Adhesion of Fibroblasts to Polymerizing Fibrin and Retraction of Fibrin Induced by Fibroblasts[1] (36425)

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The close association between fibrin strands and platelets in hemostatic plugs and thrombi is well known (1), and recent studies suggest that platelet adhesion to fibrin occurs while fibrinogen is being converted to fibrin (2, 3). The involvement of fibrin in a variety of biological processes, such as inflammation (4), tissue repair (5), and metastases of tumors (6), indicates that fibrin probably interacts with cells other than platelets. Experiments reported below confirm this with fibroblasts by demonstrating that these cells adhere to polymerizing fibrin and eventually cause retraction of fibrin.

[1] Supported in part by an Ontario Heart Foundation grant and in part by MRC Grant Nos. MA-4074 and MT-1309.

Materials and Methods. Fibroblasts, a continuous passage line of undifferentiated Earle's L cells, were kindly supplied by Dr. L. Prevec. The cells were kept in a constant exponential growth phase by daily twofold dilution with Joklik modified minimal essential medium (7). For studies with fibrin they were isolated from the culture medium using methods similar to that described for the isolation of platelets from platelet-rich plasma (8). Resuspension was in Tyrode's albumin solution and the cell count was adjusted to 1.05×10^7/ml.

Polymerizing reptilase-fibrin was prepared by incubating 9 vol of human fibrinogen (1% protein; Kabi, Stockholm) with 1 vol of 0.3% reptilase (Repilase a gift of Pentapharm, Basle; series 446) or with 1 vol of thrombin

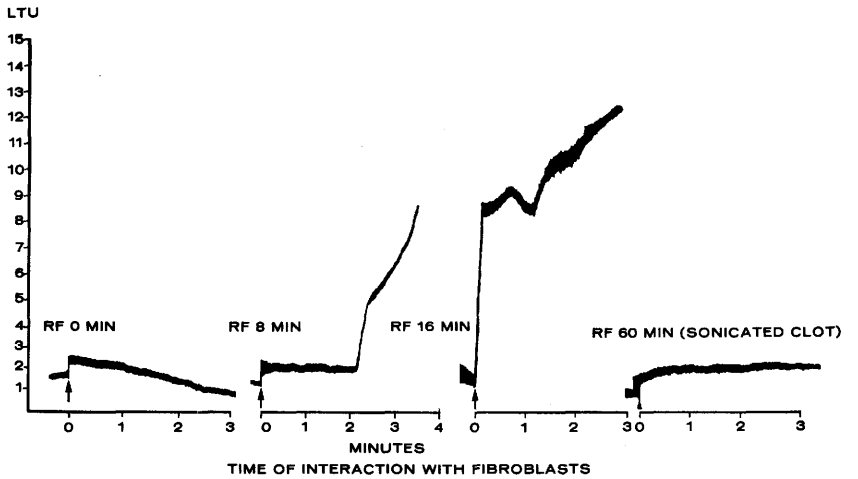


FIG. 1. Light transmission through fibroblast suspensions (10^7 cells/ml) during exposure to fibrinogen (RF 0 min), polymerizing reptilase-fibrin (RF 8 min and RF 16 min) or fully polymerized sonicated fibrin (RF 60 min). 0.4 ml aliquots of RF were preincubated for various intervals at 37° and added to 1 ml suspension as indicated by arrows. The rapid initial rise in the transparency of sample (C) is believed to be due to an immediate partial aggregation of fibroblasts by the material resulting from the prolonged incubation of fibrinogen with reptilase.

TABLE I. Changes in Light Transmission (ΔLTU) and Percentages of Total Radioactivity Becoming Associated with Fibroblasts on Incubation with Polymerizing ^{125}I-Labeled Reptilase-Fibrin (RF).

Test system	No. of expts.	ΔLTU		^{125}I-radioactivity (% of total)[a]	
		Mean	SD	Mean	SD
Fibroblasts + RF 4–6 min[b]	5	1.7	1.5	5.3	4.7
Fibroblasts + RF 10–16 min[b]	7	19.2	12.9	15.3	5.5
Fibroblasts + 0.01 *M* EDTA + RF 14 min[b]	5	0.2	0.3	2.0	2.8
Fibroblasts + 3×10^{-4} *M* PGE_1 + RF 14 min[b]	3	22.6	14.5	12.6	9.8

[a] The percentages of ^{125}I-radioactivity found in the suspending fluid sediment (containing no cells) were subtracted from the percentages of ^{125}I-radioactivity found in cell sediment. When fibrinogen alone was incubated with fibroblasts, less than 1% of the radioactivity was associated with cell sediment.

[b] Preincubation period for fibrinogen with reptilase.

(0.5 units/ml, Parke-Davis) at 37° for intervals ranging from 4 to 16 min. Interaction between fibroblasts and polymerizing fibrin was investigated by four methods: (a) by studying the changes in light transmission of a fibroblast suspension in the presence of polymerizing fibrin using the technique of Mustard *et al.* (9) (with this method the adherence of cells to each other leads to an increase in light transmission); (b) by measuring the amount of fibrin that became attached to fibroblasts using ^{125}I-fibrinogen as a precursor of fibrin (3); (c) by electron microscopy, including shadow casting (10) and thin sectioning (11) techniques; and (d) by measuring the retraction of fibrin in a system containing 0.8 ml fibroblast suspension, 0.1 ml 0.9 NaCl or an inhibitor of fibrin retraction, 0.1 ml human fibrinogen (0.25%) and 0.1 ml thrombin (50 units/ml) or 0.1 ml 3% reptilase. In some experiments 0.2 ml of reptilase–thrombin mixture was added to the system.

Results and Discussion. The light transmission of fibroblast suspensions remained unchanged during incubation with fibrinogen or fully polymerized fibrin (sonicated). In contrast, partially polymerized fibrin significantly increased the light transmission of the suspensions. Fibrinogen that had been incubated with reptilase for longer times was more effective than fibrinogen that had been incubated for shorter times (Fig. 1 and Table I). Polymerizing fibrin prepared by incubating fibrinogen with small quantities of thrombin gave similar results. Fibroblasts homogenized by sonication did not aggregate intact fibroblasts, and neither intact nor sonicated fibroblasts clotted fibrinogen over a 4-hr observation period.

The percentages of the labeled protein in the mixture adhering to fibroblasts together with the concomitant changes in light transmission are summarized in Table I. As shown, the magnitude of the change in light transmission corresponded to the amount of ^{125}I-labeled protein that became adherent to the cells. EDTA added to the fibroblast sus-

TABLE II. Retraction of Fibrin Formed in the Presence of Fibroblasts or Rabbit Platelets.

Cell suspension	Incubation time (37°) (min)	Fibrin clot vol (%): Coagulating enzymes		
		Reptilase	Thrombin	Thrombin and reptilase
Fibroblasts 1.05×10^7/ml	60	97	56	61
		(91–100)	(49–61)	(64–44)
	120	91	37	36
		(83–100)	(27–43)	(32–52)
Rabbit platelets	60	100	63	—
10^7/ml		(100–100)	(52–73)	—
	120	100	42	—
		(100–100)	(30–61)	—
	60	100	13	9.0
10^8/ml		(100–100)	(4–19)	(5–12)
	120	100	5.5	2.0
		(100–100)	(0–11)	(0– 3)

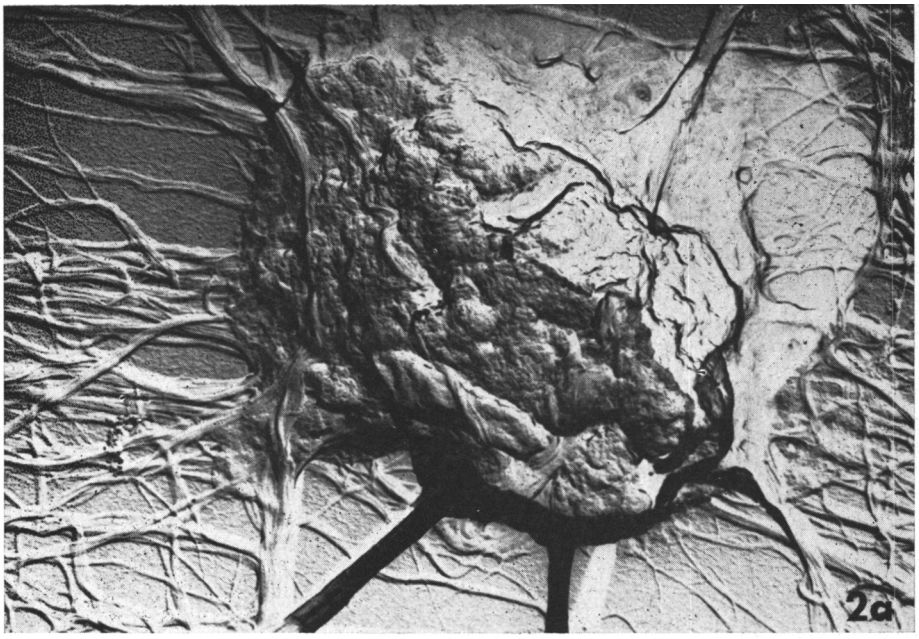
[a] Prepared according to the modified method of Ardlie, Packham, and Mustard (8) and resuspended in Tyrode's albumin solution. Mean values of three experiments and ranges in parentheses. Clot volumes were calculated from the difference between sample volume and the volume of the liquor surrounding the fibrin.

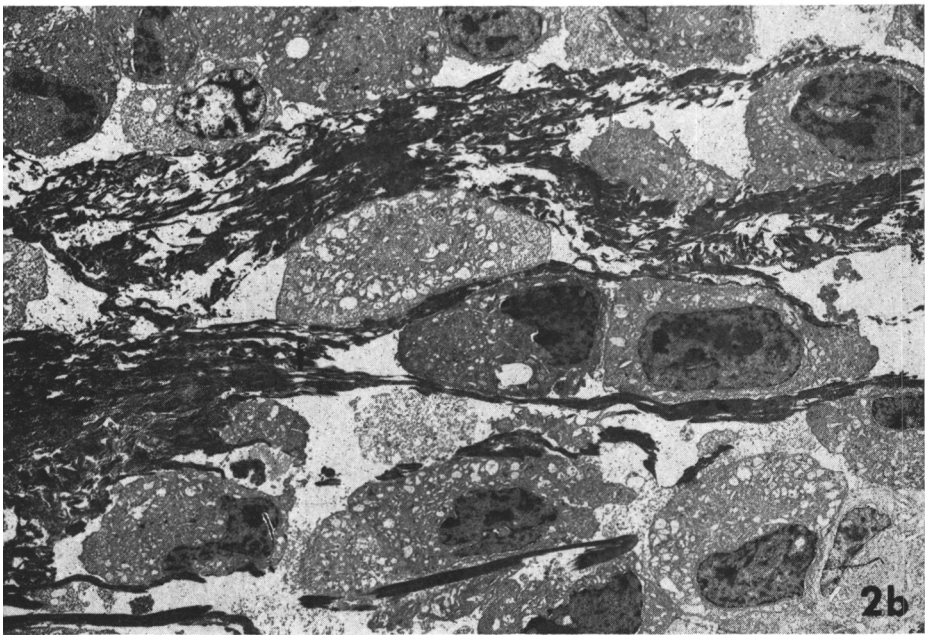
pension inhibited these changes. Prostaglandin E_1 (PGE_1, a gift of Upjohn, Kalamazoo) had no effect on the interaction of polymerizing fibrin with fibroblasts.

Electron micrographs of fibroblasts exposed to polymerizing fibrin are presented in Fig. 2. Fibroblast is shown to be entrapped in the fibrin net which covers its surface (Fig. 2a). Figure 2 b and c seem to indicate that fibrin may link cells in the aggregate.

An unexpected finding of the present studies was that fibroblasts caused retraction of fibrin formed by the action of thrombin in a manner which was in many ways similar to clot retraction produced by platelets (Fig. 3). Retraction by fibroblasts was inhibited by agents known to inhibit clot retraction by platelets, such as PGE_1 (12), EGTA (13), monoiodoacetic acid or oligomycin (14). The extent of retraction produced by 10^7 fibroblasts corresponded to that caused by approximately 5×10^7 washed pig platelets. Sonicated fibroblasts did not cause fibrin to retract. When reptilase was used as a clotting agent instead of thrombin, no retraction occurred with platelets and only very little with fibroblasts (Table II). In a system containing both reptilase and thrombin, the extent of retraction was similar to that in a system containing thrombin alone. The failure of reptilase-produced fibrin to retract with platelets has also been observed by others (15). It is noteworthy to mention that intact and sonicated fibroblasts did not cause lysis of fibrin clots, over the 24 hr observation period.

These observations suggest that fibroblasts and platelets interact with fibrin by similar mechanisms. In both instances, fibrinogen has to be in an "active" form for the interaction to take place, that is, the protein must have been acted upon by the coagulant enzyme but its reactive sites must not be consumed by polymerization. Interaction of platelets with polymerizing fibrin does not depend on platelet aggregation, and it is inhibited by calcium chelating agents but not by PGE_1 (3). The same is also true for fibroblasts (Table I). On the other hand, red cells at the concentration of 10^8 or 10^9 per ml do not adhere to polymerizing fibrin and do not cause fibrin retraction. When the fibroblasts were substituted with red cell suspension in the incubation mixture described in Fig. 1 and in Table I, there was virtually no change in light transmission and no radioactivity became associated with cell sediment (16).





Fibroblasts contain a contractile protein that is responsible for their motility and contraction by ATP (17). It is possible that this contractile protein, similarly as thrombosthenin in platelets (13), is involved in the fibrin retraction induced by the cells. The near or complete absence of fibrin retraction with either type of cell when fibrin was produced by the action of reptilase may be regarded as additional evidence for the similarity between the action of platelets and fibroblasts. Despite the adherence between reptilase-fibrin and platelets (3) or fibroblasts, this fibrin does not retract. There are two

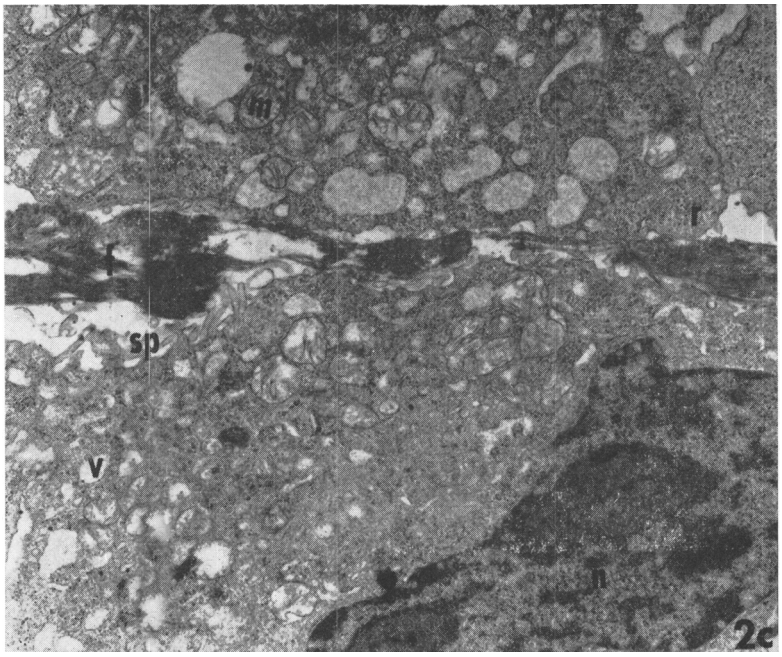


FIG. 2. Electron-microscopic appearance of fibroblasts. (a) Shadow casting, showing close association of fibrin strands with the cell. Fibrinogen solution was preincubated with reptilase for 14 min (×9,135). Exposure to polymerizing fibrin for 30 sec. (b) Association of fibrin with fibroblasts as seen by thin sectioning. Preincubation of fibrinogen with reptilase for 14 min. Exposure of cells to polymerizing fibrin for 3 min. This photograph shows interspersed fibrin (f) among fibroblasts in the aggregate (×2,415). (c) Two cells linked by fibrin: (n) nucleus, (m) mitochondrion, (v) vesicle, (r) ribosome, (sp) surface projection, (f) fibrin (×9,520). The experimental system as in (b).

possible explanations for the difference in the retraction of fibrin formed by reptilase and thrombin, respectively: (a) the fibrin may differ depending on the coagulant enzyme; it is established that thrombin splits off fibrinopeptides A and B from fibrinogen, while reptilase splits off fibrinopeptide A only (18). (b) Thrombin may stimulate platelets or fibroblasts to contract. In contrast to thrombin, reptilase does not cause platelet aggregation and the platelet release reaction (3). It is possible that neither of these cells are sensitive to reptilase.

Summary. Fibroblasts readily adhere to polymerizing fibrin, but not to fibrinogen or fully polymerized fibrin. Conditions for optimal interaction are similar to those known for the platelet–fibrin interaction. Fibroblasts retract fibrin formed by thrombin but not by reptilase. Fibrin retraction induced by fibroblasts is inhibited by agents inhibiting clot retraction induced by platelets.

We thank Dr. L. Prevec for generous supplies of fibroblasts and Messrs. A. F. Senyi, K. L. Wong and T. Bistricki for skilled technical assistance.

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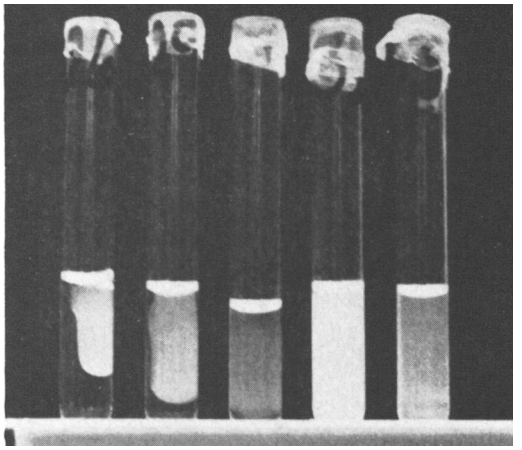

FIG. 3. Fibrin retraction induced by fibroblasts, the tubes (from the left to the right) containing the following additives: (a) 0.9% NaCl, (b) 2.5×10^{-4} *M* PGE_1, (c) 8×10^{-4} *M* monoiodoacetic acid, (d) 4×10^{-4} *M* EGTA, and (e) 8×10^{-4} *M* oligomycin. The clot volumes which were measured as explained in Table II, were after 3 hr incubation at 37° (reading from left to right): (a) 18.4%, (b) 43.5%, (c) 62.9%, (d) 85.7% and (e) 100%. The relatively high opacity of clot (d) is due to the concentration of EGTA in this sample.

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Received Nov. 3, 1971. P.S.E.B.M., 1972, Vol. 140.