1C shows examples of splenic colonies. Strikingly similar linear relations between the numbers of EM cells injected and the CFU's observed were noted, Fig. F. In the third study, the "f" factor (*15*) was found to be 0.36 and the CFU per EM cell injected was 0.223, suggesting that the majority [(0.233/0.36) = 0.62] of the EM cells must be CFU's. Since only the UEMC were sufficiently numerous (up to 80 percent) to account for the number of colonies observed and because their function has not been established, they must be considered the prime candidate stem cells.

Tissue sections of splenic colonies showed many foci of developing megakaryocytes and immature cells, Fig. 1E. The presence of Barr bodies observed in the nuclei of a large fraction of the latter cells indicate that the CFU's were derived from cells of female animals (*16*). Since the recipient mice were all males, this observation would be tantamount to direct evidence that these colonies were developed from exogenous stem cells obtained from the donor animal, a female rat.

Thus, although the exact form or morphology of the hematopoietic stem cell remains to be described, it is possible that the majority of the EM cells, that is, the UEMC, are CFU's. If true, the present technique for the separation of the EM and RM may prove useful in the study of the hematopoietic stem cell under normal as well as pathological conditions.

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11. Up to 50 percent of the calculated blood volume was removed from each rat by way of the jugular vein [J. K. Gong, C. A. Glomski, N. L. Frederiksen, A. J. Lawson, J. P. Daley, *Radiat. Res.* **65**, 83 (1976)].
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Myosin: Immunofluorescent Localization in Neuronal and Glial Cultures

Abstract. *The distribution of intracellular myosin was studied by the double antibody immunofluorescence method in primary organotypic neuronal cultures and two established neuronal and glial cell lines. An array of parallel filaments aligned with the major cellular axis and a three-dimensional subsurface network were shown to react with two different myosin antibodies. The presence of myosin-rich filaments in regions known to contain actin filaments suggests that these proteins interact to generate the motive force in nonmuscle contractile systems.*

Most theories concerning the mechanisms responsible for cellular and intracellular movement involve fibrous systems. At present, two major fibrous proteins, myosin and actin, have been implicated in cell motility. Discrete filaments of cytoplasmic actin have been isolated and shown to bind heavy meromyosin, and have been localized by immunofluorescence studies (*1*). Myosin from nonmuscle cells has been isolated and identified biochemically but its intracellular disposition and localization has not been determined (*2*). The role of these two contractile proteins in skeletal muscle has formed the basis for the general assumption that they participate in axoplasmic transport. In order to confirm and clarify the role of actin and myosin in neuronal tissues, we undertook the present study.

Since cultured neuronal tissue exhibits a high level of intracellular movement (axoplasmic flow) as well as considerable morphogenetic movement (neurite extension and radial glial migration), it serves as an excellent model for the study and localization of elements essential to motility (*3*). We studied the intracellular distribution of cytoplasmic myosin in primary cultures of chick sensory ganglia and established neuronal and glial cell lines.

Myosin from chick gizzard smooth muscle is biochemically similar to myosin isolated from embryonic chick brain (*4*). We therefore used an antibody prepared against highly purified chick gizzard myosin in a double antibody immunofluorescence study to localize myosin in cultured neuronal tissues. All experiments were repeated with an antibody prepared against human uterine smooth muscle myosin to assess further the intracellular distribution of myosin in neuronal and glial cells. Both antibodies, prepared in the laboratory of Gröschel-Stewart (*5*), have been shown to react solely with myosin and not with other contractile proteins (*6*).

Primary organotypic cultures of embryonic chick dorsal root ganglia and monolayers of murine neuroblastoma (S20) and rat Schwann cells (RN22) were grown on collagen-coated or uncoated glass cover slips either in closed Maximow chambers or in open petri dishes incubated in a humidified atmosphere of 5 percent CO_2 at 35°C (*7*). Cultures were rinsed free of media with three changes of phosphate-buffered saline (PBS), *p*H 7.6, fixed in 2 percent paraformaldehyde in PBS and air-dried after changes in graded acetone. In one series of experiments, cultures were treated with a detergent solution (0.1 percent Triton X-100 in PBS) prior to fixation to facilitate the penetration of the antibodies (*8*). Air-dried specimens were incubated for 1 hour at 37°C in moist chambers with the antibody against purified chick gizzard myosin or human uterine myosin. Control cultures were incubated with nonimmune rabbit serums and nonimmune

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rabbit immunoglobulin G. Working solutions of the antibodies (2 mg of protein per milliliter) were preabsorbed with chicken liver powder and washed human erythrocytes. After being thoroughly rinsed in PBS, all cultures were exposed to a goat antiserum to rabbit immunoglobulin G labeled with fluorescein isothiocyanate (Behring) at a 1:10 dilution in PBS for 1 hour at 37°C in moist chambers. Cover slips were mounted with glycine-glycerol buffer at *p*H 8.6 and the cultures were examined with a Zeiss Universal microscope equipped with an FITC filter package and epifluorescence and phase-contrast optics.

In both primary and established neuronal and glial cell cultures, myosin-rich fibrous elements exhibit an intense immunofluorescence which remains prominent after the cells are washed with Triton. This, and the pattern of immunofluorescence, shows that the myosin molecules are bound to detergent-resistant membranous structures. Some of these elements appear as parallel arrays of fibers aligned with the major cellular axis (Figs. 1 to 3). Higher magnification reveals an intermittent periodic fluorescence along many of the parallel fibers (Fig. 2) which may suggest regional aggregates of myosin (*9*). The occurrence of similar "interruptions" along fluorescence-labeled myosin filaments in cultured smooth muscle has been reported (*10*). We have also found a fibrillar network which binds specifically with myosin antibodies in primary cultures of chick glial cells and RN22 rat Schwann cells. This network occupies a major portion of the cytoplasm (Fig. 1, B and D, and Fig. 2). It is not limited to the cell surface but forms a three-dimensional organizational structure visible over a considerable depth of focus. A diffuse fluorescence is evident in the perinuclear region of glial cells (Fig. 1, B and D) as well as in neurites and growth cones of sensory ganglia (Fig. 3B). Within neuroblastoma cells, diffuse fluorescence is apparent in the soma and along fine cytoplasmic processes (Fig. 3D). The perinuclear region of these cells cannot be observed readily because of the overall intense reaction. The diffuse pattern demonstrated in neuroblastoma may well reflect the intrinsic mobility of this oncogenic cell type; stationary cells have been shown to exhibit a specific fibrillar arrangement (*11*). The diffuse and fibrillar fluorescence was specific for myosin since fluorescence was not observed in any of the controls. Furthermore, the fluorescence was abolished when the cultures were treated with solutions of potassium chloride at concentrations sufficient to extract myosin (*12*).

The distribution of myosin-rich structural elements shown here is similar to

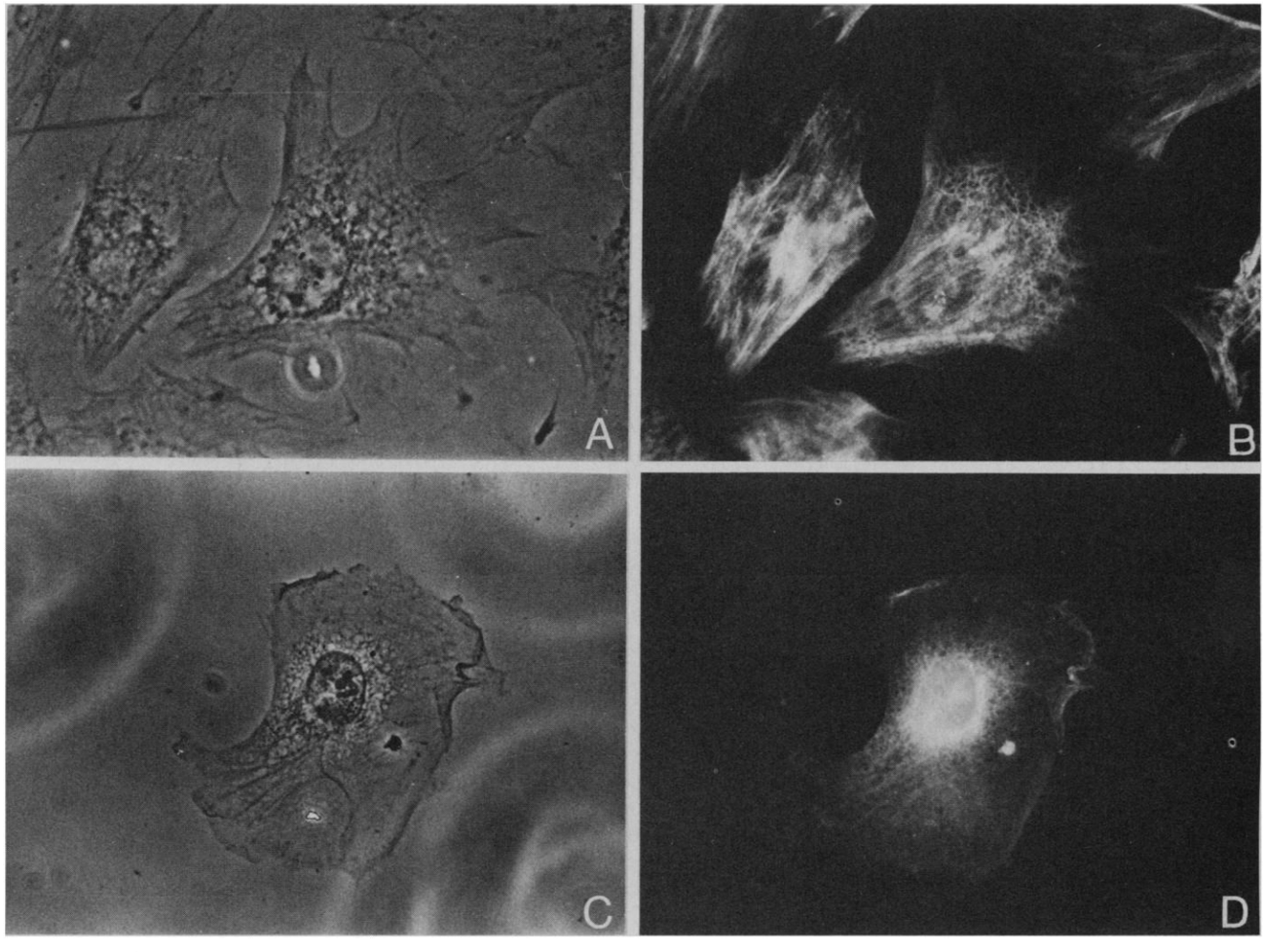
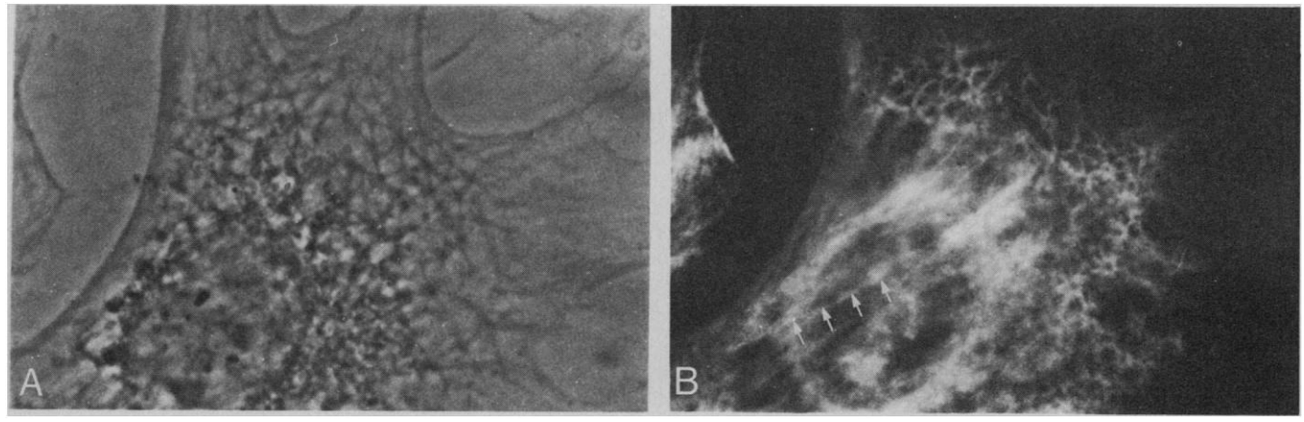


Fig. 1. Photomicrographs of cells grown in vitro on collagen-coated cover slips for 72 hours and then exposed to antibody to gizzard smooth muscle myosin for 1 hour followed by 1 hour of exposure to fluorescein isothiocyanate–labeled goat antibody to rabbit immunoglobulin G. (A and B) Dorsal root ganglia (treated with Triton X-100 to facilitate penetration of the antibodies) showing the presence of a myosin-rich filamentous network in primary nonneuronal cells as viewed with phase-contrast and epifluorescence optics, respectively (×1200). (C and D) A similar myosin-rich network in an established glial cell line (RN22) examined with phase-contrast and epifluorescence optics, respectively (×1200).

that of actin filaments as shown with immunofluorescence techniques and binding with heavy meromyosin (*13*). The myosin-rich structures seen in the present study are similar to a three-dimensional network composed of foci which bind the antibodies to actin and α-actinin and which are interconnected by tropomyosin-associated filaments (*14*).



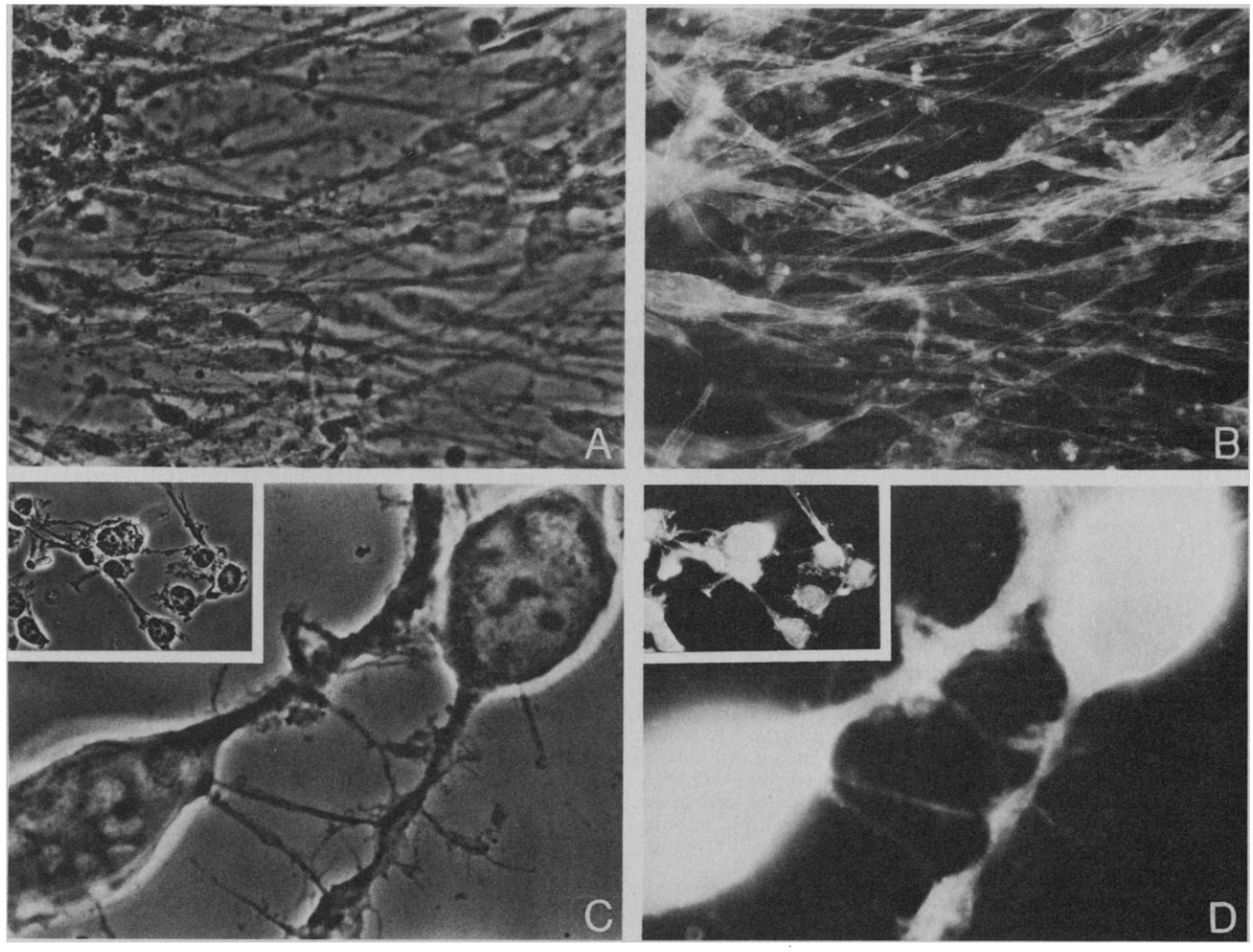


Fig. 2 (top). The same cultures as in Fig. 1, A and B, shown at higher magnifications to illustrate the details of the myosin-rich filamentous network in this primary glial cell. The arrows point to the intermittent periodic fluorescence found along many of the parallel fibers. Phase-contrast (A) and fluorescence (B) optics (×2600). Fig. 3 (bottom). Photomicrographs of cells grown in vitro on collagen-coated cover slips for 72 hours and (A and B) exposed to Triton X-100 followed by antibody to uterine smooth muscle myosin or (C and D) exposed to antibody to gizzard smooth muscle myosin for 1 hour. After antibody treatment all cultures were exposed to fluorescein isothiocyanate-labeled goat antibody to rabbit immunoglobulin G for 1 hour. (A and B) Fluorescence-labeled myosin antibody in neuronal elements of primary cultures of sensory ganglia; phase-contrast and epifluorescence optics, respectively (×1200). (C and D) Positive fluorescence in an established neuroblastoma line (S20); phase-contrast and epifluorescence optics, respectively (×3000). The widespread pattern of general fluorescence is further illustrated by the insets (×500).

The organizational similarities between the myosin-rich structures that we have found and those reported for actin is not surprising, since by analogy to skeletal muscle actin and myosin interact to produce a motive force. The widespread intracellular distribution of these proteins observed in the present study is probably necessary if they are to provide a force-generating system for intracellular and morphogenetic movements. The observed binding of myosin antibodies from human uterine muscle and chick gizzard muscle with avian and mammalian tissues suggests a conservative structure for the myosin molecule. The structural basis for the interaction of myosin and actin in neuronal and glial cells and the role of these proteins in axoplasmic flow and neurite elongation are yet to be determined. The ultrastructural localization of myosin and actin in neuronal tissues and their association with other cytoskeletal elements, such as microtubules and microfilaments, are topics for further study.

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Genetic Mapping of Xenotropic Leukemia Virus-Inducing Loci in Two Mouse Strains

Abstract. *In genetic studies of C57BL/10 and BALB/c mice, inducibility of xenotropic murine leukemia virus from tissue cultures by treatment with 5-iododeoxyuridine shows single gene segregation ratios. In both strains, the virus-inducing loci are on chromosome 1, linked to the* Dip-1 *isozyme locus, but the two may not be at allelic sites.*

Xenotropic murine leukemia viruses (MuLV's) are strains that are unable to initiate exogenous infection of mouse cells but can infect cell cultures of a number of heterologous species (*1*). They appear to constitute a single class with respect to cross-reactivity in interference and virus neutralization tests, but there is much diversity within the group (*2, 3*).

While the ecotropic (mouse-tropic) MuLV's are present as chromosomally integrated viral genomes in some inbred mouse strains and not in others (*4*), xenotropic virus genomes are probably present in all mice. This has been shown in several ways. First, xenotropic virus strains have been isolated from almost all inbred mouse strains tested, although with markedly different facility. In some mouse strains, particularly NZB, xenotropic virus is continually produced both in vivo and in embryo tissue cultures (*1, 2, 5, 6*); in other mice, such as BALB/c and C57BL, the virus can be detected at low levels in vivo and can be induced in tissue culture by treatment with 5-iododeoxyuridine (IdU) (*1, 2, 7*); in still other strains of mice, such as NIH Swiss, the virus is rarely detected in vivo and is not inducible in vitro. Second, nucleic acid homology tests with labeled DNA probes prepared from two different xenotropic MuLV strains have shown that the complete nucleotide sequences of both viruses are present in the cellular DNA of all mice tested (*8*), including the noninducible NIH Swiss. Third, MuLV glycoprotein (gp70) molecules with a tryptic digest pattern similar to that of the NZB xenotropic MuLV are found in the serum of virtually all mouse strains tested (*3*).

The marked differences in virus expression among mouse strains permit classical Mendelian segregation analysis. Genetic studies have been based on crosses between strains negative for virus expression (generally NIH Swiss) and strains with spontaneous or inducible xenotropic virus expression. When embryo cultures of backcross segregants from BALB/c were tested for IdU-inducible virus, single gene segregation ratios were observed (*7*). Similarly, the data presented below on virus induction from tail tissue cultures indicated single gene control in B10.BR, C57BL/10, and BALB/c strains. In contrast, the segregation ratios reported in crosses with NZB mice varied with the system under study. A single gene was found to regulate spontaneous release of virus by embryo cultures (*5*), while infectious center tests of adult spleen cells revealed two (or possibly three) loci that can independently give the positive phenotype (*6, 9*).

Table 1. Segregation of xenotropic MuLV induction with *Dip-1* isozyme. Mouse strains B10.BR/SgLi, C57BL/10J, and BALB/cN, which are *Dip-1*^{a} and positive for xenotropic virus induction, were mated to A/J or NFS/N mice (an inbred line derived from NIH Swiss mice); both of these are *Dip-1*^{b} and xenotropic virus induction-negative. The F_1 hybrids were crossed to A/J or NFS/N mice (or both). Tissue cultures were prepared from tail biopsy tissue (*13*) and induced with IdU (20 μg/ml for 48 hours) when the cultures were in vigorous, subconfluent condition. Cells of the mink lung cell line CCL64 (*14*) were then added. Two weeks later, fluids from the mixed cultures were harvested, frozen, and thawed; they were then tested for the presence of xenotropic virus by the focus induction test in the mink S^+L^- cell line of Peebles (*15*). *Dip-1* typing was done on kidney extract or erythrocyte hemolyzate by standard procedures (*10*).

Cross from	Number of mice				Recombinants/ total	$r \pm$ S.E.*	
	Nonrecombinant phenotypes		Recombinant phenotypes				
	Dip-1^{ab}/ X-tr$^+$	*Dip-1*^{b}/ X-tr$^-$	*Dip-1*^{ab}/ X-tr$^-$	*Dip-1*^{b}/ X-tr$^+$			
B10.BR	60	72	22	14	36/168	21 ± 3	23 ± 3
C57BL/10	19	17	7	8	15/51	29 ± 6	
BALB/c	29	28	6	1	7/64	11 ± 4	

*Recombination frequency (%) ± standard error.

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