Tissue Culture in vitro

Length of fetus, cm	Tissue	Days of culture					
		1/2	1	1 1/2	2	3	4
21	Vena cava		30 (20—38)		48 (30—96)	134 (110—190)	340 (280—480)
	Control		0—0		0—0	0 — Traces	Traces— 3,4
22	Aorta	—	10 (9—13)	—	31 (16—40)	108 (80—135)	213 (175—225)
	Control		0—0		0—0	0—0	Traces— 7,5
20	Bone marrow	—	80 (56—140)	—	168 (140—240)	275 (225—400)	443 (340—520)
	Control		0—0		0—0	0—0	0—3
23	Lung	—	12 (11—14)	—	41 (37—45)	188 (140—250)	299 (275—320)
	Control		0—0		0—0	0—0	Traces —
22	Pia mater	—	44 (22—63)	—	117 (95—145)	225 (150—300)	675 (600—750)
	Control		0—0		0—0	0—0	0—0
22	Dura mater	—	119 (110—130)	—	435 (420—450)	610 (580—640)	813 (800—830)
	Control		0—0		0—0	0—0	0—0
19	Renal cortex	6 (2—10)	119 (88—190)	205 (150—300)	448 (390—600)	—	—
	Control	0—0	0—0	0—0	Traces— 1,5		
19	Renal medulla	23 (7—40)	187 (175—195)	214 (190—265)	550 (400—750)	—	—
	Control	0—0	0—0	0—0	Traces— 1,5		

Legend to Tables 1 and 2: 1) Activity of fibrinolysis expressed in mg FDP/100 ml nutrient medium. 2) Mean values and limits of their variations in four experiments for each tissue.

TABLE 2. Inhibitory Effect of Placenta on Action of Urokinase and Fibrinolytic Activators Released by Tissue Cultures

	Time of culture, days		
	1	2	3
Urokinase 3,0 units/ml	163 (75—225)	419 (400—425)	482 (425—550)
Urokinase + placenta	17 (6—22)	86 (75—90)	110 (90—125)
Kidney	58 (30—86)	361 (225—520)	570 (480—700)
Kidney + placenta	2,5 (0—5)	31 (8—51)	75 (70—100)
Placenta	0	0	0,5
Control	0	0	2,5
Aorta	2 (0,5—4,5)	8,5 (6—10,5)	54 (32—74)
Aorta + placenta	0	0	0,8
Placenta	0	0	0,5
Control	0	0	1,5

The method described can also be used to demonstrate the release of fibrinolytic inhibitors by the placenta (Table 2). These inhibitors had an inhibitory action on plasminogen activation by urokinase and by the factors released by kidney and blood vessel tissues [3].

The method described above can be used to study the effect of hormones and drugs on the release of fibrinolytic activators by the tissues.

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