

Absence of a Blood-Brain Barrier Within Transplanted Brain Tissue?

J. M. Rosenstein (1) reported the absence of a blood-brain barrier (BBB) to blood-borne tracer and endogenous proteins within fetal rat parietal cortex transplanted to the fourth cerebral ventricle or cerebral cortex of adult rats. The author concluded that transplants of the fetal central nervous system (CNS) manifest a permanent barrier dysfunction and, therefore, are not integrated physiologically within the host CNS. The author suggested that blood-borne compounds normally excluded from the brain by the BBB would have direct access to CNS transplants and may affect neuronal function. If this report is correct, absence of a BBB in brain grafts in conjunction with a host immune response for tissue rejection could complicate the potential clinical application of human CNS transplants in the treatment of a broad range of neurological degenerative disorders, an example of which is Parkinson's disease (2); furthermore, such a finding would argue against the suggestion that blood vessels contributing to the vascular supply of grafted CNS tissue express BBB properties dictated by the grafted tissue (3).

The CNS grafts described by Rosenstein are located on or near the dorsal surface of the host brain in association with the dura mater or the choroid plexus; both contain normally leaky blood vessels (4). The intactness of the dura mater is compromised when transplanted tissue is introduced in the host CNS. Intimate contact between the grafted tissue and the dura mater or choroid plexus promotes the extracellular entry of blood-borne proteins to the graft from leaky vessels in the dura mater and choroid plexus. This possibility was not addressed in Rosenstein's report. Nor does it appear that an important control experiment was conducted—that of placing CNS grafts deep within the brain parenchyma away from the meninges and choroid plexus. We have observed (5) an extracellular spread of blood-borne tracer protein into CNS grafts (supplied with BBB vessels exhibiting interendothelial tight junctional complexes) positioned adjacent to the median eminence, an additional site in the CNS containing normally leaky blood vessels. Perfusion-fixation of the host brain within 60 seconds after intravenous administration of the tracer prevented the extracellular spread of tracer into the grafted tissue from the median eminence. Rosenstein's report provided no ultrastructural evidence

to suggest that endothelia in CNS grafts differ morphologically from typical BBB endothelia. The tracer-labeled organelles (for example, vesicles, endosomes, and dense bodies) identified by the author in the graft endothelia are associated with the endocytic process or the lysosomal system, or both. The endocytic activity of cerebral endothelia at the luminal surface in normal and grafted tissue is prominent, and organelles that sequester tracer protein that has been taken up by endocytosis are not engaged in the transcellular transport and transcytosis of the probe molecule (4–6). Extravasations of blood-borne tracer documented by the author in grafted tissue are not attributed to transendothelial transport of the tracer. Similar extravasations are evident in the CNS parenchyma of control animals injected intravenously with the tracer; the leaks may be a consequence of rupture of BBB arterioles by an elevated intra-arterial pressure induced by perfusion-fixation of the brain (4). In this context, graft vessels may be particularly sensitive (7). The author stated further that cerebral blood vessels throughout his preparations are outlined from the abluminal surface with blood-borne tracer that achieved widespread distribution within perivascular spaces after the tracer left blood vessels in the grafted tissue and circulated in the cerebrospinal fluid. However, when we injected tracer protein identical to that employed by the author systemically in control animals and animals harboring CNS grafts, we observed that the tracer labeled the cerebrovascular tree from the luminal or blood side only (5).

The absence of key control experiments in Rosenstein's study raises concern regarding the report's conclusions. These conclusions are not supported by our data on CNS grafts (5) or by preliminary data on CNS grafts presented by three independent laboratories (8).

RICHARD D. BROADWELL
Divisions of Neurological Surgery and
Neuropathology,
University of Maryland School of Medicine,
Baltimore, MD 21201

REFERENCES AND NOTES

1. J. M. Rosenstein, *Science* **235**, 772 (1987).
2. I. Madrago *et al.*, *New Engl. J. Med.* **318**, 51 (1988).
3. P. A. Stewart and M. J. Wiley, *Dev. Biol.* **84**, 183 (1981); R. C. Janzer and M. Raff, *Nature* **325**, 253 (1987); unpublished immunohistochemical data from our laboratory suggest that BBB vessels supplying CNS transplants are derived from both the

host brain and donor CNS tissue.

4. B. J. Balin, R. D. Broadwell, M. Salzman, M. El-Kalliny, *J. Comp. Neurol.* **251**, 260 (1986); B. J. Balin, R. D. Broadwell, M. Salzman, *J. Neurocytol.* **16**, 721 (1987).
5. R. D. Broadwell, H. M. Charlton, B. J. Balin, M. Salzman, *Comp. Neurol.* **260**, 42 (1987); R. D. Broadwell, B. J. Balin, A. M. Cataldo, in *Circumventricular Organs and Body Fluids* (CRC Press, Boca Raton, FL, 1987), vol. 2, pp. 61–85; R. D. Broadwell, H. M. Charlton, M. Salzman, R. Schlegel, *Soc. Neurosci. Abstr.* **13**, 1250 (1987).
6. R. D. Broadwell and M. Salzman, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7820 (1981); R. D. Broadwell, B. J. Balin, M. Salzman, *ibid.* **85**, 632 (1988).
7. We have seen occasional extravasations of blood-borne tracer in CNS grafts as well as in the host brain parenchyma. The extravasations are random, are associated with open intercellular junctions at the level of arterioles, and are seen only if the host brain is fixed by vascular perfusion at times after injection when the circulating titer of blood-borne tracer is high (less than 30 minutes).
8. I. Dusart, F. Nothias, F. Roundier, M. Peschanski, *Soc. Neurosci. Abstr.* **13**, 289 (1987); M. H. Lee, A. S. Lossinsky, A. Rabe, H. M. Wisniewski, *ibid.*, p. 289; J. C. Sorenson, J. Zimmer, A. J. Castro, *ibid.*, p. 289.

15 January 1988; accepted 25 February 1988

Response: R. D. Broadwell's commentary merits rebuttal for several reasons. First, I did in fact address differences between superficial and parenchymal grafts in my report. Second, he has essentially corroborated my major findings by indicating that ventricular and parenchymal grafts to a lesser degree contain protein exudation [(2); his note 7]. Broadwell appears to be concerned about the mechanism of protein permeability in central nervous system (CNS) grafts, but not about the data presented. Regardless of the precise mechanism, the presence of exogenous and endogenous protein in CNS tissue constitutes a blood-brain barrier (BBB) dysfunction. His commentary might leave the misconception that all CNS grafts have a completely normal BBB function; they do not. Third, to suggest that my findings could complicate potential clinical applications, at this stage, is a potentially correct but limited viewpoint of an experimental paradigm that is just beginning to be explored.

I reported that grafts in the ventricle or near brain surfaces contained extensive protein exudation. In entirely parenchymal grafts for several years I have observed a near but not complete lack of protein exudation. In serial sections of such grafts, a small and variable permeability can be measured (3). Nevertheless, the notable finding was that a graft of fetal brain that already has a BBB to protein invariably loses this privilege at least transiently and, depending on placement, permanently. Thus, the tissue could contact circulating (host) compounds to which it normally would never be exposed. That a CNS tissue graft would lack BBB properties is in direct contradiction to conventional angiogenesis concepts (4), which state that, no matter where grafted, all vessels supply-