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*).

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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. Salcman, M. El-Kalliny, *J. Comp. Neurol.* **251**, 260 (1986); B. J. Balin, R. D. Broadwell, M. Salcman, *J. Neurocytol.* **16**, 721 (1987).
5. R. D. Broadwell, H. M. Charlton, B. J. Balin, M. Salcman, *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. Salcman, R. Schlegal, *Soc. Neurosci. Abstr.* **13**, 1250 (1987).
6. R. D. Broadwell and M. Salcman, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7820 (1981); R. D. Broadwell, B. J. Balin, M. Salcman, *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.

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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-

ing CNS tissue would alter phenotypically to become cerebral-like or impermeable. I also found (*1*) that the observed permeability to protein in CNS grafts is *variable*, even when the identical experiments are performed. This finding suggests that the neovascularization process may not be a predictable one.

In fact we indicated that leaky vessels in the choroid may contribute to leakiness in an adjacent graft and stated this in our paper (*1*, page 773, paragraph 7, lines 1–12; see also *3*, *5*). Our autoradiographic studies (*5*) show that vessels can arise directly from the choroid plexus or area postrema to enter and anastomose with the nascent graft vessels. Since these normally leaky vessels do not change phenotypically, large protein exudations occur which subsequently diffuse through the graft neuropil. I believe he describes an identical physiological situation in his third-ventricle grafts (*2*) by stopping protein movement by 1 minute.

Broadwell's term "intimate contact" between graft and choroid or median eminence may not be anatomically correct. The permeable vessels of these tissues are covered by specialized epithelial cells tethered by tight junctions—the basis of the blood-cerebrospinal fluid (CSF) barrier. Assuming that there was no direct mechanical trauma (which does not occur in fourth-ventricle grafts), it is an intriguing question as to how the leaky vessels emerged from between their united cellular covering to enter the graft. Angiogenic factors emanating from the graft tissue might induce endothelial cell movement. An additional important event consequent to the vascular invasion is the potential alteration of the blood-CSF barrier (*3*). With regard to dural vessels, the issue is clearly more complicated since the dura and arachnoid are compromised. Although the arachnoid is a tightly bound layer, perhaps the dural vessels grow into the graft tissue before the tight junctions can reform (if, in fact, they do).

Contrary to what Broadwell suggests, the correct key control experiment to determine the physiological state of the vasculature and to *exclude* the possibility of altered vessels caused by exogenous protein infusion is to stain immunocytochemically for endogenous rat serum protein in the grafts, as was done in my study. The distribution of serum albumin in the graft, a well-known neuropathological test for the potential presence of vasogenic edema, mimicks that of exogenous protein in the grafts (*1*, see also *6*).

With respect to Broadwell's comment on the mechanism of tracer leakage, I suggested, but did not prove, along with several other possibilities for protein leakage, such as ischemia or immunological or astrocytic reactions, that there could be some increased endothelial transport. I believe we agree that, if some endothelial transport of protein occurs, it probably represents only a minor contribution to the observed permeability, although species differences (rat and mouse) could be involved.

With regard to the highlighting of the blood vessels, I refer the reader to the original and elegant work of Rennels *et al.* (*7*) showing outlining after *ventricular* but not vascular horseradish peroxidase (HRP) administration. In rats bearing permeable autonomic grafts, the brain vessels are lightly highlighted after short HRP circulation and much more heavily so at longer times (*8*). Because Rennels *et al.* used ventricular injection to show outlining identical to that reported to occur by Broadwell after vascular injection, it might seem logical to conclude that the precipitate of the tetramethylbenzidine method results from delivery of the material by both vascular and ventricular routes, particularly since HRP adjacent to permeable areas such as median eminence can enter the CSF.

From an experimental perspective, if CNS grafts will be used clinically, the placement is of importance in determining whether a compromised BBB will be present. My findings indicate the *possibility* of BBB permeability—a high probability in ventricular grafts and a considerably lower and variable one in parenchymal grafts. On this point, Broadwell and I are in some agreement since, in his note 7, he states there exist occasional extravasations in parenchymal grafts.

A BBB dysfunction should not necessarily be viewed in a detrimental context, but as an experimental condition in which the neural grafting paradigm can be further explored. This is suggested since it is not known directly if lack of a BBB plays a role in cerebral dysfunction (*9*). One could deliver exogenous compounds that do not cross the BBB directly to specific grafts (*10*) for a variety of biochemical or metabolic reasons. In addition, from a neuropathological perspective grafting induces extensive neovascularization, which does not occur in normal mature brain (but does, for instance, in brain tumor formation). The study of angiogenesis and permeability could yield significant data concerning mechanisms of graft function and might also be useful for the study of neurological disorders where the BBB is compromised. Finally, ventricular and parenchymal grafts (which differ in permeability) or in their neuroactive mechanisms may also differ in effectiveness in treatment of disorders.

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REFERENCES

1. J. M. Rosenstein, *Science* **235**, 772 (1987).
2. R. D. Broadwell, H. M. Charlton, B. J. Balin, M. Salcman, *J. Comp. Neurol.* **260**, 42 (1987).
3. J. M. Rosenstein and T. M. Phillips, *Progr. Brain Res.*, in press.
4. J. Folkman, *N. Engl. J. Med.* **285**, 1182 (1971); N. A. Svengaard, A. Bjorklund, J. Hardebo, U. Stenevi, *Nature* **255**, 334 (1975); P. A. Stewart and M. J. Wiley, *Dev. Biol.* **84**, 183 (1981).
5. J. M. Krum and J. M. Rosenstein, *J. Comp. Neurol.* **258**, 420 (1987); *ibid.* **271**, 331 (1988); J. M. Rosenstein, J. M. Krum, B. D. Trapp, *Brain Res.*, in press.
6. L. M. Smith and F. F. Ebner, in *Neural Regeneration and Transplantation*, G. Das and R. Wallace, Eds. (Springer-Verlag, New York, 1986), pp. 81–101.
7. M. L. Rennels *et al.*, *Brain Res.* **326**, 47 (1985).
8. J. M. Rosenstein and M. W. Brightman, *J. Comp. Neurol.* **250**, 339 (1986).
9. W. Oldendorf, *Am. J. Physiol.* **22**, 1629 (1971).
10. P. Sanberg *et al.*, *Progr. Brain Res.*, in press; P. Sanberg *et al.*, *Exp. Neurol.*, in press.

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Model of Huntington's Disease

In her article "Animals yield clues to Huntington's disease" (Research News, 11 Dec. 1987, p. 1510), Jean L. Marx reiterates the interpretation and subsequent dismissal by M. F. Beal *et al.* of our recent data presented in *Nature* (*1*). We wish to reaffirm our belief [and that of others (*2*)] that quinolinic acid demonstrates a spectrum of neurotoxicity similar to that of other excitotoxins, there being no preferential sparing of neurons containing somatostatin, neuropeptide Y, or NADPH (nicotinamide adenine dinucleotide phosphate, reduced)-diaphorase. In their original paper, Beal *et al.* (*3*) reported a study of the effects of intrastriatal injection of quinolinic acid, kainic acid, and *N*-methyl-D-aspartic acid on a variety of histochemical and neurochemical parameters. Their principal conclusion was that quinolinic acid uniquely among the excitotoxins spares somatostatin-containing neurons in a "transition zone peripheral to the injection site." GABAergic [as measured by a 50% decrease in GABA (γ-aminobutyric acid) levels (*4*)] and cholinergic neurons (as measured by a 51% decrease in the number of acetylcholinesterase-positive neurons) were similarly affected. The extent of sparing of the cells containing somatostatin was such that homogenates of whole striatum showed a 70% reduction in GABA, a 75% reduction in substance P, but no significant