duction show a variability of less than 20 percent from preparation to preparation if the age of the animal and the time from isolation to measurement of metabolic activity are kept constant.

We believe that this is the first procedure described for the isolation of a pure preparation of brain microvessels demonstrating normal metabolic capacity. Metabolic investigations of brain vessels have previously been restricted to demonstrations of enzymic activity by histochemical staining techniques (*7*). In view of the importance of the microcirculation in normal body physiology and in the pathology of several important diseases, the availability of a readily obtained, pure, metabolically active preparation of microvessels should permit this component of the vascular system to be investigated as extensively as the larger vessels. The central nervous system origin of these vessels may also permit the unique structural and metabolic features comprising the blood-brain barrier to be further elucidated (*8*).

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MIF-Like Activity in Simian Virus 40–Transformed 3T3 Fibroblast Cultures

Abstract. *Simian virus 40–transformed fibroblasts (SV3T3), as compared with their untransformed counterparts (3T3), elaborate a macromolecular product that inhibits macrophage migration and causes macrophages to aggregate and lose one type of cell coat material. The SV3T3 cells also lack this surface material relative to 3T3 cells. There may be a relation between migration inhibition factor (MIF), the cell coat, and cell migration.*

Normal macrophages, migrating from capillary tubes in culture, form a monolayer of evenly dispersed, well-separated cells with few intercellular contacts; moreover, their surfaces are coated with abundant deposits of a dense-staining material (DSM) that has recently been recognized in the electron microscope with a new osmium tetroxide–potassium ferrocyanide technique (*1*). Macrophage behavior is significantly altered by migration inhibition factor (MIF), one of a group of macromolecular mediators secreted by sensitized lymphocytes in response to specific antigen, or by normal lymphocytes exposed to certain plant lectins (*2*). Macrophages cultured with MIF-producing lymphocytes migrate only a short distance and form aggregates (*2*); they also undergo a striking loss of DSM (*1*).

There is some evidence that macrophage migration from capillary tubes, and its inhibition by MIF, has relevance to the behavior of other cell types in culture and to the more general biologic phenomenon of contact inhibition (*3*). Thus, a number of cultured lymphoid and nonlymphoid cell lines release mediators whose actions mimic those of MIF and of other lymphokines (*4*). Moreover, contact-inhibited 3T3 fibroblasts have abundant deposits of DSM-like material on their surfaces; by contrast, simian virus 40 (SV40)–transformed fibroblasts, which have lost contact inhibition, can form multilayered cell aggregates and lack dense-staining surface material (*5*). These considerations led us to predict that SV40-transformed fibroblasts might secrete large amounts of an MIF-like activity relative to untransformed cells.

To test this hypothesis cultures of BALB/c 3T3 (clone A31) (*6*), BALB/c SV3T3 (clone T2) (*7*), Swiss 3T3 and Swiss SV3T3 (*6, 8*), a concanavalin A–selected revertant line of SVT2 cells (*9*), and secondary NIH Swiss mouse embryo cells were plated in 10 ml of Eagle's minimal essential medium containing four times the usual amount of essential amino acids and vitamins (MEM × 4), 10 percent fetal bovine serum (FBS) or guinea pig serum (GPS), penicillin, and streptomycin. Cells were seeded at 1×10^6 (for log phase cultures) or 3×10^6 cells per Falcon petri dish, 100 mm in diameter (for confluent cultures), and supernatants for MIF assays were harvested 48 to 96 hours later. The growth me-

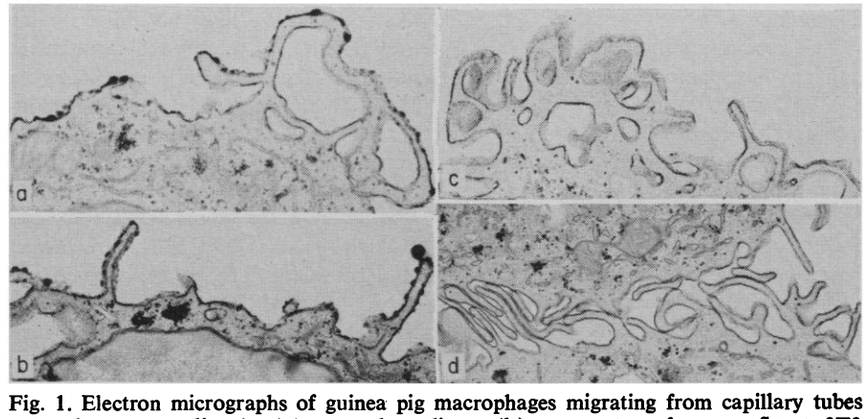

Fig. 1. Electron micrographs of guinea pig macrophages migrating from capillary tubes onto glass cover slips in (a) control medium; (b) supernatant from confluent 3T3 culture; (c) and (d) supernatant from confluent SV3T3 culture. All media contain 15 percent GPS. Abundant, dense-staining cell coat material, comparable to that observed on 3T3 cells, is observed in (a) and (b) but is absent in (c) and (d). Moreover, macrophages grown in SV3T3 culture supernatant form cellular aggregates characterized by complex interdigitations of surface folds and villi (d). Processing by the osmium tetroxide–potassium ferrocyanide technique (*1*). Magnification: (a) and (c), × 8300; (b) × 7000; and (d) × 6200.

Table 1. Macrophage migration inhibition by fibroblast culture supernatants. Culture media from each cell type were tested for MIF-like activity (*10*). Data are expressed as percentage of inhibition of macrophage migration ± standard error as compared with an appropriate control. Numbers in parentheses refer to the number of experiments. Inhibition greater than 20 percent is considered significant (*2, 10*). N.T., not tested.

Strain	Growth status (with 10% FBS)		Growth status (with 10% GPS)	
	Confluent	Log phase	Confluent	Log phase
BALB/c SV3T3	70 ± 12.5 (11)	51 ± 22.0 (3)	67 ± 12.3 (5)	N.T.
BALB/c 3T3	9 ± 7.7 (11)	16 ± 13.1 (3)	18 ± 16.0 (4)	N.T.
BALB/c SV3T3 revertant	43 ± 8.7 (2)	52 ± 3.8 (2)	N.T.	N.T.
Swiss SV3T3	49 ± 3.6 (2)	42 ± 1.3 (2)	N.T.	N.T.
Swiss 3T3	28 ± 5.1 (2)	16.2 ± 12.4 (2)	N.T.	N.T.
Secondary Swiss mouse embryo	7.0 ± 5.0 (2)	2.5 ± 1.8 (2)	N.T.	N.T.

dium was always changed 24 hours before collection of media for MIF assay. Cell culture supernatants were centrifuged (10,000*g* for 20 minutes) to remove any suspended cells, supplemented with 5 percent fresh GPS, and assayed in the standard capillary tube migration inhibition test (*10*) for MIF-like activity. The areas of migration of guinea pig peritoneal exudate cells in the various cell culture supernatants were measured by planimetry and compared with migration areas in the presence of appropriate control media. Control media were prepared by incubating portions of the same batch of medium used for fibroblast cultures in petri dishes without cells for 24 hours at 37°C. Control media used for MIF assay contained either 10 percent FBS and 5 percent GPS or 15 percent GPS, depending upon whether the fibroblasts were grown in FBS or GPS. After planimetry, macrophage viability was routinely assessed by a trypan blue dye exclusion test. Selected cultures were examined in the electron microscope (*1*).

The results summarized in Table 1 indicate that supernatants from BALB/c SV3T3 fibroblasts grown in media containing 10 percent FBS strikingly inhibit the migration of guinea pig macrophages from capillary tubes. By contrast, supernatants from both log phase and confluent BALB/c 3T3 cell cultures caused insignificant (< 20 percent) inhibition of macrophage migration. Concanavalin A–selected revertant SVT2 cells, which have regained the low saturation density characteristic of untransformed cells (*9*), also liberated an MIF-like activity, but macrophage migration was inhibited less than by supernatants from BALB/c SV3T3 cell cultures. Medium from SV40-transformed Swiss 3T3 fibroblasts was also inhibitory to macrophage migration, whereas that from Swiss 3T3 cell cultures produced only borderline inhibition of migration. Finally, normal secondary NIH Swiss mouse embryo cell cultures liberated no significant MIF-like activity into the culture medium. In contrast to Tubergen *et al.* (*4*), we found no obvious relation between production of MIF-like activity and cell division (Table 1). In all instances cell death in macrophage cultures was less than 1 percent.

The SV3T3 cells grew to higher densities (12×10^6 to 16×10^6 cells per 100-mm dish) than 3T3 cells (4×10^6 to 8×10^6 per 100-mm dish), but the differences in inhibitory activity we observed in culture media could not be attributed solely to differences in cell number. Thus, log phase BALB/c SV3T3 cell cultures contained significant amounts of MIF-like activity, whereas no such activity was detected in confluent BALB/c 3T3 cultures containing equal numbers of cells. In addition, confluent BALB/c 3T3 cells cultured for the final 24 hours in 5 ml of medium did not release detectable MIF-like activity. We have not been able to exclude the possibility that sufficient numbers of growing 3T3 cells make an MIF-like material. The minimal number of growing SV3T3 cells required to produce significant (> 20 percent) inhibitory activity was approximately 8×10^6 cells per 10 ml of medium, and at such densities 3T3 cells were invariably confluent. The problem could not be approached by serial dilutions of SV3T3 MIF-like activity because such activity, even from highly inhibitory media derived from confluent SV3T3 cells, was generally lost on twofold dilution. Growing 3T3 cells cultured at higher concentrations (6×10^6 to 7×10^6 cells per 15 ml of medium in 150-mm dishes) occasionally elaborated minimal amounts of an inhibitory activity (20 to 30 percent); however, the significance of such borderline activity is uncertain. Attempts to concentrate culture media from 3T3 cells growing at lower densities were complicated by technical difficulties described below. Even if growing 3T3 cells do elaborate an MIF-like activity, its concentration is ordinarily too low to be measured and hence probably has little physiologic significance for 3T3 cells growing under standard culture conditions.

In confirmation of Fox and Gregory (*11*), we found that fetal calf serum itself contained a factor that inhibited macrophage migration 50 to 60 percent. To avoid this complication, supernatant fluids from BALB/c 3T3 and BALB/c SV3T3 cells, grown to confluence in 10 percent GPS, were tested against a medium control containing only GPS. Similar results were obtained; BALB/c SV3T3 supernatants significantly inhibited macrophage migration, whereas BALB/c 3T3 supernatants did not (Table 1).

Lymphocytes generating MIF cause migrating guinea pig macrophages to form multicellular aggregates characterized by interdigitating villous processes and extensive areas of intercellular contact (*1*). Moreover, extramembranous DSM, abundant on uninhibited macrophages, is greatly reduced or absent on macrophage surfaces in inhibited cultures. A similar reduction in DSM was observed in macrophages whose migration was inhibited by culture in supernatants from confluent BALB/c SV3T3 cells. By contrast, macrophages cultured in supernatants from confluent BALB/c 3T3 cells exhibited abundant DSM with few intercellular contacts and were indistinguishable from those migrating in control media (Fig.1).

Preliminary characterization of the factor in BALB/c SV3T3 culture supernatants responsible for inhibiting macrophage migration revealed that the active principle was nondialyzable (18 hours at 4°C against three changes of 50 volumes of MEM) and stable after heating at 56° or 80°C for 30 minutes. After 20-fold concentration by Diaflo (Amicon, UM2 filters) membrane filtration, the MIF-like activity eluted from a Sephadex G-100 column in the albumin region and immediately beyond, in the same molecular weight range as guinea pig lymphocyte MIF (*12*). Unfortunately, complete culture medium containing 10 percent GPS acquired some capacity to inhibit macrophage migration after exposure to these Diaflo membrane filters. This nonspecific inhibitory activity was considerably less than that of BALB/c

SV3T3 supernatants and was not due to glycerol leached from the membrane filters (*13*), which were exhaustively washed prior to use. However, its presence has so far prevented precise quantitation of MIF-like activity in chromatographed SV3T3 supernatants and determination of whether highly concentrated 3T3 supernatants also possess MIF-like activity.

Productive virus infection of fibroblast cultures has previously been shown to stimulate the elaboration of lymphokine-like substances (*14*). The results reported here with 3T3 and SV3T3 fibroblasts provide the first evidence that cells may be induced to elaborate an MIF-like activity as a consequence of transformation with an oncogenic virus and associate such MIF-like activity with the absence of a newly described form of dense-staining cell coat material. Guinea pig macrophages either treated with SV3T3 MIF-like activity or cultured with lymphocytes generating MIF experience a similar loss of stainable cell coat material and undergo significant changes in their migratory properties and intercellular associations. These findings raise the possibility that the MIF-like activity secreted by SV3T3-transformed fibroblasts may also play a role in the striking alterations in cell contact behavior which accompany viral transformation.

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Barley Stripe Mosaic Virions Associated with Spindle Microtubules

Abstract. *Virions of barley stripe mosaic virus were observed attached to microtubules of the spindle apparatus. This phenomenon was found in barley cells undergoing meiosis and mitosis. The microtubules may be involved in the assembly and cell-to-cell transfer of the virus.*

Examination of ultrathin sections of various cell types of barley (*Hordeum vulgare* L., 'Atlas') which were infected with MI-1 strain of barley stripe mosaic virus (BSMV) revealed an extraordinary relationship between some virions and microtubules of spindles. In microspore mother cells undergoing meiosis (Fig. 1), and in immature cells of the ovule which were undergoing mitosis (Fig. 2), these virions appear to be attached by one end to spindle microtubules. We observed the same phenomenon in rapidly dividing root tip cells and in nondividing cells of other barley tissues (*1*). The number of virions was fewer in root tip cells and cells dividing meiotically than in dividing microspore mother and ovule cells; however, the large number of ribosomes may obscure the actual number of virions that are present. All tissues were prepared for electron microscopy according to the procedure of Mayhew and Carroll (*1*).

The rod-shaped structures in infected tissues were identified as virions of BSMV on the basis of their general morphology and staining characteristics (*2*). The rod-shaped particles were not seen in healthy tissues. The spindle microtubules of dividing barley cells were similar in structure and distribution to those observed in dividing cells of other virus-free plant species (*3*).

The association of viruses with microtubules has been reported for an adenovirus, AV5, infecting HeLa cells

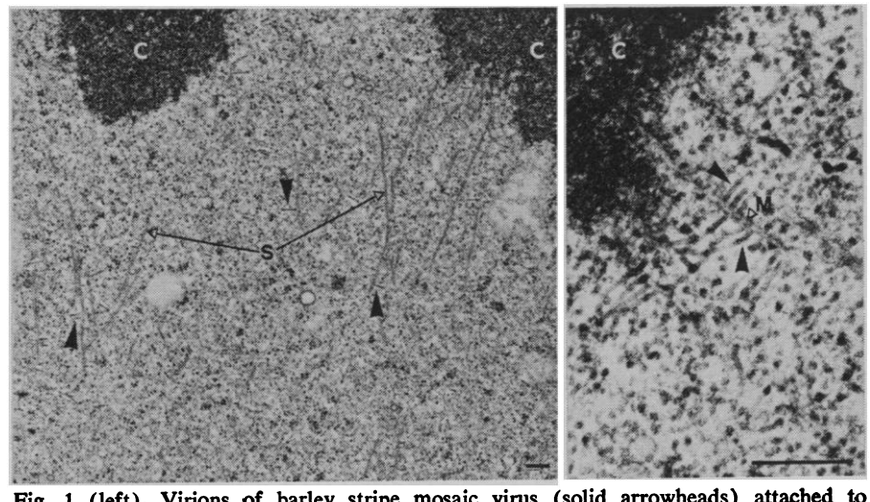

Fig. 1 (left). Virions of barley stripe mosaic virus (solid arrowheads) attached to microtubules of the spindle (*S*). Chromosomes (*C*) are also shown. Scale bar, 0.2 μm. Fig. 2 (right). Virions of barely stripe mosaic virus (solid arrowheads) attached by their ends to a spindle microtubule (*M*) which is connected to a chromosome (*C*). Scale bar, 0.2 μm.