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Greater Number of Capillary Endothelial Cell Mitochondria in Brain Than in Muscle (38889)

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The selective permeability of the brain mircrocirculatory bed (the blood–brain barrier-BBB) is widely believed to have its locus at the brain capillary endothelial cell. Whereas capillaries in other tissue are nonspecifically permeable to all small molecules by virtue of open clefts between endothelial cells, cellular fenestrae and pinocytosis, these nonspecific routes of exchange are virtually absent in brain, and transcapillary exchange probably takes place through the membranous walls and the cytoplasm of endothelial cells. Although most organic molecular exchange is probably bidirectional and net flux is determined by concentration gradients, recent studies have suggested a unidirectional flux out of the brain for potassium, iodide and organic anions (1–3). Such uphill unidirectional transport would require a source of energy in the brain capillary endothelial cell.

If the brain capillary wall performs an energy-dependent excretory function as a substantial part of its total metabolic work, it might be expected that brain capillary endothelial cells would contain more mitochondria than a tissue with nonspecifically permeable capillaries, such as skeletal muscle.

Materials and Methods. To test this hypothesis, rat brain and skeletal muscle capillaries were examined by electron microscopy and counts made of the number of mitochondria encountered in random sections. A 175-g male Wistar rat on routine diet and in apparently good health, was sacrificed, routinely perfused with glutaraldehyde, embedded, and sectioned for electron microscopy. Anesthesia was induced with 35% chloral hydrate and respiration supported with 95% O_2 and 5% CO_2. Two tenths milliliter heparin and 0.8 ml 1% $NaNO_2$ were injected intracardiac, and a cannula was placed in the ascending aorta. The right atrium was cut, and the rat perfused with 500 ml 1.25% glutaraldehyde and 1% paraformaldehyde in 0.135 *M* phosphate buffer at pH 7.2. The animal was cooled for 4 hr in a plastic bag at 4°. Six blocks were taken from cerebellar vermis and six blocks from the vastus medialis muscle; washed in dextrose buffer, secondarily fixed with 1% buffered osmium tetroxide, dehydrated, and embedded in epon resin 812. Thick sections were used to select capillaries; thin sections were stained with both uranium and lead.

Electron micrographs were made from randomly selected cerebellar (n = 26) and vastus medialis muscle (n = 28) capillaries

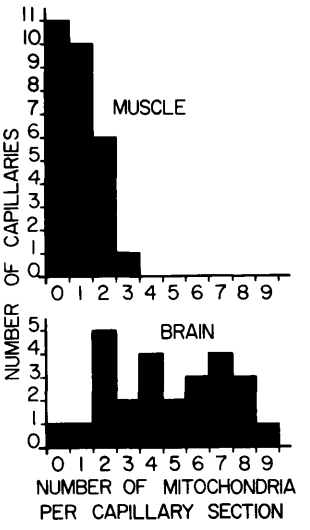

FIG. 1. A histogram showing distribution of electron microscopic mitochondrial counts in brain vs muscle capillaries. Eleven of the 28 muscle capillary sections showed no mitochondria, 10 showed one, and none showed more than three. The brain capillaries showed mitochondrial counts ranging from 0 to 9. The difference between the two tissues is significant, $P < 0.001$.

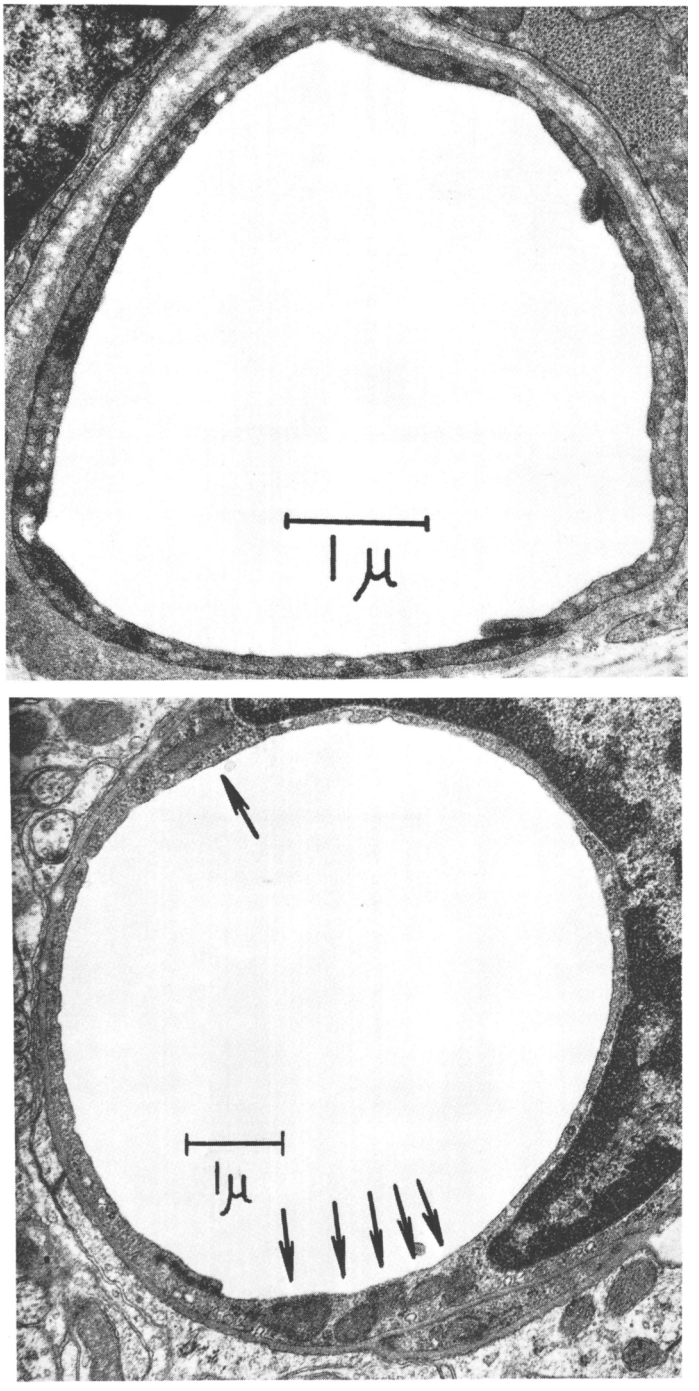

FIG. 2. (a) Capillary from the vastus medialis muscle of a rat. Note the absence of mitochondria in cytoplasm of endothelial cell. The average muscle capillary examined in this study showed less than one mitochondrion per section. Note also large number of pinocytotic vesicles. (b) Capillary from lobule V of rat cerebellar vermis. A segment of the endothelial cell nucleus is not seen, but there are no pertinent data missing. The cerebellar endothelial cell contains six mitochondria (↓) and very few pinocytotic vesicles. The number of mitochondria seen here is fairly representative of the average number (4.7) of mitochondria found in the randomly chosen cerebellar capillaries studied here.

thus representing a total of 54 different capillaries. Only capillaries cut at approximately right angles to the direction of the flow were selected.

The numbers of mitochondria lying central to the endothelial cell basement membrane were counted in order to exclude mitochondria within the pericyte which appears in some sections immediately external to the basement membrane. Criteria for mitochondrial identification were: (1) a surrounding bilaminar membrane, and (2) an internal cristal structure.

Results. The capillary endothelial cell mitochondrial counts per section were 4.7 $\pm$ 2 in brain and 0.82 $\pm$ 0.86 in muscle. Thus, the brain capillary cells contained approximately 5.6 times more mitochondria than muscle. A representative brain capillary section showing six mitochondria and a similarly representative muscle capillary section showing zero mitochondria are shown in Fig. 2. As indicated in Fig. 1, about 39% of the 28 muscle capillary sections showed no mitochondria. Only one cerebellar capillary showed no mitochondria.

Discussion. Because the mitochondrion is the source of most cellular energy, these findings support the hypothesis that the brain capillary cell is performing substantially more metabolic work than muscle capillary cells. It is reasonable to suggest this apparent extra brain capillary work capacity is, in some part, related to energy-dependent transcapillary transport.

If it is assumed that the number of mitochondria per cut section of cell is approximately directly proportional to cell work being performed and that the major function being carried out by brain capillaries, but not by muscle capillaries, is active transport, the extra brain capillary mitochondria observed here suggest that approximately 80% of the work being performed by brain capillaries is active transport of solutes from brain extracellular fluid into blood plasma or from blood plasma into brain extracellular fluid. The other 20% of the brain capillary cell metabolic work could well be its internal cellular maintenance which it carries out in common with muscle capillary endothelial cells.

If active transport functions of the brain capillary are important in the optimization of the composition of the brain's neuronal fluid environment, a possible teleological basis for the BBB becomes apparent. Were the brain capillary bed nonspecifically permeable, as in other tissues such as muscle, unidirectional BBB pumping would be very inefficient. To generate and maintain an ionic concentration gradient in the steady state (such as exists for potassium across the BBB), the brain capillary bed must have both an energy-dependent pump to transport the solute up a concentration gradient into plasma and, to make this pumping effective, it must abolish nonspecific leakage to prevent the solute from passively diffusing back from plasma into brain extracellular fluid. The mechanisms by which the normal brain capillary cells form tight, impermeable intercellular junctions, form no cellular fenestrae, and virtually stop pinocytosis are unknown, but these structural characteristics of the brain capillary must greatly enhance any unidirectional pumping of solutes by BBB.

By visual inspection the brain and muscle endothelial mitochondria did not appear obviously different in size or structure but planimetric estimates of mitochondrial cross-sectional areas in muscle and brain capillaries would more rigorously establish tissue differences. Although the present data support the view that there are several times as many mitochondria in cerebellar capillary endothelial cells than in vastus medialis skeletal muscle, clearly more sections from other regions and other organs must be examined before these observations can be generalized.

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1. Ahmed, A., and Van Harreveld, A. J., J. Physiol. **204**, 31 (1969).
2. Bradbury, M., Segal, M. B., and Wilson, J., J. Physiol. **221**, 617 (1972).
3. Wolfgram, L. I., Katzman, R., and Escriva, A., Neurology **24**, 772 (1974).

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