Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 5597–5601, September 1982
Developmental Biology

Differentiation-dependent stimulation of neovascularization and endothelial cell chemotaxis by 3T3 adipocytes

(angiogenesis/development/fat cells/heparin)

JOHN J. CASTELLOT, JR.*, MORRIS J. KARNOVSKY*, AND BRUCE M. SPIEGELMAN†

*Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115; and †Sidney Farber Cancer Institute and Department of Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

Communicated by Arthur B. Pardee, June 21, 1982

ABSTRACT **3T3 cells that have undergone adipose differentiation *in vitro* secrete factor(s) that stimulate angiogenesis (neovascularization) *in vivo*. When medium containing 0.5% fetal calf serum was conditioned by 3T3-F442A adipocytes, it stimulated angiogenesis when placed on the chicken chorioallantoic membrane. Control medium or medium conditioned by preadipocytes did not stimulate angiogenesis, even at much higher doses. Thus, the production of the angiogenic activity is strongly dependent upon differentiation of the adipocytes. The degree of the angiogenic response to adipocyte-conditioned medium was potentiated by heparin; heparin added to unconditioned medium or to preadipocyte-conditioned medium was not angiogenic. The adipocyte-conditioned medium also strongly stimulated, in a differentiation-dependent fashion, the motility of aortic and capillary endothelial cells in a modified Boyden chamber assay. Checkerboard analysis indicated that 75% of the motility-stimulating activity was chemotactic in nature. The chemotactic activity has an apparent specificity for endothelial cells, in that chemotaxis of smooth muscle cells and fibroblasts was stimulated to a much lesser extent. These results, in conjunction with our previous demonstration of an endothelial cell mitogen produced by 3T3 adipocytes [Castellot, J. J., Jr., Karnovsky, M. J. & Spiegelman, B. M. (1980) *Proc. Natl. Acad. Sci. USA* 77, 6007–6011] indicate that the differentiation of these cells is closely linked to the production of factors that stimulate angiogenesis *in vivo* and growth and chemotaxis of endothelial cells *in vitro*.**

The development of new blood vessels (neovascularization, or angiogenesis) occurs during many important biological processes, including normal embryonic development, wound healing, inflammation, and neoplasia. The new blood vessels arise from the endothelial cells of preexisting capillaries and small vessels (1, 2). Because neovascularization appears to involve stimulation of endothelial cell protease activity, motility, and proliferation, much attention has been focused on factors that stimulate these processes *in vitro* (3).

Little is known about neovascularization during normal tissue development. As a model we have examined interactions *in vitro* between vascular endothelial cells and 3T3-F442A adipocytes (4). This system was chosen because adipose tissue becomes highly vascularized during embryological development (5), and the 3T3-F442A preadipocytes undergo differentiation *in vitro* in a manner highly analogous to adipocyte differentiation *in vivo* (6).

We have previously shown that 3T3 adipocytes produce a potent mitogenic activity that is specific for vascular endothelial cells (7), suggesting that it plays a role in angiogenesis. Production of the mitogenic activity is differentiation dependent in that adipocytes secrete much more of the mitogen than do preadipocytes. Because proliferation of endothelial cells is only a part of the angiogenesis process, we have investigated whether differentiating adipocytes also stimulate endothelial cell chemotaxis *in vitro* and neovascularization *in vivo*. We now report that 3T3 adipocytes secrete, in a differentiation-dependent fashion, factor(s) that stimulate endothelial cell chemotaxis *in vitro* and neovascularization *in vivo*. Thus, 3T3 adipocytes apparently produce the signals required to trigger new blood vessel development, and the expression of this information is closely linked to the differentiation of the adipocytes.

MATERIALS AND METHODS

Heparin was obtained from Elkins–Sinn (Cherry Hill, NJ) or Upjohn. All other chemicals were obtained from Sigma unless otherwise noted. Bovine adrenal capillary endothelial cells were obtained from Bruce Zetter (Children's Hospital Medical Center, Boston, MA). Bovine aortic endothelial cells (BAEC) were isolated and characterized as described (8–10). They were grown in Dulbecco's modified Eagle's medium (DME medium) supplemented with 10% heat-inactivated (56°C, 30 min) calf serum, penicillin at 100 units/ml, and streptomycin at 100 μg/ml. Baby hamster kidney 21/c13 (BHK) cells were grown in DME medium supplemented with 10% calf serum, penicillin at 100 units/ml, and streptomycin at 100 μg/ml. Rat aortic smooth muscle cells (SMC) were isolated and characterized as described (11). They were grown in RPMI 1640 medium (KC Biological, Lenexa, KS) supplemented with 20% fetal calf serum, penicillin at 100 units/ml, and streptomycin at 100 μg/ml. 3T3-F442A cells were obtained from Howard Green (Massachusetts Institute of Technology, Cambridge, MA). To minimize adipose differentiation, 3T3-F442A stocks were grown in DME medium supplemented with 9% cat serum, 1% calf serum, penicillin at 100 units/ml, and streptomycin at 100 μg/ml (12). All cells were grown at 37°C in a 10% CO_2/90% air atmosphere.

Adipocyte-conditioned medium was collected from 3T3-F442A cells that were grown and maintained for 6–7 days after confluence in DME medium containing 10% fetal calf serum, at which time adipose differentiation had reached a maximum as determined by both biochemical and morphological criteria (13). Cells were washed three times in DME medium, and 10 ml of DME medium containing 0.5% fetal calf serum was placed on each 100-mm tissue culture dish. After 48 hr, the conditioned medium was removed and filtered through a 0.22-μm pore diameter filter (Nalge). This filtration had no effect on either the angiogenic or chemotactic activities. The conditioned medium was stored at −20°C for up to 6 months, or at 4°C in the dark

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: DME medium, Dulbecco's modified Eagle's medium; BAEC, bovine aortic endothelial cells; BHK, baby hamster kidney 21c/13; SMC, smooth muscle cells.

for up to 2 months, with little loss of activity. Preadipocyte-conditioned medium was obtained from 3T3-F442A cells grown and maintained 6–7 days after confluence in DME medium containing 9% cat serum and 1% calf serum. Very little adipose differentiation occurs under these conditions (12). The medium conditioned by preadipocytes and its subsequent handling was exactly as described for 3T3-F442A adipocytes.

To assay the angiogenic activity in adipocyte- or pre-adipocyte-conditioned medium, we used the chicken chorioallantoic membrane assay developed by Folkman (14). Briefly, a 1.5 × 3 cm window was made in the eggshell at day 8 or 9 after fertilization, such that the chorioallantoic membrane dropped away from the shell. The window was covered with cellophane tape and the eggs were returned to the incubator. The test substance was dried in 5-μl or 10-μl aliquots in the center of a 13-mm plastic coverslip (Lux) to form a spot 3–5 mm in diameter. In some experiments, heparin was added to the test substance before drying it on the coverslip (final heparin concentration, 5 μg per coverslip). The coverslip was placed (spot side down) on the chorioallantoic membrane on day 10. After 48 hr, eggs were examined for the growth of new vessels toward the spot on the coverslip. The strength of the angiogenic response was graded on a scale of 0 to 5+, as described by Folkman (15).

Chemotaxis assays were performed with 25-μl modified Boyden chambers (Neuro Probe), as described (16–18). Briefly, 5-μm or 8-μm pore diameter filters (Nuclepore) were soaked in 0.5 M acetic acid overnight. The filters were washed with distilled water and incubated for at least 16 hr in a 100 μg/ml gelatin solution. The gelatin-coated filters were air-dried and then incubated in a 10 μg/ml fibronectin solution (Collaborative Research, Waltham, MA) for 1–2 hr and then air-dried. The test substance (which always contained 0.5% fetal calf serum) was placed in the bottom chamber. All fetal calf serum used was from the same serum lot. The filter was inserted, and freshly trypsinized cells (5×10^4 per assay) in DME medium containing 0.5% fetal calf serum were added to the top chamber. The chambers were incubated for 4 hr at 37°C in a 10% CO_2/90% air atmosphere. The filters were removed and fixed in methanol for 15 min at room temperature. Cells on the upper filter surface were carefully removed by rubbing with a cotton swab. The filters were stained with Giemsa stain and mounted on slides. The number of cells on the lower filter surface was determined by counting at least ten ×430 fields in a light microscope. To distinguish between chemotaxis and chemokinesis, we performed a checkerboard analysis (16), in which cells are exposed to the motility-stimulating substance both in the presence and in the absence of a gradient. In these experiments, different concentrations of conditioned medium were placed in the top and bottom chambers. In assays in which conditioned medium was added to the top chamber, the trypsinized BAEC were taken up directly into the desired concentration of conditioned medium.

RESULTS

Angiogenic Activity of Adipocyte-Conditioned Medium. To determine whether adipocytes produced all the factors required to stimulate new blood vessel growth *in vivo*, we tested the ability of adipocyte-conditioned medium to stimulate neovascularization in the chicken chorioallantoic membrane assay (14). When DME medium controls containing 0.5% fetal calf serum were tested for angiogenic activity, few positive responses were seen at 5, 25, or 100 μl per egg (Table 1). The same medium conditioned by 3T3 adipocytes for 48 hr gave a positive angiogenic response in one out of seven eggs when tested at 5 μl per egg. However, as the dose of adipocyte-conditioned medium was increased, a marked positive response was obtained (>70% positive; Table 1). The positives at 48 hr were usually 2+, according to the scoring system of Folkman, in which negative responses are given a 0, and positive eggs are rated from 1+ to 5+ (15). When the adipocyte-conditioned medium-stimulated positive eggs were allowed to develop for 3 or 4 days, the strength of the angiogenic response increased to 3+ and 4+. Three different batches of conditioned medium were used to obtain the results in Table 1. Good reproducibility between batches was found, especially at the higher concentrations.

Table 1. Angiogenic activity of adipocyte-conditioned medium

Test substance	Positive responses		
	5 μl	25 μl	100 μl
DME medium control	0/9	3/29	3/20
DME medium control + heparin	0/8	4/19	5/22
Adipocyte-conditioned medium	1/7	20/26	16/17
Adipocyte-conditioned medium +heparin	6/10	21/22	24/25
Preadipocyte-conditioned medium	ND	1/10	3/11
Preadipocyte-conditioned medium + heparin	ND	1/7	4/12

The indicated quantities (per egg) of test substance were tested for their ability to stimulate new blood vessel growth in the chicken chorioallantoic membrane assay. Samples containing heparin have 5 μg of heparin per egg. The number of positive responses (numerator) and the total number of eggs tested with the particular test substance (denominator) is given. ND, not done.

Taylor and Folkman recently observed that heparin potentiated the angiogenic activity of tumor cell extracts but was not angiogenic by itself (19). We therefore examined the effect of heparin on the angiogenic activity of adipocyte-conditioned medium (Table 1). When added to DME medium controls containing 0.5% fetal calf serum, heparin at 5 μg per egg produced only a slight increase in positive responses. However, 5 μl of adipocyte-conditioned medium containing this amount of heparin produced a weak (1+) positive response in 6 out of 10 eggs, compared with 1 out of 7 positives when heparin was not present. Heparin added to higher concentrations of adipocyte-conditioned medium increased the strength of the response to 2+ and 3+. Thus, heparin potentiated the angiogenic activity of adipocyte-conditioned medium but did not induce neovascularization by itself.

A positive response in the chorioallantoic membrane assay could also be produced by inflammation; thus, it is important to rule out this artifact. Because inflammatory responses in this system generally occur 3 or more days after the coverslips are implanted (15, 20), we minimized this possibility by scoring the eggs after 48 hr. In addition, randomly selected positive eggs were fixed and prepared for histological examination of the neovascularized region. No evidence of inflammation was detected in any of the samples. To determine if differentiation of the 3T3-F442A cells was necessary for production of the angiogenic activity, we tested the ability of conditioned medium from 3T3-F442A cells in the preadipose state to stimulate neovascularization (Table 1). Only a small increase in positive responses was observed at 25 and 100 μl per egg. When heparin was added to either 25 μl or 100 μl of preadipocyte-conditioned medium, only a slight further increase in the number of positive responses was observed (1 out of 7 and 4 out of 12, respectively). These results, when compared to those obtained with adipocyte-conditioned medium, indicate that 3T3-F442A cells must differentiate in order to produce the angiogenic activity. The difference observed between adipose and preadipose states does not

reflect a simple difference in the ratio of cells to medium, because there is no significant difference between preadipocytes and adipocytes in cell number or amount of protein per dish when insulin is not present (7), as in these experiments.

Chemotactic Activity of Adipocyte-Conditioned Medium. Because an important part of neovascularization is the movement of endothelial cells towards the target tissue (1), we determined if 3T3 adipocytes secrete a motility-stimulating activity for endothelial cells by using the modified Boyden chamber method (16, 17). This system was used because a relatively large number of samples can be quantitatively assayed in a single experiment, and because chemotaxis can be distinguished from chemokinesis by the use of checkerboard analysis (16). This system does not distinguish, however, between a fluid-phase gradient or a bound gradient.

When DME medium controls containing 0.5% fetal calf serum were placed in the bottom chamber, some cells (64 cells per mm^2) moved to the lower filter surface after 4 hr (Fig. 1). This represents the background due to random locomotion of the cells. When conditioned medium from 3T3-F442A adipocytes was placed in the bottom chamber, the number of cells crossing to the lower filter surface was dramatically increased. Fig. 1 also shows the dose response of the motility-stimulating activity and represents the average of several batches of adipocyte-conditioned medium. There was an average net increase of 280 cells per mm^2 on the lower filter surface at the highest concentration of conditioned medium used. As the concentration of conditioned medium decreased, the number of cells on the lower filter surface decreased proportionately. Because neovascularization occurs via motility of small vessel endothelial cells, these experiments were repeated with bovine adrenal capillary endothelial cells, and similar results were obtained (Fig. 1).

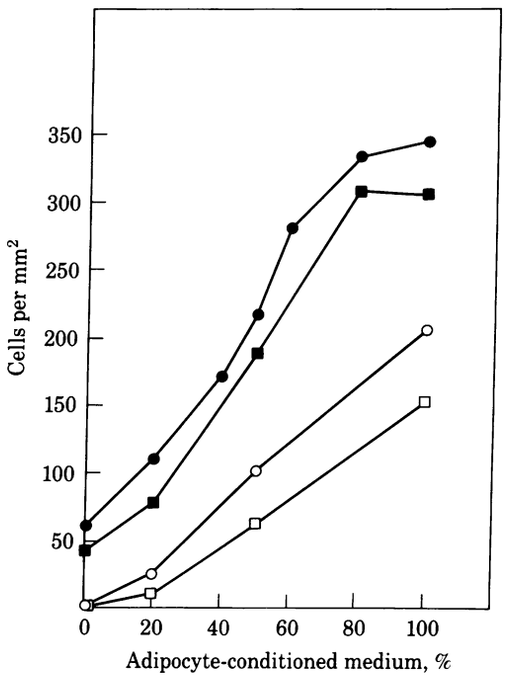

FIG. 1. Dose response of motility-stimulating and chemotactic activities in adipocyte-conditioned medium. The activity of the indicated concentrations of adipocyte-conditioned medium in 0.5% fetal calf serum/DME medium was assayed on BAEC (●) and bovine adrenal capillary endothelial cells (■). The number of cells per mm^2 that passed through the membrane pores to the lower filter surface was determined by counting random high-power fields in a light microscope. The net chemotactic activity of adipocyte-conditioned medium toward BAEC (○) and bovine adrenal capillary endothelial cells (□) was determined by correcting for random locomotion and chemokinetic movement, as measured by checkerboard analysis (see Fig. 2).

The movement of cells across the filter in response to adipocyte-conditioned medium could be due to a stimulation of chemokinesis [the random locomotion of cells in response to a chemical stimulus (21)] or chemotaxis [the directed locomotion of cells in response to a chemical stimulus (21)]. We tested this possibility by performing a checkerboard analysis (16), in which cells are exposed to the chemotactic agent both in the presence and in the absence of a gradient (Fig. 2). As previously noted (Fig. 1), when no adipocyte-conditioned medium was present in the top chamber, increasing numbers of cells migrated to the lower filter surface as the concentration of adipocyte-conditioned medium in the bottom chamber increased. When no conditioned medium was present in the bottom chamber, the number of cells on the lower filter surface remained essentially constant or decreased slightly as the concentration of conditioned medium in the top chamber increased (Fig. 2). When equal concentrations of adipocyte-conditioned medium were present in the top and bottom chambers, there was a net chemokinetic movement of cells to the lower side of the filter (Fig. 2). Correcting for random movement by subtracting the serum background and chemokinetic movement from the total motility, the net increase in BAEC on the lower filter surface due to chemotaxis is approximately 200 cells per mm^2 when the maximal gradient is present (100% conditioned medium in the bottom chamber and no conditioned medium in the top chamber). The stimulation of cell motility by adipocyte-conditioned medium thus appears to be largely chemotactic but also contains a significant chemokinetic component. Whether or not these two activities are the result of the same factor(s) is unknown.

To test if differentiation of the 3T3-F442A cells was required for production of the motility-stimulating activity, we assayed the ability of conditioned medium from 3T3-F442A preadipocytes to stimulate BAEC motility (Fig. 3). Preadipocyte-conditioned medium caused a maximal net increase of approximately 80 cells per mm^2 on the lower filter surface, substantially less than the 280 cells per mm^2 increase seen with adipocyte-conditioned medium. Similar results were obtained with bovine adrenal capillary endothelial cells (Fig. 3). The production of the motility-stimulating activity thus appears to be differentiation dependent. It is possible that the preadipocyte-conditioned

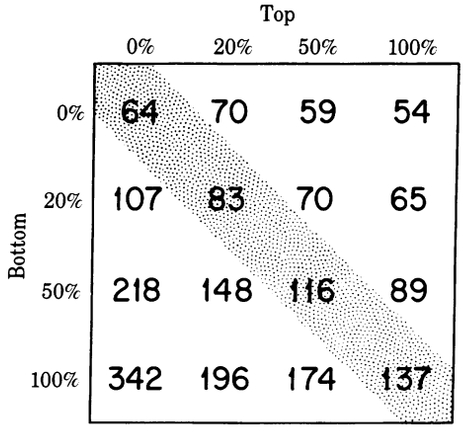

FIG. 2. Checkerboard analysis of motility-stimulating activity in adipocyte-conditioned medium. The number of BAEC per mm^2 crossing the membrane to the lower filter surface was scored as a function of adipocyte-conditioned medium concentration on the top and bottom of the filter. DME medium + 0.5% fetal calf serum is referred to as 0%.

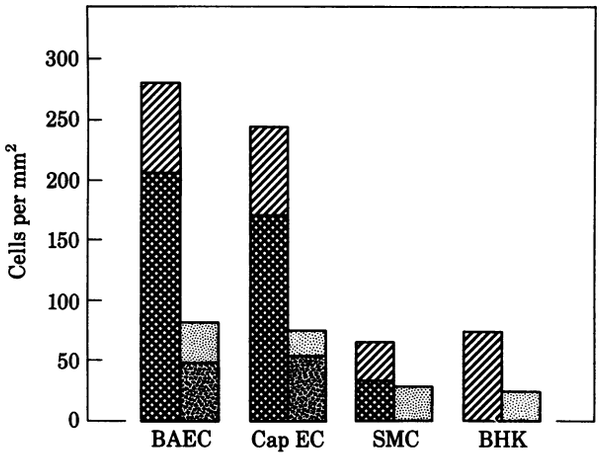

FIG. 3. Motility-stimulating and chemotactic activities of adipocyte-conditioned medium and preadipocyte-conditioned medium towards different cell types. The motility-stimulating activity of adipocyte-conditioned medium (hatched bars) and preadipocyte-conditioned medium (bars with small uniform stippling) on the indicated cell types was assayed. The data are presented as cells per mm² crossing the membrane to the lower filter surface. The background due to random migration of cells in DME medium + 0.5% fetal calf serum has been subtracted from these data. For BAEC, this background was 64; for bovine adrenal capillary endothelial cells (Cap EC), 43; for SMC, 57; and for BHK fibroblasts, 76. The chemotactic activity of adipocyte-conditioned medium (cross-hatched bars) and preadipocyte-conditioned medium (bars with two sizes of stippling) was determined by correcting for the chemokinetic activity as measured by checkerboard analysis. This analysis was not performed for preadipocyte-conditioned medium on BHK cells or SMC, or for adipocyte-conditioned medium on BHK cells.

medium is less stimulatory because the preadipocytes do not secrete either the chemotactic or the chemokinetic activity. We tested this possibility by performing a checkerboard analysis on preadipocyte-conditioned medium (Fig. 4). Although the overall stimulation of BAEC motility by preadipocyte-conditioned medium is low, making analysis more difficult, it also appears to consist of both chemotactic and chemokinetic components in roughly the same proportion as adipocyte-conditioned medium. When the chemotactic components are compared by subtracting random background and chemokinesis, the preadipocyte-conditioned medium produced a net increase of 50 cells per mm² due to chemotaxis (Fig. 4), whereas adipocyte-conditioned medium stimulated a net increase of 200 cells per mm² due to chemotaxis (Fig. 2; both calculated at 100% conditioned medium). Thus, differentiation of 3T3 adipocytes caused a 4-fold increase in production of the chemotactic activity.

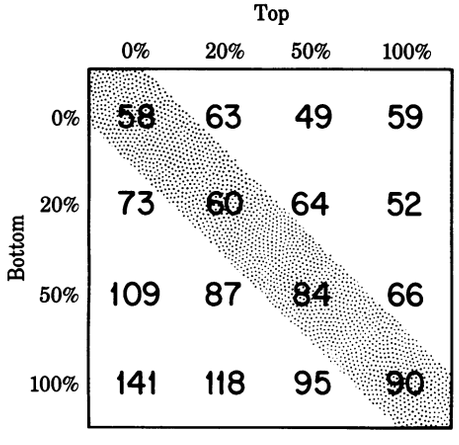

FIG. 4. Checkerboard analysis of chemoattractant activity in preadipocyte-conditioned medium. BAEC per mm² crossing to the lower filter surface were scored as a function of preadipocyte-conditioned medium concentration on the top and bottom of the filter.

It is important to know whether the chemotactic activity produced by adipocytes is specific for endothelial cells. To do this, we assayed the ability of adipocyte-conditioned medium to stimulate directed motility of BHK fibroblasts and rat aortic SMC. Adipocyte-conditioned medium caused a net increase of approximately 70 cells per mm² of both cell types on the lower filter surface (Fig. 3). Conditioned medium from preadipocytes was even less stimulatory (Fig. 3). Checkerboard analysis indicates that the stimulation of SMC motility also consists of both chemotactic and chemokinetic components (data not shown). Adipocyte-conditioned medium thus appears to have much more chemotactic activity toward endothelial cells than toward these other cell types. Although we cannot rule out the possibility that under different assay conditions (e.g., coating the filters with different substrates) these other cell types could be strongly stimulated by adipocyte-conditioned medium, it should be noted that the collagen–fibronectin substrate used has been shown to be an optimal or near-optimal substrate for the response of fibroblasts and SMC to other chemotactic factors (17, 18).

DISCUSSION

Neovascularization appears to consist of at least three distinguishable processes involving endothelial cells: (*i*) changes in attachment to the extracellular matrix and to each other (1); (*ii*) movement towards the tissue being vascularized (1); and (*iii*) proliferation to provide cells for new vessels (1–3, 15). This paper reports that 3T3-F442A cells produce, in a differentiation-dependent manner, factor(s) that stimulate neovascularization *in vivo* (Table 1) and endothelial cell chemotaxis *in vitro* (Figs. 1 and 2). Although there are numerous reports of substances that stimulate endothelial cell growth *in vitro* or neovascularization *in vivo* (see ref. 3 for a review), an angiogenic activity secreted as a consequence of the differentiation of the producer cell type has not been demonstrated previously, to our knowledge. This behavior resembles the angiogenic response to adipocyte differentiation during embryological development (5).

We have previously shown that adipocytes produce a potent mitogenic activity for capillary and aortic endothelial cells *in vitro* (7). Production of the mitogenic activity is differentiation dependent and specific for endothelial cells (7). Rifkin and coworkers recently demonstrated that conditioned medium from adipocytes, but not from preadipocytes, strongly stimulated both plasminogen activator and collagenase activities in capillary endothelial cells (22). These protease activities were not stimulated in aortic endothelial cells. This is in contrast to the mitogenic (7) and chemotactic activities, which are observed with both capillary and aortic endothelial cells. Thus, 3T3 adipocytes produce factor(s) that stimulate endothelial cells to undertake three processes *in vitro* that are likely to be directly related to the ability of adipocytes to stimulate neovascularization *in vivo*—increases in protease activity, chemotaxis, and proliferation. All of these activities, like angiogenesis in *in vivo* assays, are dependent on the differentiation of the adipocytes. Although the absolute necessity of, and relationship between, these various components of neovascularization have not been extensively explored, it seems reasonable to assume that each must occur to stimulate new blood vessel proliferation *in vivo*.

The neovascularization of the chicken chorioallantoic membrane induced by adipocyte-conditioned medium was potentiated by heparin, although heparin by itself did not stimulate new vessel growth (Table 1). The potentiation of angiogenesis

by heparin was observed by Taylor and Folkman (19), using tumor cell extracts. The observations reported here indicate that this is a general effect and suggest a role for heparin or related species in developmental neovascularization. The mechanism of the heparin effect is unknown, but the heparin may function by stimulating a rate-limiting step in neovascularization or by inactivating an endogenous inhibitor of angiogenesis, thus allowing more rapid vessel growth. It may also potentiate neovascularization by aiding in the formation of the gradient of adipocyte-conditioned medium released from the coverslip on the chorioallantoic membrane.

The adipocyte–endothelial cell system we have described has several important features. First, because the production of adipocyte-derived angiogenic factor(s) is closely linked to the cell differentiation process, this system offers the opportunity to study the relationship between gene expression, differentiation, and the control of tissue vascularization. Second, because proliferation, chemotaxis, and protease activity in endothelial cells are all strongly stimulated by adipocyte-conditioned medium, it should be possible to determine if a single adipocyte-derived factor stimulates all of these processes, or if angiogenesis requires several distinct molecular signals. Finally, comparison of the biochemical properties of the adipocyte-produced factors with angiogenic factors derived from other sources should provide insights into the relationship between different types of normal and pathological neovascularization.

We gratefully acknowledge Drs. John M. Robinson and Heikki Seppa for their many helpful discussions. We thank Ms. Mary Hagney for her expert histological preparations of chorioallantoic membranes. This work was partially supported by National Institutes of Health Grant HL 17747 to M.J.K.

1. Ausprunk, D. & Folkman, J. (1977) *Microvasc. Res.* **14**, 53–65.
2. McAuslan, B. & Hoffman, H. (1979) *Exp. Cell Res.* **119**, 181–190.
3. Auerbach, R. (1981) in *Lymphokines*, ed. Pick, E. (Academic, New York), Vol. 4, pp. 69–88.
4. Green, H. & Kehinde, O. (1976) *Cell* **7**, 105–113.
5. Wasserman, F. (1965) *Handb. Physiol.* **5**, 87–100.
6. Green, H. (1978) in *10th Miami Symposium on Differentiation and Development*, eds. Ahmad, F., Schultz, J., Russell, T. R. & Werner, R. (Academic, New York), pp. 13–33.
7. Castellot, J. J., Jr., Karnovsky, M. J. & Spiegelman, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6007–6011.
8. Jaffe, E., Nachman, R., Becker, C. & Minick, C. (1973) *J. Clin. Invest.* **52**, 2745–2756.
9. Booyse, F., Sedlak, B. & Rafelson, N. (1975) *Thromb. Diath. Haemorrh.* **34**, 825–839.
10. Gimbrone, M. (1974) in *Progress in Hemostasis and Thrombosis*, ed. Spaet, T. (Grune & Stratton, New York), Vol. 3, pp. 1–28.
11. Hoover, R., Rosenberg, R., Haering, W. & Karnovsky, M. J. (1981) *Circ. Res.* **47**, 578–583.
12. Kuri-Harcuch, W. & Green, H. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 6107–6109.
13. Spiegelman, B. M. & Green, H. (1980) *J. Biol. Chem.* **255**, 8811–8818.
14. Folkman, J. (1974) *Cancer Res.* **34**, 2109–2113.
15. Folkman, J. (1975) in *Cancer*, ed. Becker, F. F. (Plenum, New York), Vol. 3, pp. 355–388.
16. Zigmond, S. H. & Hirsch, J. G. (1973) *J. Exp. Med.* **137**, 387–410.
17. Postlethwaite, A. E., Snyderman, R. & Kang, A. H. (1976) *J. Exp. Med.* **144**, 1188–1203.
18. Grotendorst, G. R., Seppa, H. E. J., Kleinman, H. K. & Martin, G. R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3669–3672.
19. Taylor, S. & Folkman, J. (1982) *Nature (London)* **297**, 307–312.
20. Auerbach, R. (1974) in *Concepts of Development*, eds. Lash, J. & Whittaker, J. R. (Sinauer Associates, Stamford, CT), pp. 261–271.
21. Wilkinson, P. C. & Allan, R. B. (1978) in *Leukocyte Chemotaxis*, ed. Gallin, J. I. (Raven, New York), pp. 1–23.
22. Rifkin, D. B., Gross, J. L., Moscatelli, D. & Jaffe, E. (1982) in *Pathobiology of the Endothelial Cell*, eds. Nossel, H. & Vogel, H. (Academic, New York), pp. 191–197.