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30 October 1984; accepted 28 January 1985

Matrix-Driven Translocation of Cells and Nonliving Particles

Abstract. *Cells of metazoan organisms produce and react to complex macromolecular microenvironments known as extracellular matrices. Assembly in vitro of native, compositionally nonuniform collagen-fibronectin matrices caused translocation of certain types of cells or polystyrene-latex beads from regions lacking fibronectin into regions containing it. The translocation process was not due to diffusion, convection, or electrostatic distribution effects, but may depend on nonequilibrium phenomena at the interface of contiguous collagen matrices formed in the presence and absence of fibronectin or particles. Extracellular matrix formation alone was sufficient to drive translocation by a biophysical process that may play a role in cellular migration during embryogenesis, as well as in other types of tissue reorganization such as inflammation, wound healing, and tumor invasion.*

Extracellular matrix components such as the collagens, fibronectin, laminin, hyaluronic acid, and proteoglycans influence cellular biosynthetic activities, shapes, and motility during interactions with one another and with different types of cells (*1*). It has usually been considered that cellular translocation results from forces generated within cells that are interacting with their matrices (*2*). The matrix would impart speed and direction to this motile activity by a combination of chemokinetic (*3*), chemotaxic (*4*), and route-opening (*5*) effects. However, cells with no intrinsic motile behavior, and even nonliving particles such as polystyrene beads, can be selectively conveyed along neural crest pathways in embryos into which they have been artificially introduced (*6*). We now report that matrices constructed from collagen and fibronectin generate a driving force that is capable of translocating living cells and nonliving particles several millimeters in a matter of minutes.

Chick embryo cells of various types were mixed with a gelling solution of type I collagen (*7, 8*). Contiguous with the cell-containing gel was a second gel (legend to Fig. 1), poured at the same time and containing different fibronectins (3 to 50 μg/ml). At an appropriate fibronectin concentration in the second gel (in excess of a threshold value between 3 and 6.25 μg/ml), the cells moved as much as 6 mm into the originally cell-free region in about 10 minutes. The rate of translocation (defined as movement over distances of at least 1 mm) varied with the concentration of collagen but not with that of fibronectin once its threshold level had been reached.

Primary gels containing chick limb bud precartilage mesenchyme cells (*9, 10*) (2.5×10^6 cells per milliliter) were formed adjacent to secondary gels containing chick cellular, chick plasma, or human plasma fibronectin (12.5 μg/ml), or no fibronectin. Translocation began 10 to 15 seconds after the gels were poured, and continued for as much as 20 minutes. Over a period of 5 to 20 minutes, cells from the border regions, including those from about 1 mm away from the interface, moved into the secondary gels containing chick cellular or human plasma fibronectin, with the farthest moving cells traversing more than 5 mm. Movement then stopped and no further change was observed in 2 days of observation. In the presence of any of several biologically active preparations of chick plasma fibronectin (*11*) or in the absence of fibronectin, no translocation took place during the 2-day period (displacement of the cell front from the original boundary was less than 0.25 mm). This was the case when concentrations of chick plasma fibronectin were more than eight times the threshold level for chick cellular or human plasma fibronectin (Fig. 1). These experiments were repeated 30 to 50 times for each fibronectin, with six separately prepared batches of rat tail tendon collagen, and there was no variation in the qualitative results. The rate of translocation depended on the collagen concentration. If the collagen concentration was higher or lower than the optimal value of about 1.7 mg/ml, translocation of cells was slower or did not occur.

Individual cells and groups of cells translocated at speeds of more than 5 μm/sec (Fig. 2). Furthermore, the path taken by each moving cell was unidirectional, with none of the random walk characteristics that would be expected for a stochastic process such as diffusion. Cells did not move in opposite directions.

Different cell types varied in their responses to matrices containing different fibronectins. Chick limb bud precartilage cells translocated in response to human plasma fibronectin and chick cellular fibronectin, but not in response to chick plasma fibronectin. Chick embryo fibroblasts responded to the human fibronectin, but not to either chicken fibronectin. In contrast, cells obtained from hearts of 5-day chick embryos did not respond to any of the fibronectins (*12*).

Two observations suggested that the translocating cells were not moving under their own power. (i) The observed rates of translocation were more than an order of magnitude greater than the most rapid cellular movement previously described in vitro (*2*); (ii) cells translocating in collagen gels showed none of the changes in cellular morphology associated with motile behavior, as assessed by Nomarski differential interference contrast microscopy.

Polystyrene latex beads are conveyed along neural crest pathways in embryos by a driving force arising from the extracellular environment (*6*). We therefore introduced such beads, instead of cells, into collagen gels as the primary gel systems (Fig. 3, a and b). When human plasma fibronectin was present in the secondary gel there was massive translocation of the beads; when it was absent there was no translocation. The same threshold concentration of fibronectin (between 3 and 6.25 μg/ml) was necessary here as in the cell-containing system. The beads were not translocated in response to chick plasma fibronectin, and only to a small extent with chick

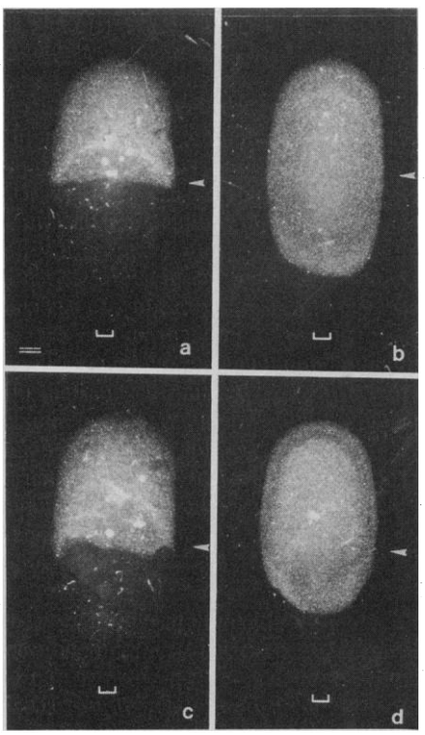
cellular fibronectin; this finding is also in agreement with the relative effectiveness of fibronectins in the cellular systems assayed.

Clumps of as many as six 6-μm polystyrene latex beads translocated as rapidly as individual beads. However, 0.2-μm polystyrene latex beads did not translocate (Fig. 3c), indicating that there was a lower limit to the size of translocatable particles. Apart from size, the composition and surface characteristics of the inert particles (Table 1 and Fig. 3d) were determining factors in their ability to undergo matrix-driven translocation. Heparin-coated beads or beads of derivatized polystyrene latex bearing a negative charge (either sulfonated or carboxylated) could be translocated; as dextran sulfate coated beads also have a negative charge, charge does not appear to be the main determinant of the translocatability of the beads. It seems more likely that the ability to interact with a specific fibronectin domain is the characteristic feature of responsive cells or beads (*13*).

Under optimal translocation conditions, macroscopic movement of a front of cells or beads across the boundary between the primary and secondary gels began at about 10 seconds after the gels are poured. The onset of translocation and the subsequent contour of the front were independent of the order in which gels were poured. To consider possible mechanisms for the driving force, we examined the kinetics of collagen fibrillogenesis in the two regions relative to the time course of translocation (Fig. 4, a and b). The gel mixtures assayed were identical to those used in the translocation experiments except that no cells or beads were present. Neither the time of onset nor the overall rate or extent of fibrillogenesis differed between fibronectin-lacking or fibronectin-containing collagen gels (Fig. 4b). We cannot exclude subtle differences in fibril organization between the two types of preparation (*14*).

Measurements during the earliest phase of fibrillogenesis of type I collagen matrices (Fig. 4b, inset) show that a brief, rapid increase in optical density was followed by a pause. The initiation of the subsequent continuous rise in optical density at about 10 seconds was coincident with the onset of translocation.

Thus, the period of maximal translocation corresponded to the period of maximal fibrillogenesis of both types of gel. A large component of the driving force for translocation probably arises from the active process of matrix formation (*15, 16*). The conditions under which the gels form in these experiments were physiological with respect to salt concentration



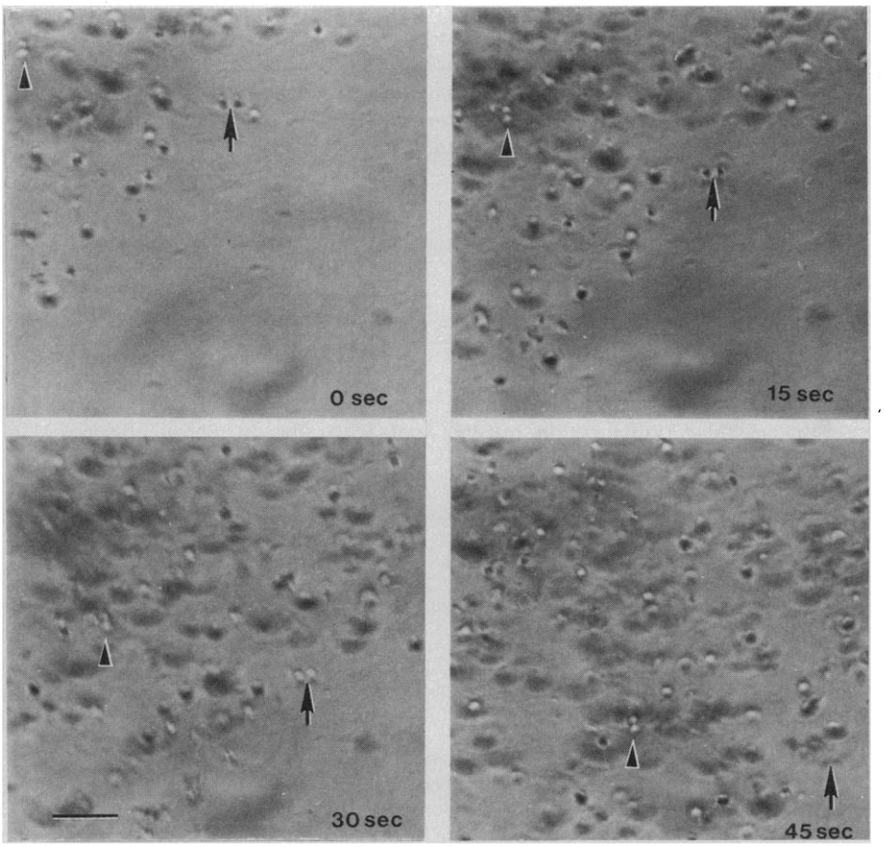


Fig. 1 (left). Translocation of chick limb mesenchymal cells (*9*) in response to chick cellular fibronectin and human plasma fibronectin, but not chick plasma fibronectin. Type I collagen was extracted from the tails of young adult rats as described (*7, 8*). The acetic acid extract was adjusted to a protein concentration of 3 to 5 mg/ml and dialyzed for 2 days against two changes of 1/10 strength Ham's F-12 medium (Gibco) without bicarbonate. Primary gels were made by starting with 0.7 ml of the collagen solution on ice, adding 0.1 ml of 10× Ham's F-12, 0.1 ml of sodium bicarbonate (11.76 mg/ml), and 0.1 ml of fetal bovine serum or distilled water, and rapidly mixing with 1 ml of cells in Ham's F-12 (with or without serum) at 5×10^6 cells per milliliter. Secondary gels were made as described above but with 1 ml of medium or medium plus fibronectin instead of the cell suspension. A drop of primary gel was poured on a tissue culture dish contiguous with a drop of secondary gel containing (a) medium alone, (b) chick cellular fibronectin, (c) chick plasma fibronectin or (d) human plasma fibronectin (fibronectin concentration, 12.5 μg/ml). Chick cellular and human plasma fibronectins (BRL) and four different lots of chick plasma fibronectin, confirmed to be active in cell agglutination, attachment, and spreading assays (*11*) were used. Gels were incubated at room temperature or 37°C. Gels were photographed through a dissecting microscope, illuminated from below by a fiber optics ring light. Square brackets indicate lower limit of secondary gel. Arrowheads represent position of original boundary between primary and secondary gels. Scale bar, 1 mm. Fig. 2 (right). Unidirectional translocation of mesenchymal cells in response to human plasma fibronectin (12.5 μg/ml). Translocation across the boundary between primary and secondary gels began after about 10 seconds. Movement was monitored by phase-contrast microscopy, and photographed at 15-second intervals. The arrowhead and arrow point to corresponding groups of cells in each frame. Scale bar, 50 μm.

and pH. The fibrils formed in these circumstances were similar to those found in tissues (native) (*7*). Gelling occurred in the absence of native fibril formation if either salt or pH was not at physiological levels (*7*). When gels were not formed at physiological salt or pH conditions, translocation of cells or beads was completely eliminated as determined in 15 experiments. In addition, the matrix-driven translocation of limb bud precartilage mesenchyme cells or 6-μm polystyrene latex beads did not occur with the following polymeric matrices: agarose (0.2 percent or 0.4 percent), methylcellulose (1 percent), and type I + type II collagen in a 25:1 ratio (*17*).

We conclude that matrix-driven translocation accompanies fibrillogenesis of type I collagen and is likely to be thermodynamically dependent on this process. Moreover, the nature of the fibrils formed, whether similar or different in the two gel regions, is an essential factor in promoting the effect (*18*).

The unidirectionality and great speed of bead and cell movement preclude diffusion as the mechanism of translocation from the primary to the secondary gel. Moreover the failure of the 0.2 μm-polystyrene latex beads to translocate under conditions permissive for translocation of the larger beads argues against the possibility that formation of fluid or matrix convection currents at the boundary between primary and secondary gels might be responsible for the conveyance of particles from one region to another. To test these possibilities more directly we added Eosine Y (anionic), methylene blue (cationic), or alizarin (uncharged at neutral pH) to primary gels; secondary gels contained human plasma fibronectin (12.5 μg/ml). In no case did any apparent movement of colored material occur across the gel boundary within the first 15 minutes (Fig. 5, a to c). After more than an hour of observation, the only apparent change at the boundary was a fuzziness resulting from diffusion of the stains.

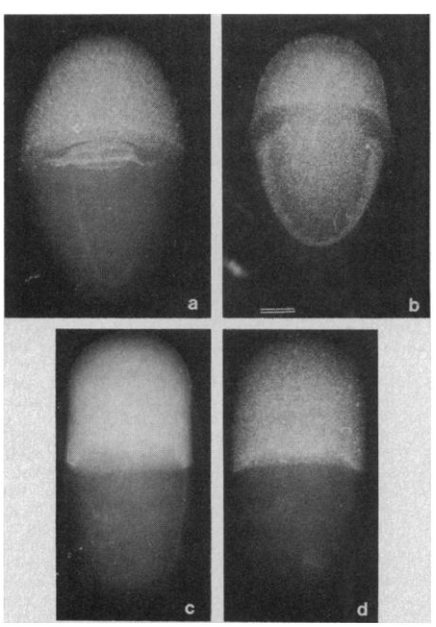
Fibronectin-free matrix material surrounding the moving particles appeared to move across the original boundary along with the particles (Fig. 2). To confirm this, alizarin was added to the primary gel in the presence of 6-μm latex beads. The matrix of the primary gel, as well as beads, was rapidly transported across the interface (Fig. 5d).

The results of our experiments do not support mechanisms for matrix-driven translocation that depend on diffusion of beads or cells (which would in any case be orders of magnitude slower than diffusion of stain molecules), or on any disturbance at the boundary of the two forming matrices resulting in convection currents. They also exclude explanations in terms of a Donnan-type redistribution

Table 1. Inert particles are selectively translocated by extracellular matrices. Primary gels contained the particle types shown and secondary gels contained human plasma fibronectin (25 μg/ml). Polystyrene latex beads (6 μm; Polysciences) were used as supplied, or were sulfonated by incubation for 30 seconds with concentrated sulfuric acid. Sulfonated beads were washed three times with medium before they were added to the primary gel. Other types of polystyrene latex beads (carboxylated or 0.2 μm) were used as supplied (Polysciences). Latex beads (6 μm) were coated with human plasma fibronectin as described by Bronner-Fraser (*6*) or by incubation with poly-L-lysine (Sigma, 0.5 mg/ml) for 30 minutes. Poly L-lysine–coated beads were subsequently coated by incubation for 30 minutes with heparin or dextran sulfates of molecular weights ranging from 5,000 to 500,000 (12.5 mg/ml, Sigma). At least 20 replicates of each experiment were performed with no differences in outcome.

Translocated	Not translocated
	Sephadex 6-25 (Dextran)
	Bio-Beads SM-2 (Styrene-divinylbenzene)
	Bio-Gel P-60 (Polyacrylamide)
*Polystyrene latex beads**	
Not coated	Not coated, 0.2 μm
Sulfonated	Fibronectin coated
Carboxylated	Poly-L-lysine coated
Heparin coated	Dextran sulfate coated

*Unless otherwise noted, 6-μm beads were used.



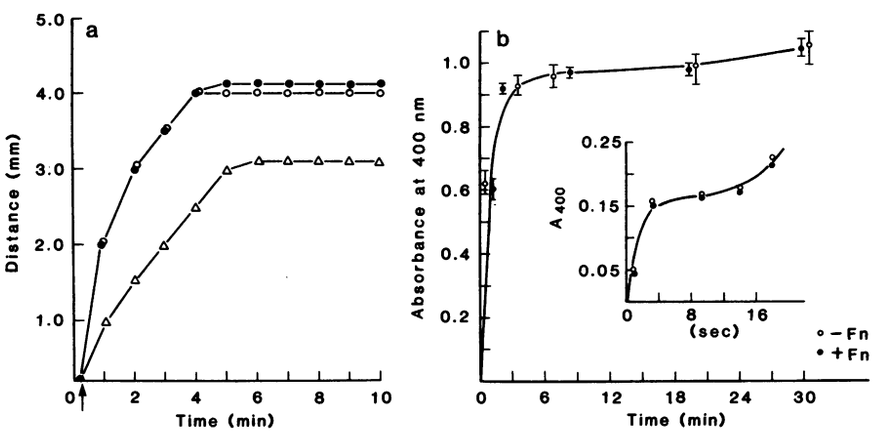


Fig. 3 (left). Selective translocation of inert particles into human plasma fibronectin–containing gels (12.5 μg/ml). Polystyrene latex beads were sonicated for 15 seconds and suspended in a primary gel (legend, Fig. 1) in place of cells. (a) Primary gel contained 6-μm beads, secondary gel contained medium; (b) primary gel contained 6-μm beads, secondary gel contained fibronectin; (c) primary gel contained 0.2-μm beads, secondary gel contained fibronectin; (d) primary gel contained poly-L-lysine–coated 6-μm beads, secondary gel contained fibronectin. Scale bar, 2 mm. Fig. 4 (right). Relationship between matrix-driven translocation and collagen fibrillogenesis. (a) Distance translocated from the original position of a primary-secondary gel boundary was measured at the tip of the moving bead front in three preparations similar to that in Fig. 3b. Preparations poured at the same time, such as those indicated by the open and filled circles, usually have the same translocation kinetics. Open triangles indicate gels that were poured several minutes later than gels used for experiments denoted by circles. About 15 such kinetic measurements were made, and in each case the pattern of translocation became stabilized after 5 to 10 minutes when the collagen concentration was optimized for rapid translocation (about 1.7 mg/ml). Arrow indicates the time of initiation of translocation. (b) Neutralized collagen mixtures at physiological ionic strength, containing or lacking human plasma fibronectin (12.5 μg/ml) were prepared as in Fig. 4A. Mixtures were placed in quartz cuvettes, and absorbance at 400 nm was monitored in a Zeiss PM-6 spectrophotometer (*37*). There were no significant differences in rate or extent of collagen fibrillogenesis in the presence or absence of fibronectin between three pairs of preparations assayed over 30 minutes or two pairs of preparations assayed over 20 seconds (inset).

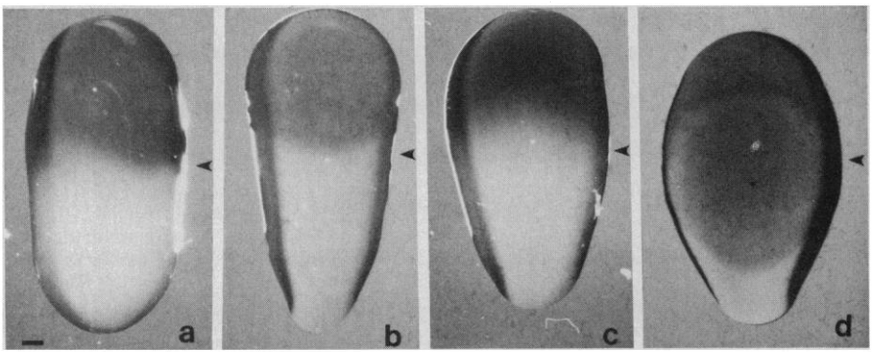


Fig. 5. Organic stains are not translocated by extracellular matrices in the absence of translocatable particles. Eosine Y (a), alizarin (b), or methylene blue (c) were mixed with primary gels lacking cells or beads, and formed contiguous with secondary gels containing human plasma fibronectin (12.5 μg/ml). Photographs shown were taken after 20 minutes. In (e) a primary gel containing alizarin also contained 6-μm polystyrene latex beads. Arrowheads indicate original position of primary-secondary gel interface. Scale bar, 1 mm.

of charged particles in a heterogeneous polymeric environment (*19*), an otherwise conceivable mechanism in light of potential differences in collagen fibril organization across the primary-secondary gel boundary. A passive electrostatic mechanism of this sort would promote the redistribution of the neutral or anionic dyes, as well as the 0.2-μm polystyrene latex particles (which have a larger surface to mass ratio than the translocatable 6-μm particles of identical composition). It appears that the active process promoting movement of cells or beads and their associated matrices across the boundary depends on several factors, including native type I collagen fibrillogenesis, a disparity in an appropriate fibronectin at the boundary, and the presence on the fibronectin-free side of particles of appropriate size and surface characteristics (*13*).

The unidirectionality of cell and particle translocation in our system appears to be due to the vectorial nature of the fibronectin concentration disparity at the gel interface. The penetration of the particle-containing matrix into the fibronectin-containing matrix (Fig. 5d) suggests that unidirectional translocation could result from nonequilibrium processes similar to those described for other polymeric systems (*20*). It is possible that interaction of appropriate cells or particles with the interposed matrices results in their mutual conveyance across the boundary by the formation of similar "dissipative structures" (*21*) in the system described here (*22*).

The process that we have called matrix-driven translocation is capable of translocating living cells and nonliving particles at high velocities; the effects described here could potentially result in major changes in cellular patterns in embryos within minutes. Because different types of cells respond selectively in an all-or-none fashion to matrices containing different types of fibronectin, this effect appears to be a biophysical process with biological discriminatory power.

We do not know whether matrix-driven translocation plays a role in vivo or is simply a novel chemimechanical property of biological macromolecules. However, developing and adult organisms could exploit this process for cellular translocation by the construction of matrices of appropriate composition. The oriented translocation of cells during embryogenesis (*23–28*), wound healing (*29*), tumor invasion (*30*), and inflammation (*31*) is poorly understood, and may, in part, involve matrix-driven effects. Primary mesenchyme (*23*), mesenchyme of the area vasculosa (*32*), primordial germ cells (*23*), neural crest cells (*32, 33*), and the invading mesenchymal cells of the primary stroma of the developing cornea (*27, 28*) migrate through fibronectin-rich matrices during embryogenesis. It has been proposed that these cells move under their own power (*2*) by "contact guidance" (*34*) on extracellular matrix tracks. However, these processes could also involve matrix-driven translocation. This is particularly compatible with cases such as the precartilage mesenchymal condensations of the developing vertebrate limb (*26, 35, 36*) in which fibronectin-rich matrices also contain type I collagen (*38*).

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17. Primary gels consisted of matrices containing either chick limb precartilage mesenchymal cells or 6-μm polystyrene latex beads. Secondary gels consisted of the same matrices containing human plasma fibronectin (12.5 to 50 μg/ml) in medium, or medium alone. Type I collagen was gelled by simultaneously adjusting *p*H and salt to physiological levels as in the legend to Fig. 1 (native), adjusting only the *p*H (low salt), or adjusting only the salt (low *p*H). For the type I and type II collagen mixture, 50 μl of type II collagen (approximately 1 mg/ml) in 1/10 strength medium was added to 0.25 ml of type I collagen (5 mg/ml) in 1/10 strength medium. Low melting point agarose (Réactifs, IBF, France; 0.8 percent or 0.4 percent in medium) was melted at 70°C, cooled to 50°C, and mixed with an equal volume of cells or fibronectin in medium. Beads clumped if placed directly in agarose. Methylcellulose (0.25 ml, 2.1 percent) was mixed with 0.17 ml of 3X medium, and 0.08 ml of cells or beads in medium or 0.08 ml of fibronectin in medium. Fibronectin-dependent translocation was considered to have occurred if the cells or particles had moved farther into a secondary gel containing fibronectin after 1 hour or after 1 day than into a corresponding secondary gel lacking it. Ten or more replicates of each experiment were performed with no variations in qualitative outcome.
18. Our method of preparing type I collagen (*7, 8*) does not exclude the possible presence of noncollagenous acetic acid–soluble nondialyzable components of the rat tail tendon, which could have contributed to the formation of the translocating matrices.
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22. Macroscopic disturbances do not occur at the boundary of the interposed collagen matrices in the absence of translocatable cells or particles (Fig. 5). Nevertheless, local effects at the boundary, such as the diffusion of fibronectin into the primary gel, could trigger macroscopic organized behavior by a "reactive" component, such as cells or beads capable of binding fibronectin. The mean square distance traveled by a diffusible substance, such as the fibronectin dimer, obeying Fick's law, is $\overline{\Delta X^2} = 2DT$. A reasonable estimate for the diffusion coefficient (D) of fibronectin is 2×10^{-7} cm^2/sec (*35*). This was also confirmed by M. Rocco and L. Zardi. *J. Cell Biol.* **97**, 324 (Abstr.) (1983). Therefore, during the time (T) of 10 seconds, after which translocation begins, fibronectin molecules could have diffused an average of 2×10^{-3} cm (one cell diameter) into the primary gel. The time course of translocation initiation (Fig. 4a) is therefore consistent with a reaction-diffusion process involving fibronectin (*35*).
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39. We thank K. M. Yamada and S. K. Akiyama (NCI, NIH) for gifts of fibronectins, T. Linsenmayer for the type II collagen, A. Springer for advice with photography and for discussions, and H. Godfrey, R. Melamede, and W. Ross for suggestions. Supported in part by a grant from the New York Medical College Intramural Research Program, and NIH by grant HD18148.

2 July 1984; accepted 14 February 1985

High-Affinity Uptake of Serotonin into Immunocytochemically Identified Astrocytes

Abstract. *Primary cultures of astrocytes from neonatal rat brain were incubated with tritiated serotonin. After fixation they were stained by immunofluorescence for the astrocyte-specific marker glial fibrillary acidic protein and processed for autoradiography. Silver grain density was increased over cells positive for glial fibrillary acidic protein and was reduced to background levels when sodium was omitted from the medium or the specific inhibitors of serotonin uptake fluoxetine and chlorimipramine were present. The results indicate that mammalian astrocytes can take up serotonin by a sodium-dependent, high-affinity system previously thought to be the exclusive property of serotonergic nerve endings.*

The action of most neurotransmitters released in the central nervous system is thought to be terminated mainly through reuptake by nerve endings (*1*). However, many synapses are surrounded by astrocytic processes (*2*), which could also serve as sites of uptake. Indeed, astrocytes have active systems for taking up amino acid transmitters. Many of the studies showing this were done with cultured glial cells (*3*), but uptake of exogenous labeled amino acids by astrocytes

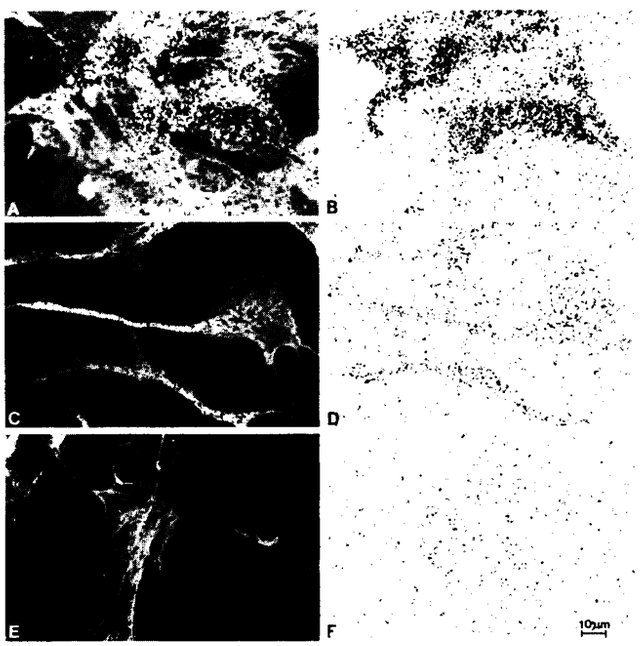

Fig. 1. Glial fibrillary acidic protein immunofluorescence (A, C, and E) and bright-field autoradiography of [^{3}H]serotonin uptake (B, D, and F) in the presence of Na^+ (A to D) and in medium in which Na^+ was replaced by choline (E and F). The cells in (A) and (B) lack processes and those in (C) and (D) are process-bearing. Primary astrocyte cultures (5 weeks in culture) were prepared from the cerebral cortices of newborn rat pups after removal of the meninges (*19*). The cells were grown on pairs of glass cover slips in 60-mm plastic dishes (Corning) in Eagle's basal medium with 10 percent fetal calf serum, supplemental vitamins and amino acids, and penicillin and streptomycin (*19*) and were used within 6 weeks. For combined autoradiography and immunocytochemistry the growth medium was removed and the cells were washed three times in buffered medium containing the following (in millimoles per liter): Na^+ (145), K^+ (4.5), Mg^{2+} (0.4), Ca^{2+} (1.3), Cl^- (127), SO_4^{2-} (0.4); HCO_3^- (25), and glucose (10) (*p*H 7.4). The cells were first incubated for 20 minutes in the same medium with $10^{-4}M$ pargyline and $10^{-5}M$ ascorbate with additions or changes as indicated in a 5 percent CO_2 and 95 percent air atmosphere at 37°C. The cells were then incubated in 2 ml of the medium containing 0.3 μM [^{3}H]serotonin (specific activity, 9 to 23 Ci/mmol; Amersham) for a further 30 minutes. To stop uptake the cells were rapidly washed seven times with 2 ml of ice-cold phosphate-buffered saline (PBS). The cells were then fixed in 4 percent paraformaldehyde and 0.25 percent glutaraldehyde in 0.1M cacodylate buffer for 15 minutes at 0°C. The cover slips in the dish were then rinsed five times with 2 ml of ice-cold PBS to remove the fixative, put into acetone at −10°C for 3 minutes to render their plasma membranes permeable to antibodies, and rinsed twice in PBS. They were then incubated for 30 minutes with a 1:200 dilution of monoclonal mouse antibody (immunoglobulin G) to human GFAP (Amersham), rinsed three times in PBS, incubated for 30 minutes with a 1:20 dilution of a rhodamine-labeled rabbit antibody to mouse immunoglobulin G, and rinsed three times in PBS. The cover slips were mounted with Fluoromount on microscope slides (cells facing up) and air-dried at 4°C overnight. The slides were dipped in Kodak NTB2 at 41°C, air-dried for 1 hour, and left at 4°C for 11 days. Then the slides were developed in Dektol (1:2 dilution) for 2 minutes, placed in 1 percent acetic acid for 30 seconds, fixed in Kodak fixer for 4 minutes, and finally rinsed in three water baths for 5 minutes each. The cells were viewed with a Nikon Labophot microscope with a ×50 oil-immersion lens, a xenon 75-W light source, and appropriate filters for rhodamine epifluorescence. Controls in which antibody to GFAP was absorbed with purified bovine GFAP showed no detectable fluorescence.