The homogenized sponge is centrifuged and the supernatant is evaporated. The crude extract is dissloved in 300 ml of fresh water in a finger bowl in which a goldfish is placed, and the behavior of the fish is timed and recorded. Control experiments are done simultaneously.

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Isolated Brain Microvessels: A Purified, Metabolically Active Preparation from Bovine Cerebral Cortex

Abstract. *A purified, metabolically active preparation of brain microvessels was isolated from bovine cerebral cortex by using a simple procedure involving mild disruption of the tissue by homogenization and trapping of the vessels on nylon sieves. This preparation permits in vitro metabolic and structural studies of small blood vessels.*

Knowledge of the metabolism and structural composition of isolated blood vessels is important in studies of the biochemistry and pharmacology of this organ system, which is a primary site for a wide range of pathologic disturbances, including arteriosclerosis and diabetes mellitus. The microvessels, defined as those with diameters less than 300 μm, are frequently affected by such diseases to as great an extent as larger vessels like the aorta (*1*). However, only the latter have been the subject of extensive biochemical investigations (*2*). The difficulty of readily obtaining sufficient quantities of microvessels for experimentation is one reason for this disparity. The development in our laboratory of simple isolation procedures involving mild homogenization and sieving for obtaining morphologically intact and metabolically active preparations of kidney glomeruli and retinal blood vessels suggested that similar techniques might be useful for the isolation of microvessels from other tissues (*3*). The successful application of these techniques to the isolation of microvessels from cerebral cortex provides a readily obtained preparation of small blood vessels suitable for metabolic investigations of the microcirculatory system in general as well as those aspects peculiar to vessels of the central nervous system.

Figure 1 shows a typical preparation of brain microvessels at low (Fig. 1a) and higher (Fig. 1b) magnification. Gray matter is obtained from the cerebral cortex of bovine brain. For optimum preservation of metabolic activity, the vessels should be isolated promptly from brains removed from the animals immediately after slaughter, and the isolated vessels should be transported to the laboratory in cold oxygenated buffer to minimize the effects of anoxia on the tissue. Pieces of cortical tissue are homogenized in Earle's balanced salt solution buffered with HEPES (*4*) (1 : 1 by volume) with ten vertical strokes of a hand-held loosely fitting Teflon pestle in a smooth glass tube (*3*). The homogenate is poured over a 153-μm nylon sieve (*5*), and the material remaining on the sieve after washing with buffer is rehomogenized, resieved, and washed as before. A highly enriched preparation of microvessels with the appearance of fine threadlike strands is caught by the sieve, while the bulk of the nonvascular tissue of a fine granular nature is not retained. The vessels are freed of any adhering or accompanying nonvascular contamination either by homogenizing them with two or three additional strokes, resieving, and rewashing, or by "combing" through a suspension of the preparation in a petri dish with a piece of 210-μm nylon mesh. The vessel strands

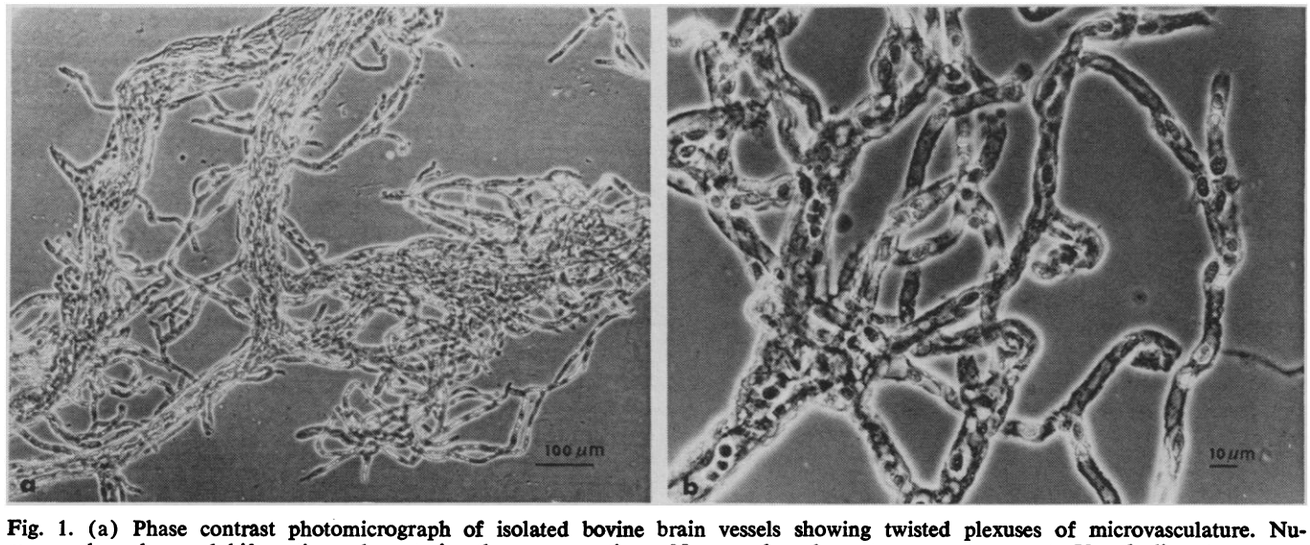

Fig. 1. (a) Phase contrast photomicrograph of isolated bovine brain vessels showing twisted plexuses of microvasculature. Numerous branches and bifurcations characterize these preparations. Nonvascular elements are not present. Vessel diameters range from 6 to 80 μm (× 85). (b) Phase contrast photomircograph at higher magnification showing vascular arborization in isolated bovine brain vessels. Clusters of red blood cells occupy vessel lumina. Endothelial cell nuclei are particularly evident at this magnification (× 350).

float and adhere to the "comb" and can be washed off into another dish with a stream of buffer, leaving behind the granular contaminants, which settle to the bottom of the dish and do not stick to the comb. The purity of the vessels must be monitored during isolation if uniformly clean preparations are to be obtained. This is easily done by observing the suspended preparation in a petri dish during the latter stages of isolation with a binocular dissecting microscope at a magnification of 25 to 50. In this way a highly purified preparation consisting almost entirely of isolated vascular trees is readily obtained. A typical preparation yielded 850 mg of brain vessels from 50.6 g of cerebral cortex (wet weights).

Vessels prepared in this manner are shown by phase contrast microscopy to be virtually devoid of nonvascular material. They appear as large masses of contiguous vessels (Fig. 1a), which exhibit extensive dichotomous branching and numerous bifurcations. Vessel diameters range from 6 to 80 μm, and the vessels usually remain associated in discrete vascular arborizations which resemble in vivo pampiniform vessel plexuses. The smallest vessels in the preparation are therefore within the size range of true capillaries (*1*). At higher magnifications (Fig. 1b) endothelial cell nuclei are easily visualized and aggregates of red blood cells cluster within the vessel lumina. The absence of nonvascular material is particularly evident at this magnification.

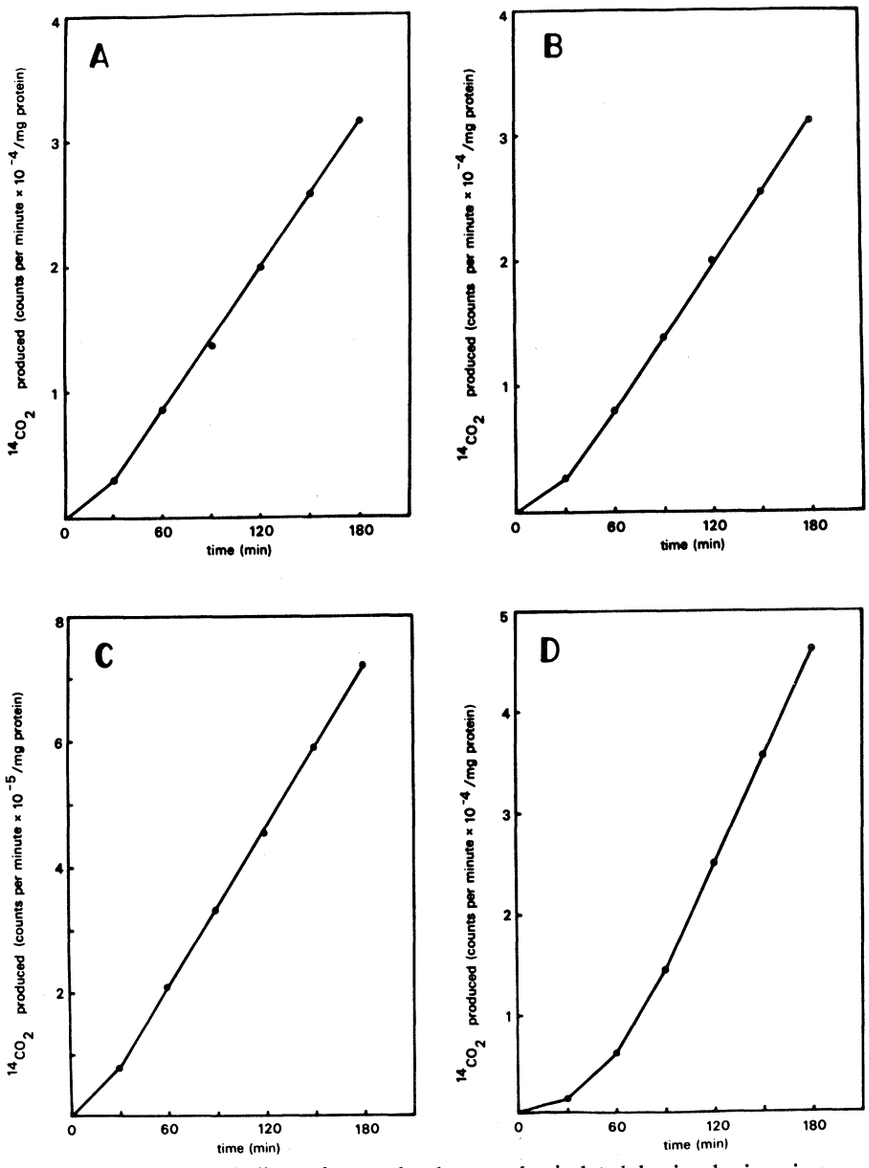

Fig. 2. Oxidative metabolism of several substrates by isolated bovine brain microvessels. The $^{14}CO_2$ production from ^{14}C-labeled substrates was measured by continuous trapping of $^{14}CO_2$ evolved from reaction mixtures in an oxygen atmosphere at 37°C. Incubation flasks contained 48 to 72 μg of vessel protein and 0.2 μc of the appropriate ^{14}C-labeled substrate in 0.51 ml of incubation buffer: (A) D-[^{14}C]glucose (uniformly labeled); (B) sodium [1-^{14}C]pyruvate; (C) L-[^{14}C]glutamic acid (uniformly labeled); and (D) [1-^{14}C]oleic acid. Control experiments not shown in this figure include incubations with buffer alone and with bovine blood dilutions designed to give concentrations of blood elements equivalent to those in the vessel preparations (300 to 3000 red blood cells). These controls yielded no significant $^{14}CO_2$ production in excess of background from identical amounts of the radioactive substrates.

The metabolic activity of the isolated brain vessel preparations is demonstrated by their ability to linearly consume oxygen and to produce CO_2 with several substrates. The vessels exhibit an endogenous rate of oxygen consumption (10.9 nmole or 0.278 μl of O_2 per milligram of protein per minute) that is markedly stimulated by the addition of succinate (10.8 m*M*) (32.6 nmole or 0.830 μl mg^{-1} min^{-1}). Measurements were made at 37°C in a thermostated cell containing 305 μg of vessel protein in 1.8 ml of buffer; a Clark-type oxygen electrode was used. The oxygen consumption of the brain vessels in the presence of endogenous substrate alone is five to ten times greater than corresponding values reported for a number of different large vessel preparations and approaches values obtained for highly metabolically active tissues such as kidney and liver (*6*). The energy metabolism of microvascular tissue, at least in the brain, may not be as disadvantageous as that of the larger vessels, when compared with that of other organs. Addition of the mitochondrial uncoupling agent 2,4-dinitrophenol ($3 \times 10^{-4}M$) to vessels with succinate as substrate produced a small but definite increase in oxygen consumption (36.7 nmole or 0.933 μl mg^{-1} min^{-1}), indicating that the respiration of the cells had been uncoupled by this agent.

The integrity of the major energy-producing pathways—glycolysis, the citric acid cycle, and fatty acid oxidation—in the isolated microvessel preparations is evidenced by the linear production of $^{14}CO_2$ from ^{14}C-labeled glucose, pyruvate, glutamic acid, and oleic acid over a period of several hours (Fig. 2). The maintenance for 3 hours of a linear rate of fatty acid oxidation, a pathway which is particularly sensitive to cellular damage, indicates the intactness of subcellular compartmentation and the coupled nature of oxidative metabolism in this vascular tissue preparation. The rates of oxygen consumption and $^{14}CO_2$ pro-

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-

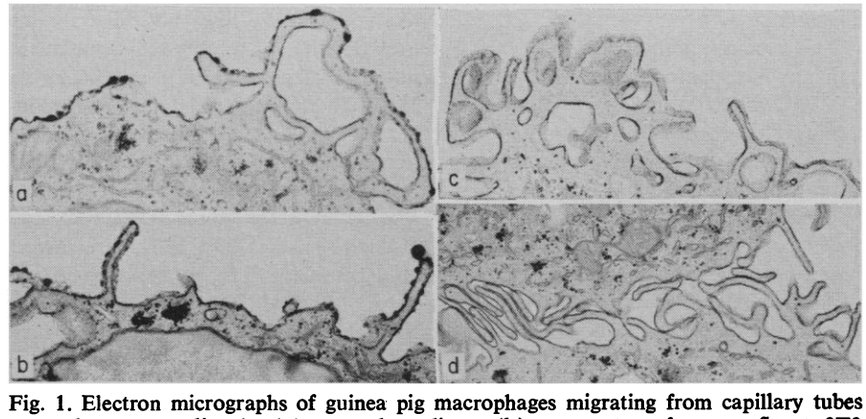

Fig. 1. Electron micrographs of guinea pig macrophages migrating from capillary tubes onto glass cover slips in (a) control medium; (b) supernatant from confluent 3T3 culture; (c) and (d) supernatant from confluent SV3T3 culture. All media contain 15 percent GPS. Abundant, dense-staining cell coat material, 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.