15. Circumsporozoite precipitation (CSP) tests [J. Vanderberg, R. Nussenzweig, H. Most, *Milit. Med.* **134**, 1183 (1969)] were performed as described (*9*). Twenty-five sporozoites were examined and the number with granular precipitates on the surface (2+ reaction) or with a long threadline filament at one end (4+ reaction) determined. The CSP score was the number of sporozoites with 2+ reactions multiplied by 2, plus the number of sporozoites with 4+ reactions multiplied by 4. A score of 8 was considered positive.
16. Inhibition of sporozoite invasion of hepatoma cells (ISI) [M. R. Hollingdale, E. H. Nardin, S. Tharavanij, A. L. Schwartz, R. S. Nussenzweig, *J. Immunol.* **132**, 909 (1984)] was performed as described (*9*). Assays were performed in triplicate at a serum dilution of 1:20. The percent ISI = [100][1 − (number of sporozoites that entered in the presence of test serum) ÷ (number of sporozoites that entered in the presence of control serum)]. The four sera with the highest ISI (Table 2) were serially diluted to determine the last dilution at which sporozoite invasion was inhibited ≥50% (ISI-50). The ISI-50 was >1:300 but <1:1000 in two sera, and <1:300 in the other two.
17. Although monoclonal antibodies to CS protein repeat epitopes react in the four assays, the target antigens for the ELISA (purified protein from the repeat region of the CS protein), IFAT (air-dried sporozoites), and ISI and CSP (live sporozoites) are different, and these assays may measure antibodies to different epitopes. In fact, when sera from all 83 individuals were compared, there was a significant correlation only between the results of the IgG IFAT and IgG ELISA ($r = 0.45$, 95% confidence interval = 0.27–0.61, $P < 0.00001$) and the IgG IFAT and ISI ($r = 0.30$, 95% confidence interval = 0.11 to 0.48, $P = 0.0028$).
18. Concentrations of IgG subclass antibodies to $R32tet_{32}$ were determined as described (*9*). Dilutions of purified myeloma proteins of each of the four subclasses were used to establish standard curves. At the 1:50 serum dilution used in these assays, the minimum detectable concentrations of IgG1, IgG2, IgG3, and IgG4 antibodies to $R32tet_{32}$ were 0.75, 0.95, 0.95, and 1.6 μg/ml, respectively. (Myeloma proteins and antibodies to subclasses were provided by R. Wistar, Jr., and C. Cole.)
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Astrocytes Block Axonal Regeneration in Mammals by Activating the Physiological Stop Pathway

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Regenerating sensory axons in the dorsal roots of adult mammals are stopped at the junction between the root and spinal cord by reactive astrocytes. Do these cells stop axonal elongation by activating the physiological mechanisms that normally operate to stop axons during development, or do they physically obstruct the elongating axons? In order to distinguish these possibilities, the cytology of the axon tips of regenerating axons that were stopped by astrocytes was compared with the axon tips that were physically obstructed at a cul-de-sac produced by ligating a peripheral nerve. The terminals of the physically obstructed axon tips were distended with neurofilaments and other axonally transported structures that had accumulated when the axons stopped elongating. By contrast, neurofilaments did not accumulate in the tips of regenerating axons that were stopped by spinal cord astrocytes at the dorsal root transitional zone. These axo-glial terminals resembled the terminals that axons make on target neurons during normal development. On the basis of these observations, astrocytes appear to stop axons from regenerating in the mammalian spinal cord by activating the physiological stop pathway that is built into the axon and that normally operates when axons form stable terminals on target cells.

IN THE ADULT MAMMALIAN CENTRAL nervous system (CNS), regenerating axons typically grow relatively short distances—less than 1 mm. The restriction of axonal elongation in the adult CNS is not a limitation of the intrinsic capacity of axons to elongate (*1, 2*); but rather, cellular elements of the CNS, particularly astrocytes, appear to restrict the elongation of axons (*3, 4*).

The effects of mature mammalian astrocytes on axonal elongation can be studied at the transitional zone between the dorsal root and the dorsal root entry zone of the spinal cord (*1, 5–8*). By transecting the axons in the dorsal root, it is possible to study their regeneration from the root into the spinal cord without directly injuring the cord. Trauma to the cord results in the formation of a connective tissue scar that contains fibroblasts and a dense collagenous matrix (*1, 4*), which has been shown to prevent axonal elongation (*1, 9*). In the dorsal root regeneration model, there is no connective tissue scar and axonal growth in a purely astroglial environment can be studied.

Regenerating axons in the dorsal root are surrounded by a substrate that contains Schwann cells and their basal laminae. This milieu is similar to that in a peripheral nerve and supports the active elongation of regenerating axons, which grow until they reach the dorsal root transitional zone. At this interface between root and spinal cord, the regenerating axon tips encounter a substrate that consists almost entirely of reactive astrocytic processes. Here, among these processes, many of the growth cones stop and form stationary axon terminals that remain in place for a year or more (*6–8*). These observations suggest that mature astrocytes can stop the intrinsic tendency of axons to elongate through their effects on the axon tip.

By what mechanisms do astrocytes stop axonal elongation? Normally, a physiological sequence is activated in the axon tip when it makes synaptic contact with an appropriate postsynaptic neuron in the CNS (*10–12*). One important part of this physiological pathway is the disassembly of cytoskeletal polymers (neurofilaments and microtubules) that are continuously transported into the stationary axon tip. Specifically, proteases that degrade the neurofilaments are selectively activated in the presynaptic terminal (*11–14*); if these proteases are inhibited by the protease inhibitor leupeptin, neurofilaments accumulate in the axon terminal (*15*).

Axons can also be stopped from elongating without fully activating the physiological pathways that degrade the neurofilaments. If axons are physically obstructed from elongating by being forced to grow into a cul-de-sac that is produced by tightly ligating a peripheral nerve, the trapped axon tips fill with neurofilaments and other axonally transported structures (*13, 16*). This

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indicates that simply blocking the forward progress of the axon is not sufficient to activate the physiological pathway that normally controls the transport of neurofilaments and other transported structures into the axon terminal.

Neurofilament accumulation in the axon tip is an index of the activity of the metabolic mechanisms that remove the cytoskeleton from a stationary axon terminal (*13*). To determine if adult astrocytes activate these metabolic mechanisms when they stop axonal growth or if they physically obstruct regenerating axonal elongation without activating the metabolic mechanisms, we compared the axon tips of regenerating axons stopped by astrocytes at the dorsal root transitional zone with those that were physically obstructed by a ligation neuroma, which is formed by tightly ligating a peripheral nerve or dorsal root. We show that neurofilaments do not accumulate in the stationary axon terminals that contact reactive astrocytes in the dorsal root transitional zone.

Adult rat lumbar (L5) dorsal root axons were completely transected 5 mm from the

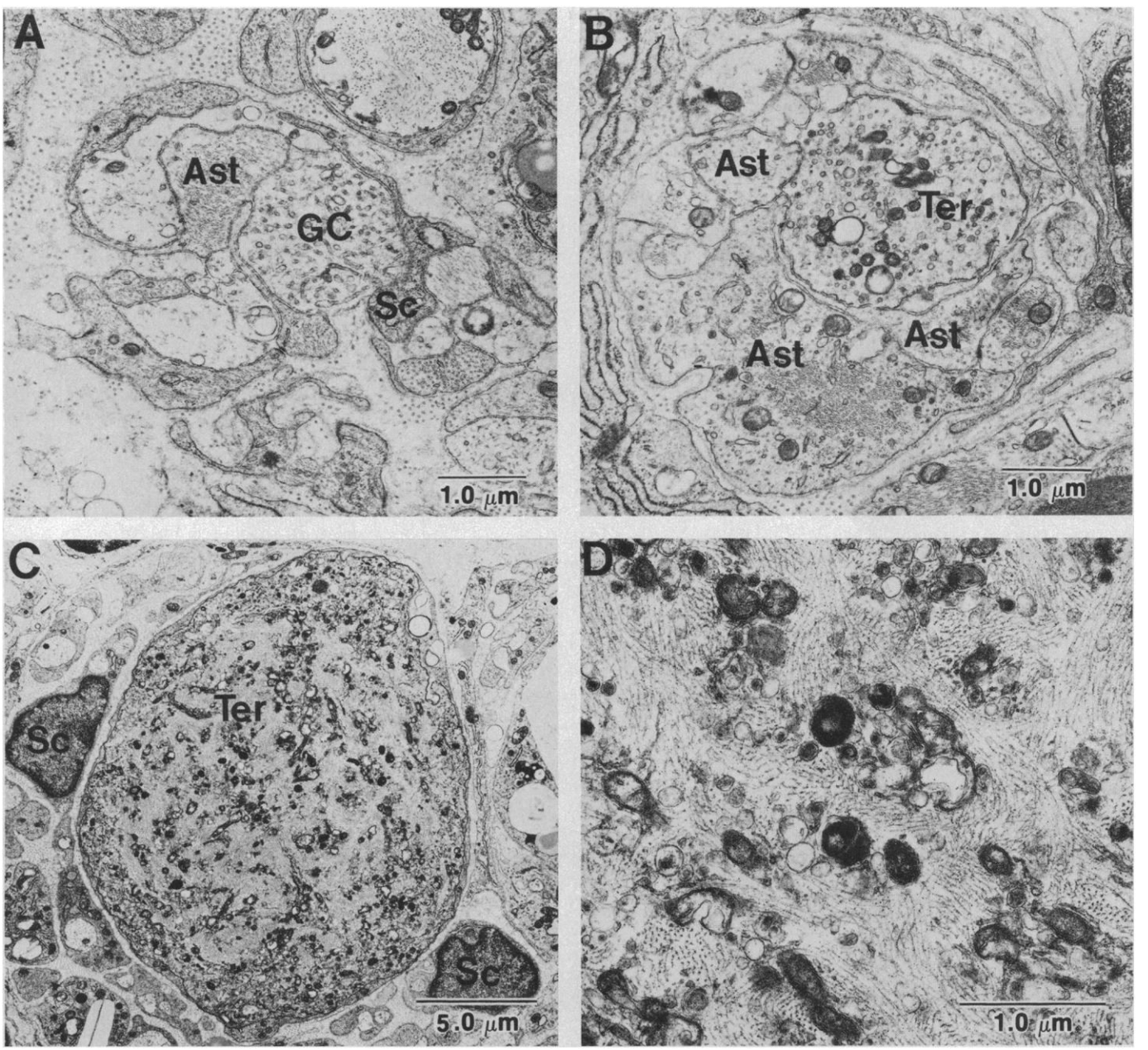

Fig. 1. (**A**) Growth cone (GC) of regenerating dorsal root axon observed in the vicinity of the root transitional zone. This axon tip has the typical features of a growth cone; it has a clear cytoplasm and a well-developed system of tubulovesicular membranes. Note that the growth cone is in close apposition to both astrocytic (Ast) and Schwann cell (Sc) processes. Scale bar, 1.0 μm. (**B**) Typical axon terminal (Ter) in the CNS side of the root transitional zone. This stopped axon tip is characterized by small (35 to 40 nm in diameter) agranular vesicles, occasional dense-cored vesicles, and numerous, small, normal-appearing mitochondria. Notably, the cytoplasm of these terminals contains few, if any, neurofilaments and microtubules. These terminals were completely encapsulated by astrocytic processes, which were easily identified by their numerous intermediate filaments. Scale bar, 1.0 μm. (**C**) A large dorsal root axonal terminal within the dorsal root away from the astrocytes of the root transitional zone. These stopped axonal tips were packed with neurofilaments and membranous organelles and were surrounded by Schwann cells and the connective tissue matrix of the root endoneurium. Moreover, these swollen terminals were the same as those that formed at a cul-de-sac in ligated peripheral nerves and dorsal roots. Scale bar, 5.0 μm. (**D**) High magnification of a part of the terminal in (C). Neurofilament bundles cut in every plane of section are the predominant cytological feature of these terminals. Scale bar, 1.0 μm.

spinal cord by crushing the root with Dumont no. 5 forceps twice in the same place for 10 seconds. After 3 weeks to 3 months, the treated animals were anesthetized and perfused intracardially with a fixative. Ligation neuromas were formed in hypoglossal and saphenous nerves and in dorsal roots by tightly ligating the nerve with 6.0 suture. After 3 to 6 weeks, the animals were anesthetized and perfused with the same fixative. The perfusion solution consisted of 5.0% paraformaldehyde, 2.5% glutaraldehyde, and 7.0% sucrose in 0.1*M* phosphate buffer (*p*H 7.4). The spinal cords were processed by removing the dorsal quadrant of the cord, containing the root entry zone of the crushed L5 root, which was cut into 1-mm-thick slabs. Tissues were then fixed by immersion for 2 hours in the same fixative, washed for 0.5 hour in buffer, and "post-fixed" in 2.0% OsO_4 for 1 hour. After washing in distilled water, the tissue was stained en bloc in 0.5% aqueous uranyl acetate for 3 hours, dehydrated in a graded series of ethanols, and embedded in Maraglas. In 1-μm-thick sections stained with toluidine blue, the dorsal root transitional zone was identified, and thin sections of this region were cut, placed on 200-mesh hexagonal grids, and routinely stained. The grids were examined and photographed on a JEOL 100CX transmission electron microscope.

Light microscopic analyses of horseradish peroxidase–filled dorsal root axons confirmed studies showing that the axons regenerate through the dorsal root into the transitional zone of the spinal cord (*1, 5–7, 16*). At the transitional zone, axons showed a number of reproducible growth patterns: some axons turned around and grew back into the dorsal root. A small number of axons grew through this zone and continued for a few hundred micrometers before stopping in the dorsal horn. Many of the axons stopped at the transitional zone; these axons formed a discrete row of terminals at the junction between the dorsal root and the spinal cord, the root-cord interface.

At 3 weeks after transection, some growth cones were observed in the region of the dorsal root where astrocytic processes project from the spinal cord into the root (Fig. 1A). These growth cones often were apposed to glial processes but were not completely surrounded by astrocytic processes. Axon tips that had penetrated farther into the dorsal root transitional zone were completely enveloped by astrocytic processes (Fig. 1B). These axo-glial terminals were present in the dorsal root transitional zone at 3 weeks and at 3 months, when regeneration is effectively complete. These results support the proposal that regenerating sensory axons form stable terminals on astrocytes at the dorsal root entry zone (*6–8*).

All of the axo-glial terminals were small (1.0 to 2.0 μm in diameter) and had relatively smooth contours, which suggests that they had few, if any, filopodia. The axo-glial terminals contained small agranular vesicles, a few dense-cored vesicles, tubulovesicular profiles, and normal mitochondria. The cytoplasm between these membranous organelles was relatively unstructured and had few, if any, neurofilaments or microtubules. These results indicate that neurofilaments did not accumulate in the axo-glial terminals.

In contrast with the axo-glial terminals at the transitional zone, a few (less than 1.0%) large swollen axon terminals were found in the dorsal root among the collagenous connective tissue elements (Fig. 1, C and D). These terminals, which were 10 to 15 μm in diameter, were packed with neurofilaments and a variety of membranous organelles including dense bodies. Furthermore, these abnormal terminals were similar to those that formed when axons regenerated into a cul-de-sac produced by tightly ligating a peripheral nerve. Both in the dorsal root and in the cul-de-sac, the large swollen terminals were enwrapped by Schwann cells that were, in turn, surrounded by the collagenous matrix of the endoneurium. Thus, neurofilaments will accumulate in the terminals of regenerating dorsal root axons that have been stopped from elongating by a connective tissue obstacle.

Our results indicate that astrocytes stop axons that are regenerating through physiological pathways within axons. During elongation, axons extend filopodia from their tips. When an axon tip contacts a target cell, filopodial extension is reduced and eventually ceases. In this way, the target cell stops the exploratory behavior of the axon tip. Just as with stationary axon terminals on target cells, the axo-glial terminals that contacted astrocytes had few, if any, filopodia. Apparently, adult mammalian astrocytes can inhibit the extension of filopodia from elongating axon tips. Our results also indicate that after the astrocytes have stopped the axon tip from advancing, they activate the intrinsic axonal mechanisms that prevent slowly transported cytoskeletal polymers from accumulating in the stationary axon terminal. On this basis, we propose that astrocytes stop axonal elongation through their effects

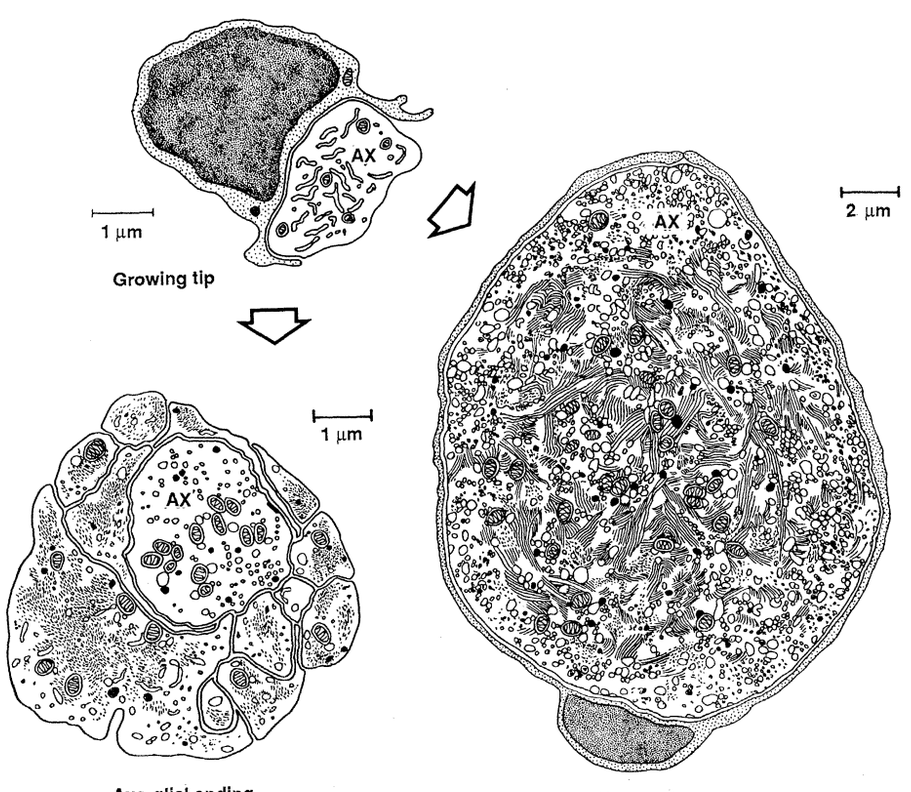

Fig. 2. Summary drawing depicting the two mechanisms by which regenerating axons (AX) can be stopped. In one case, the axo-glial ending, the growth cone is stopped by activating the physiological stop pathway within the axon. This pathway operates during the development of axon terminals on target cells, and we propose that astrocytes can also stop axonal elongation through this pathway. Axonal elongation can also be stopped by physically obstructing axonal elongation. In this case, the neuromitic ending, connective tissue elements block the forward progression of the growth cone, and it is converted to a neurofilament-swollen terminal packed with membranous organelles.

on the intrinsic physiological pathways that normally operate when axons form synaptic contacts with target neurons or peripheral receptors.

These observations and others indicate that regenerating axons can be stopped by two different mechanisms—(i) by activating the physiological stop pathway that is built into the axon and (ii) by physically obstructing the advance of the axon tip (Fig. 2). In the adult mammalian spinal cord, both target neurons and astrocytes have the potential to stop axonal growth because of their capacity to activate the physiological stop pathway. Just as with target cells, astrocytes may contribute to the formation of neural connections by controlling the intrinsic tendency of axons to elongate. In the mature CNS, astrocytic processes envelop a substantial part of the surface of synaptic terminals; one of their roles may be to prevent axon terminals from wandering away from their synaptic contacts (*17*).

For effective regeneration in the CNS, axons must reconnect with their targets. The first and essential step in the sequence of reconnection is elongation of the axons. To promote axonal elongation within the substratum of the CNS, two different kinds of environmental factors must be considered. If the spinal cord of an adult mammal is transected, a connective tissue scar forms in the damaged region, and swollen axon terminals that are often filled with neurofilaments have been observed in contact with the scar (*9*). In this case, preventing or removing the connective tissue obstacle is clearly essential for axonal regeneration. This, however, may not be sufficient to promote axonal regeneration. In addition, the tendency of the mammalian astrocytes to limit the motility of the axon tip through the physiological stop pathway must also be considered.

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Eosinophils Cocultured with Endothelial Cells Have Increased Survival and Functional Properties

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Human peripheral blood eosinophils, cells often associated with allergic and parasitic diseases, were maintained in vitro for at least 14 days when they were cocultured with bovine endothelial cells and for at least 7 days when cultured with either bovine or human endothelial cell–derived conditioned medium. The cocultured eosinophils became hypodense and generated about three times as much leukotriene C_4 upon activation with calcium ionophore and killed about three times as many antibody-coated larvae of *Schistosoma mansoni* as freshly isolated normodense eosinophils. That these cells can be maintained in vitro by coculture with endothelial cells, and the surprising finding that the cocultured eosinophils have biochemical, cytotoxic, and density properties similar to those of eosinophils in patients with allergic and other disorders, will facilitate investigation of the regulation and role of these cells in health and disease.

EOSINOPHILS, WHICH ACCOUNT FOR 3 to 6% of the circulating granulocytes in humans, are thought to remain in the bloodstream for 6 to 12 hours before they enter connective tissues where they survive for several days (*1*). On the basis of density-gradient sedimentation, two populations of eosinophils (designated normodense and hypodense) have been identified (*2–4*). Hypodense eosinophils are prominent in the peripheral blood of patients with eosinophilia such as that associated with helminthic infections (*2–4*). Eosinophils are known to generate leukotriene C_4 (LTC_4) upon activation of the 5-lipoxygenase pathway (*5–7*) and to kill antibody-coated targets such as larvae of *Schistosoma mansoni* (*8, 9*). Both functions are enhanced in hypodense eosinophils (*4, 10*) and can be augmented in vitro in normodense eosinophils upon exposure to recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) (*11*). We now report that eosinophils can be maintained in vitro by coculture with endothelial cells. The cocultured eosinophils produce more leukotrienes in response to calcium ionophore and kill more antibody-coated larvae of *S. mansoni* than freshly isolated cells.

When human peripheral blood eosinophils were cultured for 7 days at a density of 5.0×10^5 cells in 2 ml of enriched RPMI 1640, >99% ($n = 5$) of the cells died as assessed by uptake of Trypan blue. However, in a representative experiment when rep-

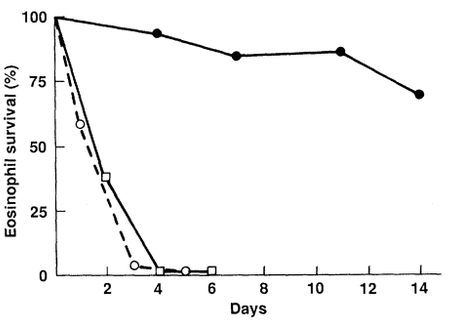

Fig. 1. The survival of eosinophils when cultured in enriched RPMI 1640 (○) or when cultured in enriched RPMI 1640 in the presence of endothelial cells (●) or 3T3 fibroblasts (□). Human eosinophils were isolated under sterile conditions to a purity of 88 ± 11% (mean ± SD, $n = 11$) according to a modification (*7, 29*) of the method of Vadas *et al.* (*9*), and residual contaminating erythrocytes were eliminated by hypotonic lysis. The results are typical of the eight and four experiments done on cells cultured for 7 and 14 days, respectively.

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