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My colleagues and I investigated the sites and mechanisms of aluminum (Al) and manganese (Mn) distribution through the blood–brain barrier (BBB). Microdialysis was used to sample non–protein-bound Al in the extracellular fluid (ECF) of blood (plasma) and brain. Brain ECF Al appearance after intravenous Al citrate injection was too rapid to attribute to diffusion or to transferrin-receptor–mediated endocytosis, suggesting another carrier-mediated process. The brain:blood ECF Al concentration ratio was 0.15 at constant blood and brain ECF Al concentrations, suggesting carrier-mediated brain Al efflux. Pharmacological manipulations suggested the efflux carrier might be a monocarboxylate transporter (MCT). However, the lack of Al ^{14}C-citrate uptake into rat erythrocytes suggested it is not a good substrate for isoform MCT1 or for the band 3 anion exchanger. Al ^{14}C-citrate uptake into murine-derived brain endothelial cells appeared to be carrier mediated, Na independent, pH independent, and energy dependent. Uptake was inhibited by substrate/inhibitors of the MCT and organic anion transporter families. Determination of ^{26}Al in rat brain at various times after intravenous ^{26}Al suggested a prolonged brain ^{26}Al half-life. It appears that Al transferrin and Al citrate cross the BBB by different mechanisms, that much of the Al entering brain ECF is rapidly effluxed, probably as Al citrate, but that some Al is retained for quite some time. Brain influx of the Mn^{2+} ion and Mn citrate, determined with the *in situ* brain perfusion technique, was greater than that attributable to diffusion, suggesting carrier-mediated uptake. Mn citrate uptake was approximately 3-fold greater than the Mn^{2+} ion, suggesting it is a primary Mn species entering the brain. After Mn^{2+} ion, Mn citrate, or Mn transferrin injection into the brain, brain Mn efflux was not more rapid than that predicted from diffusion. The BBB permeation of Al and Mn is mediated by carriers that may help regulate their brain concentrations. ***Key words:*** **aluminum, b.End5 cells, blood–brain barrier, brain efflux, brain endothelial cells, brain influx, *in situ* brain perfusion, manganese, microdialysis, rat. *Environ Health Perspect* 110(suppl 5):699–704 (2002).**
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Aluminum and Manganese As Neurotoxicants

Aluminum (Al) and manganese (Mn) are neurotoxicants that have the potential to contribute to neurodegenerative disorders (*1–5*). The contributory role of Al in the dialysis encephalopathy syndrome has been shown. Avoidance of the major Al sources contributing to the syndrome has greatly reduced this problem, although occasional outbreaks still occur (*6*). There has been concern about the suggested role of Al in Alzheimer disease (AD) since the initial report of elevated brain Al in victims of this condition (*7*). Some studies have shown a small positive correlation between drinking-water Al concentration and dementias, including AD [reviewed by Yokel (*2*)]. However, the role of Al in AD etiology is highly controversial because of the conflicting results of studies of brain Al of AD victims, the association of drinking-water Al and the risk for AD, and other reported end points of Al toxicity. As a result of continued concern about the neurotoxicity of Al, the U.S. Environmental Protection Agency has put Al on its contaminant candidate list, the U.S. Food and Drug Administration recently implemented labeling requirements for Al in large- and small-volume parenterals, and Canada established operational guidance limits for drinking-water Al on the basis of the precautionary principle (*8–10*).

Manganese can produce a parkinsonism-like syndrome (*11,12*). This has occurred in miners after prolonged exposed to manganese dioxide by inhalation (*13*), families drinking well water contaminated by buried dry-cell batteries [cited by Hudnell (*11*)], workers in dry-cell battery factories, and perhaps from environmental exposure (*14*). There is currently concern about airborne exposure from the use of Mn in fungicides such as ethylenebis(dithiocarbamato)manganese (Maneb; Cerexagri, Inc., King of Prussia, PA, USA) and in the fuel-additive octane-enhancer methylcyclopentadienyl manganese tricarbonyl (MMT) (*11*). The use of MMT in the United States has been permitted by a court decision (Ethyl Corporation v. Carol M. Browner, Administrator of the United States Environmental Protection Agency, and the United States Environmental Protection Agency. 1995. Case No. 94-1516, U.S. Court of Appeals for District of Columbia Circuit, Washington, DC), although MMT has apparently not been extensively used as a fuel additive in the United States since the decision. In contrast to Al, for which no mammalian essentiality has been shown, Mn is essential, required for brain development and function (*15*).

There was very little published information on the sites and mechanisms of brain Al entry prior to our initiation of such studies, and nothing on efflux from the brain. My colleagues and I have elucidated the predominant site and have investigated the rates and mechanisms of blood–brain barrier (BBB) permeation of Al, as well as the duration of Al retention in the brain. Brain Mn uptake has been attributed to a carrier-mediated process [reviewed by Aschner and colleagues (*16,17*)]. However, the studies were not conducted under conditions that controlled the Mn species, leaving uncertainty about the Mn species that enter the brain. There have been no reports of studies of Mn efflux from the brain.

The Sites and Mechanisms of Metal Distribution into the Brain

The potential routes that substances may distribute from the nasal cavity into the brain include absorption into systemic circulation followed by permeation through the BBB or choroid plexuses, and absorption from the nasal cavity into olfactory nerves followed by neuronal distribution directly into the brain. The ability of Al to enter the brain via this latter route has been addressed in two preliminary studies (*18,19*). In contrast, Mn has been shown to slowly enter the brain via this route, although quantitation (bioavailability) has not been reported (*20,21*).

Al and Mn may enter the brain from blood, either through the choroid plexuses or the BBB. These routes of distribution

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between blood and brain are shown diagrammatically in Figure 1. There is a choroid plexus in each of the four cerebral ventricles of the brain. They synthesize most of the cerebrospinal fluid (CSF) that fills the brain ventricles and the subarachnoid space that surrounds the brain and spinal cord. The total surface area of the choroid plexuses is approximately 10 cm^2. Brain atlases of the rat and human show brain regions as far as 1 mm away from the nearest component of the CSF compartment.

The BBB provides the other route of distribution from blood to brain. The anatomical basis of the BBB is primarily attributed to the tight junctions between the cerebral microvascular endothelial cells that line the microvessels that perfuse the brain. Additional impediments to permeation through the BBB come from the absence of fenestrations and the low transcytotic activity of the endothelial cells, the pericytes that surround 30% of their surface, and the astrocyte foot processes that cover 99% of the surface of the endothelial and pericyte cells. Substances must either diffuse through the membranes of these cells or be transported by cell membrane carriers to penetrate the BBB. The surface area of the 400 miles of brain capillaries that are the site of the BBB is roughly 12 m^2, about 1,000-fold that of the choroid plexuses. Visual examination of electron micrographs suggests that no cell in the brain is >30–40 µm from the nearest microvessel (BBB site) (*22*). There is much greater opportunity for rapid exchange between blood and brain through the BBB than through the choroid plexuses and CSF compartment.

The potential mechanisms of distribution of substances across the BBB are the same as those across any cell membrane: diffusion, carrier- and receptor-mediated transport by facilitated diffusion, and active transport. The roles of lipophilicity (hydrogen bonding potential, polar surface area) and molecular weight (size) as predictors for diffusion of small molecules across the BBB have been well described (*23,24*), providing the opportunity to estimate the permeability rates of Al, Mn, and their complexes across the rat BBB from their octanol/buffer partitioning coefficient and molecular weight (*23*). Studies have identified carrier-mediated transport of some metals through the BBB, including copper as an L-histidine complex, iron by transferrin-receptor–mediated endocytosis (TfR-ME), mercury as a cysteine complex, and zinc as an amino acid complex (*25*). These suggest the possibility that Al, Mn, and/or their complexes may cross the BBB by carrier-mediated transport. We conducted studies in rats and in cells derived from the BBB to elucidate the distribution of Al and Mn into and out of the brain and the mechanisms mediating these processes. Animal work was approved by the University of Kentucky Institutional Animal Care and Use Committee and was conducted following the Guiding Principles in the Use of Animals in Toxicology of the Society of Toxicology (*26*).

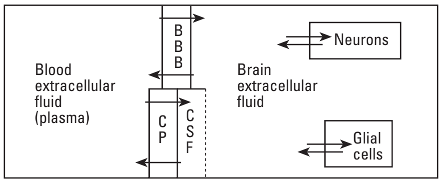

Figure 1. The routes of distribution between plasma and brain: through the BBB and the choroid plexus (CP). Arrows show membranes through which distribution might occur by carrier-mediated transport in addition to diffusion. The dashed line depicts the absence of a membrane barrier to distribution between the CSF and brain ECF.

The Predominant Site of Aluminum Distribution into and out of the Brain

We initially studied Al distribution across the rat BBB using microdialysis probes implanted in the frontal cortex, lateral ventricle, and blood (jugular vein). This provided the ability to sample the extracellular fluid (ECF) in those compartments and therefore in the fluids on both sides of the BBB and a choroid plexus. Microdialysis provides a reflection of unbound substances in the ECF. It was used because it enables repeated sampling and therefore determination of distribution across the BBB and choroid plexus at multiple time points within the same subject. The substance concentrations in dialysates exiting the brain or CSF compared with blood were used to generate a brain:blood ratio (BBr).

It has been pointed out that consideration of the chemical species of a metal in a biofluid indicates the species available to distribute across membranes, such as the BBB, and that might serve as substrates for carriers (*25*). Speciation calculations conducted by Wesley Harris [cited by Yokel and McNamara (*27*)] indicated that 91% of plasma Al is Al transferrin and 7–8% is Al citrate, whereas 90% of Al in brain ECF was predicted to be Al citrate and only 4% Al transferrin. Al citrate was administered in the microdialysis studies. The Al concentration in the dialysate exiting the frontal cortex microdialysis probe reached a maximal steady value in the first 5-min sample after Al citrate was given as an intravenous bolus, indicating quite rapid distribution of Al from blood to brain ECF (Figure 2A). The microdialysis probe in the frontal cortex was some distance from the cerebral ventricles where Al might enter the CSF from a choroid plexus. Maximal penetration of substances through brain parenchyma is thought to be <1 mm (*28*), perhaps in part due to a bulk flow of ECF from brain parenchyma toward the cerebral ventricles (*29*). The appearance of the maximal value within 5 min in the frontal cortex suggests entry through the BBB. However, there is evidence of rapid distribution of solutes from CSF to the brain ECF surrounding the microvasculature. This distribution is believed to occur through the paravascular space (*30*). Comparison of the Al concentration in the dialysates obtained from the frontal cortex versus the CSF and comparison of the Al BBr calculated from brain/blood and CSF:blood ratio for each sample interval (Figure 2B) revealed a higher Al concentration, and higher BBr, in the frontal cortex than in CSF. This supports the suggestion that Al enters the brain from blood through the BBB rather than through the choroid plexuses because entry through the choroid plexus and diffusion through the brain would not be expected to produce a higher Al concentration in the frontal cortex

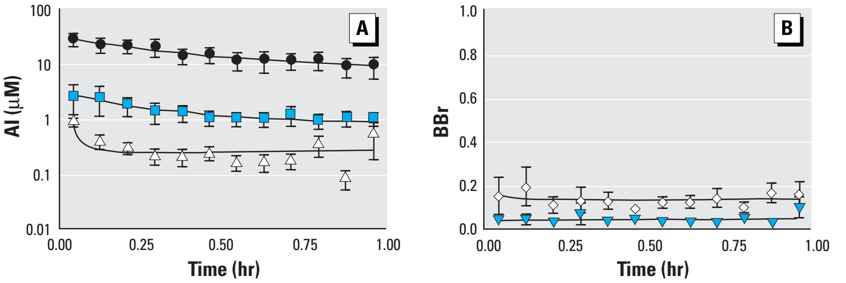

Figure 2. Aluminum concentration determined by atomic absorption spectrometry in three compartments of rats simultaneously and repeatedly using microdialysis after a bolus intravenous Al injection. (*A*) Al concentration in dialysates exiting from microdialysis probes implanted in blood ECF (plasma; circles), brain ECF (squares), and the lateral ventricle CSF (triangles), after an intravenous bolus injection of 0.5 mmol Al citrate/kg. (*B*) BBr of Al for the results shown in *A*: brain ECF:blood (diamonds) and CSF:blood (inverted triangles). Values shown are mean ± SEM of at least three animals at each time. Lines show two-term nonlinear regressions in *A* and one-term nonlinear regressions in *B*. From Allen and Yokel (*53*). Published with permission of Blackwell Publishing.

than in the CSF. The frontal cortex BBr was consistently <1, suggesting a process other than diffusion was mediating Al transfer across the BBB.

Further evidence of carrier-mediated distribution of Al across the BBB was obtained based on the calculated plasma Al concentration in the microdialysis studies (1 mM), the rate of Al citrate flux through membranes (4×10^{-16} mol/cm^2/sec) (*31*), the brain capillary surface area (240 cm^2/g brain) (*32*), and brain ECF volume (0.15 mL/g brain). Calculating the amount of Al that would diffuse across the BBB in 5 min from 1 mL of blood into 0.15 mL of ECF provided an estimate of the resultant Al concentration in the brain ECF. When compared with the blood Al concentration, this produced a BBr of 0.00003. This is much less than the observed BBr of approximately 0.15.

To further assess Al distribution across the BBB from the relationship between brain and blood ECF Al concentrations, Al citrate was bolus dosed and infused at various rates. This achieved quite constant Al concentrations in the sampled compartments, shown by the consistent Al concentration over time in the dialysates exiting microdialysis probes in the brain and blood. Plasma and brain ECF Al concentration increased proportionally to the increase in the Al infusion rate (Figure 3A), suggesting linear (concentration independent) Al distribution and elimination. However, the BBr was consistently approximately 0.15, significantly less than 1 (Figure 3B). These results support the hypothesis that Al BBB permeation is carrier mediated because at steady state the concentration of Al in blood ECF (plasma) × the brain influx rate must equal the concentration of Al in brain ECF × the brain efflux rate. Because the concentration of Al in blood ECF was greater than the concentration of Al in brain ECF, the brain influx rate must be less than the efflux rate. Therefore, BBB permeation of Al cannot be attributed solely to diffusion.

Efflux of Al from the brain also likely occurs across the BBB rather than through CSF. In these studies it was necessary to administer doses of Al citrate that exceeded physiological Al and citrate concentrations and exceeded the metal-binding capacity of transferrin. These high concentrations were necessary to reliably quantitate increases in Al above its background concentration, considering the low relative recovery of Al citrate (~3.25%) by microdialysis (*33*). The calculated plasma Al concentration at the highest Al citrate infusion rate was approximately 1 mM. Because the Al was introduced as Al citrate, and it is estimated transferrin could not have complexed more than 15% of the Al in plasma at the lowest Al blood level and only 2% at the highest, it is likely that most Al in plasma and essentially all in brain ECF, where the transferrin concentration is <1% of that in plasma, was associated with citrate. The Al recovered by microdialysis could not have been Al transferrin because transferrin is too large to penetrate the membrane of the microdialysis probes we used. The marker of BBB permeability included in most of these studies, 4-trimethylammonium antipyrine (*34*), did not indicate that BBB integrity was significantly compromised. These results suggest Al citrate can enter the brain from blood through the BBB by a mechanism other than diffusion.

Mechanism(s) of Aluminum Distribution across the BBB

Many carriers have been reported at the BBB (*35*). A review of these carriers suggests some candidates that might mediate Al citrate transport across the BBB, including TfR-ME. However, Al citrate can also enter the brain by one or more other mechanisms. Assuming that TfR-ME clears Al from blood into brain ECF across the BBB at the same rate as it clears iron [3×10^{-3} mL blood/gm brain/hr (*36*)], the Al that could enter brain ECF from blood in 5 min would result in a BBr of 0.0004 based on estimated plasma ECF Al concentrations up to 1 mM from our microdialysis results. The observed BBr was much greater than this value. Calculation of Al speciation at pH 7.4 showed approximately 1.25 and 29% of the Al would be present as $\mathrm{Al(H_{-1}citrate)^-}$ and $\mathrm{Al(H_{-1}citrate)(OH)^{2-}}$, respectively, in the presence of 1 mM citrate. The percentage of these species would increase at lower Al citrate concentrations (*37*). In these complexes there is a single, noncomplexed, ionized carboxylate group. The presence of this monocarboxylate suggested that Al citrate may be a substrate for the monocarboxylate transporter (MCT) at the BBB. At least one MCT isoform, MCT1, has been shown to be expressed at the BBB. The reported rate of substrate transfer by the MCT across the BBB, 60 nmol/g brain/min, could transport 40,000 ng Al, as the citrate, into 1 mL brain ECF in 5 min after the intravenous bolus Al injection described above if Al citrate serves as a comparably transported substrate. This is about 15 times the estimated rate of appearance of Al in brain ECF that we observed. This calculation is based on the observed 2.8 mM Al in frontal cortex dialysate within 5 min of intravenous Al citrate injection and an estimated 3.25% relative recovery, yielding an estimated 2,520 ng Al/mL ECF.

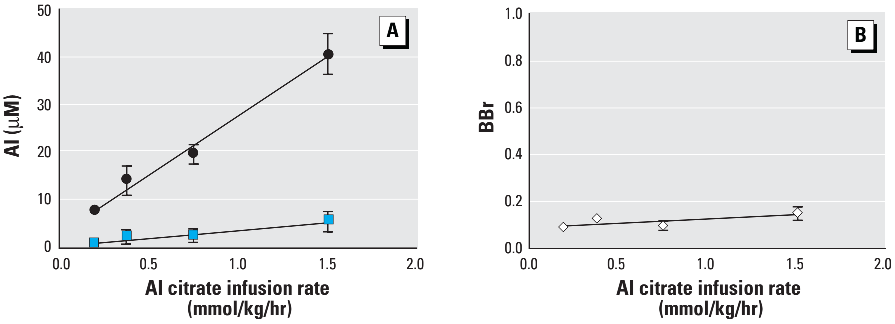

Figure 3. Steady-state Al concentrations determined by atomic absorption spectrometric analysis of microdialysis samples from the brain and blood of rats during intravenous infusion of four Al citrate concentrations. (*A*) Steady-state Al concentration in dialysate exiting microdialysis probes implanted in blood ECF (plasma; circles) and brain ECF (squares) at various infusion rates of Al citrate. (*B*) Steady-state BBr of Al for the results shown in *A*. Values shown are means ± SEM of five animals at each infusion rate. Lines show linear regressions. From Allen et al. (*54*).

To test the hypothesis that MCT1 mediates Al citrate transport across the BBB, substances were included in the dialysate of the microdialysis probe implanted in the frontal cortex of rats that were receiving an intravenous infusion of Al citrate. These substances were expected to diffuse from the dialysate into the brain to achieve sufficient concentrations to produce local effects in the same brain region where BBB function was being studied. Addition of 15 µM $\mathrm{CN^-}$ or 10 µM 2,4-dinitrophenol, as metabolic inhibitors, 1 M pyruvate as a substrate for the MCT, and a pH 10.2 dialysate or 1 mM carbonylcyanide-*p*-(trifluoromethoxy)phenylhydrazone to reduce proton availability and proton gradients significantly increased the BBr to approximately 1 (*38,39*). Similar increases in the BBr were not observed in the contralateral frontal cortex nor was the integrity of the BBB significantly compromised. These results suggest inhibition of an energy-dependent process, competition for MCT transport, and reduction of protons inhibited carrier-mediated Al citrate transport across the BBB, leaving only diffusion mediating its BBB permeation. The results were consistent with the hypothesis that Al citrate is a substrate for MCT-mediated transport across the BBB. To further test this hypothesis, studies were conducted with the rat erythrocyte because it expresses MCT1 and the band 3 anion exchange transporter. However, the uptake of a known substrate, ^{14}C-lactate, was not inhibited by Al citrate, nor was there significant uptake of Al ^{14}C-citrate. These results suggest Al citrate is not an effective substrate for either MCT1 or the band 3 anion exchange transporter.

To characterize, and perhaps identify, the carrier(s) mediating Al citrate transport across the BBB, Al citrate uptake studies were conducted using b.End5 cells, an immortalized cell line derived from murine brain endothelial cells. Al ^{14}C-citrate uptake was slow and reasonably linear for 4 hr. Uptake of Al ^{14}C-citrate was approximately 70% greater than ^{14}C-citrate uptake. Uptake after 1 hr achieved an intracellular Al citrate concentration of approximately 25% of the medium concentration. In comparison, diffusion was calculated to produce an intracellular Al citrate concentration of approximately 1% of the medium concentration. These results suggested carrier-mediated uptake.

Al ^{14}C-citrate uptake into b.End5 cells was pH independent, sodium independent, and energy dependent and was inhibited by numerous nonspecific substrates and inhibitors of MCT and organic anion transporters. Uptake was concentration dependently inhibited by two relatively specific organic anion transporter substrates (*37*). Comparison of the characteristics of the carrier(s) mediating Al citrate uptake to those of carriers described at the BBB suggests Al citrate transport may be mediated by an organic anion transporter such as an MCT isoform and/or an organic anion–transporting polypeptide (oatp). Further work elucidating which of the MCT and oatp isoforms are expressed at the BBB and the identification of isoform-selective inhibitors that could be used in future studies of Al citrate uptake into brain endothelial cells or the brain would advance the ability to identify the BBB Al citrate transporter(s).

Some Aluminum Persists in the Brain for a Long Time

We conducted a study to ascertain the residence time of Al in the brain. Administration of the stable, ubiquitous isotope of Al, ^{27}Al, is not well suited to determine the brain half-life of Al because brain Al concentrations are relatively small and do not greatly increase in response to Al exposure. Therefore, an elevation above, and subsequent decrease toward, the endogenous Al concentration is difficult to reliably determine. The natural abundance of ^{26}Al is extremely small. ^{26}Al can be quantified with exquisite sensitivity (~1×10^6 atoms) as the ^{26}Al:^{27}Al isotopic ratio by accelerator mass spectrometry (AMS), enabling the study of Al toxicokinetics at physiologically relevant Al exposures (*40*). A disadvantage is the cost of AMS analysis of ^{26}Al (~\$200/sample). We gave rats intravenous ^{26}Al transferrin or ^{26}Al citrate and terminated them from 4 hr to 256 days later to determine the percentage of Al in blood that enters the brain, the time course of Al efflux from the brain and whether brain Al clearance is enhanced by repeated chelation therapy with desferrioxamine, a clinically-useful Al chelator. The peak brain ^{26}Al concentration, approximately 0.005% of the ^{26}Al dose per gram of brain, was similar after ^{26}Al transferrin and ^{26}Al citrate dosing and was similar to some previous, smaller, short-term studies (*41*). The comparable results from Al citrate and Al transferrin are probably due to the change in the Al ligand from citrate to transferrrin, the preferred ligand, within minutes because the administered Al citrate dose was only about 1% of the plasma transferrin metal-binding capacity. The half-life of brain ^{26}Al could not be accurately calculated but was estimated to be about 150 days. The brain half-life was roughly 55 days in rats that received desferrioxamine injections three-times weekly, demonstrating brain Al retention in compartments from which it can be mobilized (Figure 4). It is difficult to extrapolate these results to the human because of the lack of comparable metal half-life determinations in rat versus human brain or sufficient insight into allometric scaling from rat to human brain for metals. The residence of Al in the brain and other soft tissue organs may reflect the residence of Al in bone, which contains approximately 50–70% of the Al body burden. The bone ^{26}Al half-life is prolonged in the rat (*42*).

Manganese Citrate Enters the Brain by a Carrier-Mediated Process

We recently began studies of Mn distribution across the BBB. Brain uptake of small-molecular-weight Mn species was previously attributed to a carrier-mediated process, but the work was not conducted under conditions that controlled the Mn species (*43,44*). The experimental approach we employed was to compare brain influx and efflux rates determined in the rat to BBB permeation rates predicted for capillary diffusion to ascertain if there was evidence for carrier-mediated influx or efflux of Mn. To study brain Mn uptake under conditions where better control of the Mn species could be maintained, we used ^{54}Mn species and the *in situ* brain perfusion technique (*45*), as modified by Allen and Smith (*46*). Brain influx of the Mn^{2+} ion and Mn citrate, which were calculated to represent 40 and 12%, respectively, of non–protein-bound Mn in plasma (*47*), were studied, as well as Mn transferrin. The brain uptake rates determined in the intact rat were compared with the estimated brain capillary diffusion rates of these Mn species, which were calculated from the their molecular weight and lipophilicity (Figure 5). Lipophilicity was determined as the partitioning coefficient between octanol and an aqueous phase. The estimated brain capillary diffusion rates were then calculated from the relationship between molecular weight and lipophilicity for substances that diffuse through the BBB (*23*) times the brain capillary surface area (240 cm^2/g brain). The estimated brain capillary diffusion rates of the Mn ion, Mn citrate, and Mn transferrin ranged from 1.5 to 2.8×10^{-5} mL/sec/g (*48*).

The average observed brain uptake rates of the Mn citrate, Mn ion, and Mn transferrin into the nine brain regions sampled were 25, 8.6, and 5.7×10^{-5} mL/sec/g,

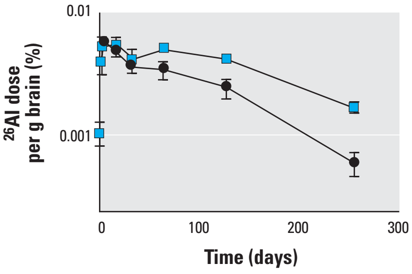

Figure 4. Brain ^{26}Al determined by AMS in rats terminated at various times after intravenous administration of ^{26}Al transferrin. The rats received an injection of saline 3 times weekly (squares) or 0.15 mmol/kg desferrioxamine (circles). Values are the means ± SEM of results from four to six rats and are shown as the percentage of the injected ^{26}Al per gram of brain. From Yokel et al. (*41*) with permission of Oxford Press.

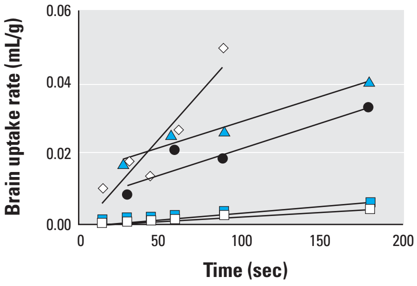

Figure 5. The brain (parietal cortex) uptake rates for three Mn species determined after their intravenous infusion as ^{54}Mn in the rat, using the *in situ* brain perfusion method. The rat received an intra-arterial perfusion for a variable duration (shown) of one of the three Mn species: Mn citrate (diamonds), Mn transferrin (triangles), or Mn^{2+} ion (circles). Values are mean from 7–11 rats per data point except for Mn citrate (diamonds), $n = 4$ for 90 sec. The symbols for Mn transferrin and Mn citrate at 30 and 60 min are shown slightly to the left and right, respectively, of their true values to enable their visualization. Squares show upper and lower range of brain uptake predicted for these three Mn species for capillary diffusion. Lines are best-fitted linear regressions of the means.

respectively, suggesting carrier-mediated uptake of each Mn species. To verify that Mn was entering the brain, compared to being adsorbed onto or taken into but not through brain endothelial cells, the capillary depletion method was used to separate brain capillary cells from the rest of the brain (*49*). Only 8–25% of the Mn was associated with capillary cells, suggesting the *in situ* brain perfusion studies were showing Mn distribution across the BBB (*47*).

Brain Manganese Efflux Does Not Appear to Be Carrier Mediated

The efflux of Mn out of the brain across the BBB was determined using an established method (*50*). Efflux through brain capillaries (the BBB) was calculated from the product of the volume of distribution of the Mn within the brain, from which it can efflux to blood, times the brain elimination rate constant. The volume of Mn distribution in the brain was determined from uptake of ^{54}Mn as the ion, citrate, and transferrin into rat parietal brain slices versus time. In contrast to brain slice uptake of *para*-aminohippurate, which reached a plateau at approximately 60 min (*51*), uptake of these Mn species continued to increase for up to 180 min, suggesting continued brain cell uptake. The brain elimination rate constant of these three Mn species was determined from the percentage of ^{54}Mn remaining in the brain at various times after injection as ^{54}Mn ion, citrate, or transferrin into the parietal cortex, compared with the percentage of ^{14}C-sucrose or ^{14}C-dextran, which are expected to very slowly efflux from the brain by diffusion across the BBB. The Mn species did not efflux from the brain more rapidly than sucrose or dextran. Taken with the parietal slice uptake results, the lack of brain efflux suggests Mn continues to be taken up into brain cells over time and is not transported out of the brain by carrier-mediated processes (*52*).

Carriers Mediate the Permeation of Aluminum and Manganese across the BBB

The results of the studies reviewed herein suggest that there are carriers at the BBB that mediate the uptake of Al and Mn into the brain. This may be a beneficial process for Mn, an essential element for brain metabolism. However, Al is not essential and, like Mn, is neurotoxic when sufficient brain concentrations are achieved. The results suggest that there is a carrier-mediated mechanism to protect the brain from Al, by effluxing it across the BBB into blood. It does not appear that a similar protective mechanism is present for Mn. Further work is necessary to identify the carriers mediating transport of these metals across the BBB.

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