

BLOOD–BRAIN BARRIER TO PERTECHNETATE FOLLOWING DRUG-INDUCED HYPOTENSION

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There is some disagreement on the effects of profound hypotension and ischaemia on the blood–brain barrier (BBB). In a study in the gerbil, of the penetration of the BBB by Evan's Blue–albumin following clamping of one carotid artery [1], it was found that no breakdown of the BBB occurred with less than 30 min occlusion, longer periods of ischaemia producing breakdown which occurred in some animals only at 5–29 h after restoration of blood flow. In contrast, dogs subjected to hypotension to a mean cerebral perfusion pressure of 30–45 mm Hg showed extensive breakdown of the barrier to Evan's Blue–albumin complex immediately after the end of the hypotensive episodes [2]. Differentiating between the effects of ischaemia and infarction, Petito and colleagues [3] concluded that the BBB remains impermeable to large molecules for 15–30 min after reperfusion following ischaemia without infarction. In the same model Sage, Van Uitert and Duffy [4] showed that, in contrast to large molecules, a small molecule (leucine) did leak through the BBB 15 min after reperfusion. Some of these differences may result from the dissimilar procedures used for producing ischaemia, or species differences, small molecules seeming more sensitive than large ones to breakdown of the BBB.

If breakdown of the BBB to large molecules is widespread following hypotension [2], it might be useful to develop a probe molecule technique for clinical use as a sensitive diagnostic and prognostic test of brain injury in unconscious man.

SUMMARY

The integrity of the blood–brain barrier (BBB) was examined in rabbits, in terms of the partition of ^{99m}Tc -pertechnetate ($^{99m}\text{TcO}_4^-$) between brain and blood, following intracarotid injection of hypertonic arabinose, hypertension (168 mm Hg and 10% inspired carbon dioxide), or hypotension (< 20 mm Hg for 15 min). Corrections were made for changes in tissue blood contents, using chromium-51 as a red cell marker. In control animals the mean brain: blood ratio was 0.038 (range 0.027–0.052). Following arabinose there was a five-fold increase in mean BBB permeability (mean brain: blood ratio 0.192 (0.070–0.378)). There was no change after hypertension and carbon dioxide (mean ratio 0.034) or after hypotension (mean ratio 0.032), despite an increase in cerebral extracellular potassium. Examination of other tissues showed no change in the $^{99m}\text{TcO}_4^-$ tissue/blood partition in heart muscle in any study but, following hypotension, ratios in the kidney (mean ratio 1.63) and, to a lesser extent, the liver (mean ratio 1.37) had increased, suggesting an abnormality of active transport under these conditions. We conclude that, while $^{99m}\text{TcO}_4^-$ tissue/blood partitioning revealed osmotic disruption of the BBB, profound hypotension with evidence of brain cell damage did not change BBB permeability to the same marker. Hypotension may influence active transport of this ion in liver and kidney.

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We chose the probe molecule ^{99m}Tc -pertechnetate ($^{99m}\text{TcO}_4^-$, mol. wt 163 Daltons) for two reasons: the radionuclide has a short half-life, rapid elimination from the body, and is readily available and easily detected; and it has been shown [4] that a small molecule is even more sensitive to BBB breakdown than is albumin.

Although there are a number of techniques used to detect brain injury *in vivo*, all are invasive and impractical for clinical use. The technique for measuring the change in extracellular potassium is well established and we have used this as an indicator of brain injury following hypotension. To evaluate the permeability model, we have compared the partitioning of $^{99m}\text{TcO}_4^-$ between blood and brain and other tissues, in control animals, and following osmotic disruption of the BBB, acute hypertension or profound hypotension.

MATERIALS AND METHODS

Thirteen New Zealand White rabbits (mean weight 3.9 kg (range 2.8–5.3)), were anaesthetized with Hypnorm (fluanisone 3.3 mg kg⁻¹ + fentanyl 0.1 mg kg⁻¹) 0.33 ml kg⁻¹ i.m. Cannulae were inserted into an artery and a vein in the ear. Tracheotomy was performed and the animals were paralysed with pancuronium 4 mg and the lungs ventilated artificially to an airway pressure of 15–20 cm H₂O with 3–4 cm H₂O of PEEP, using 50% nitrous oxide in oxygen. Anaesthesia was maintained by a continuous infusion via the ear vein of Hypnorm 0.1 ml kg⁻¹ h⁻¹ and pancuronium 1 mg kg⁻¹ h⁻¹. Saline 0.9% was also given at 5 ml kg⁻¹ h⁻¹, with 3 ml kg⁻¹ h⁻¹ boluses of hydroxyethyl starch (American Hospital supply) if required to maintain blood volume and arterial pressure. Airway pressure and arterial pressure were measured continuously using Statham pressure transducers and a Devices carrier amplifier with signals recorded on a Devices multichannel recorder.

The animals were allocated to four groups. *Group 1* were controls.

Animals in *group 2* were subjected to osmotic disruption of the BBB with 30 ml of arabinose 1.8 mol litre⁻¹ injected slowly over 30 s into the internal carotid artery [5]. This vessel was dissected out and clamps placed on the external carotid artery and the artery to the pinna on the side of injection.

Hypertension was induced in *group 3* animals using an i.v. bolus of angiotensin II, 20 µg kg⁻¹ [6]. To potentiate the effects of angiotensin II, the lungs were ventilated during this period with a nitrous oxide in oxygen mixture containing 10% carbon dioxide.

Craniotomy was performed in *group 4* animals and a potassium ion sensitive electrode was

inserted 1 mm into the parietal cortex. The electrodes detected the extracellular potassium throughout the course of the study, their signals being processed by a high impedance amplifier and recorded on a Watanabe MC6623 recorder [7]. The potassium electrodes were calibrated [7] using six dilutions of potassium chloride in sodium chloride, to maintain a constant chloride concentration of 150 mmol litre⁻¹ and potassium ion concentration of 1, 3, 5, 10, 25 and 50 mmol litre⁻¹. The cationic balance was maintained at 150 mmol litre⁻¹ with sodium ions. Calibrations were carried out before each insertion into the brain and after termination of the study, the difference in calibrations being approximately 1 mmol litre⁻¹. In every case there was a large increase in potassium ion concentration to more than 50 mmol litre⁻¹ on final cardiac arrest induced with i.v. potassium chloride. Hypotension was induced with an infusion of trimetaphan 10 mg kg⁻¹ h⁻¹ i.v., 1% inspired halothane and a variable infusion of sodium nitroprusside (total dose 1.65–6.25 mg kg⁻¹). Fine adjustment was achieved by blood letting (up to 10 ml kg⁻¹). Mean arterial pressure (MAP) was reduced steadily, over a period of 25–30 min, to between 17 and 20 mm Hg. It was then maintained at this value for 15 min. This pressure was that found, by preliminary studies, to produce an abrupt increase in cortical extracellular potassium concentration.

In each group of animals two radiolabelled molecules were used. Red cells were labelled *in vitro* with chromium-51 to quantify the blood volume in tissues sampled at the end of the study. A 10-ml blood sample was taken from each animal at the start of the study and chromium-51, 1 MBq was added with citrate dextrose solution. After 30 min, the blood was centrifuged and the supernatant removed. The cells were washed, re-suspended and re-centrifuged three times. The red cells were re-constituted with 0.9% saline to a volume of 10 ml and injected back into the animal, together with $^{99m}\text{TcO}_4^-$ 100 MBq 5 min before any experimental manoeuvre. Fifteen minutes after a manipulation was finished, the anaesthetized animal was killed with a potassium injection, a blood sample being taken ante-mortem. Tissues were removed, weighed and counted in a well counter to quantify chromium-51 and technetium-99m. In each case the $^{99m}\text{TcO}_4^-$ count for the tissue sample was corrected for the blood content of each tissue as measured by the

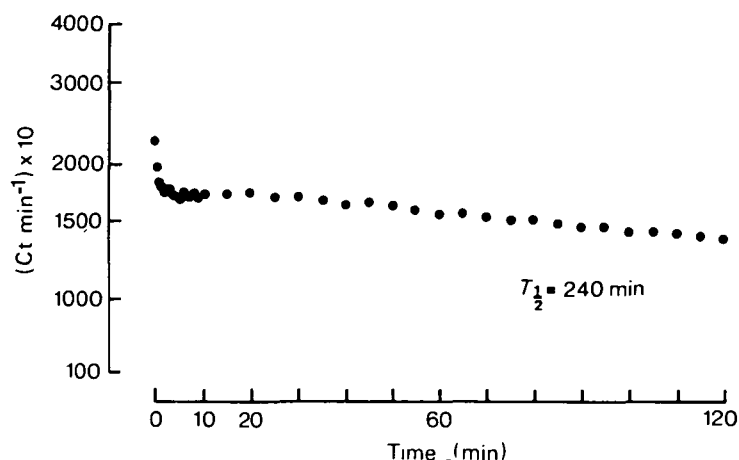


FIG. 1. Typical decay curve for external scintillation counts, over the praecordial region.

TABLE I. Brain: blood partition ratios ($\times 100$) for pertechnetate in individual rabbits

Group	Tissue	
	Left brain	Right brain
Control		
1	2.8	2.7
2	5.1	5.2
3	3.5	3.4
Osmotic opening of BBB		
1	6.6	16.0
2	13.0	8.6
3	37.8	33.0
Hypertension		
1	3.5	3.1
2	2.5	3.8
3	5.0	2.8
Hypotension		
1	3.8	1.4
2	3.4	4.9
3	2.9	2.8
4	2.1	1.8

chromium-51. A corrected $^{99m}\text{TcO}_4^-$ tissue: blood partition ratio was calculated.

Data were analysed using the Randomization test for two independent samples [8].

RESULTS

Clearance of pertechnetate

An external scintillation detector over the rabbit's heart was tuned to detect technetium-99m and this showed a clearance half-life of approximately 4 h (fig. 1).

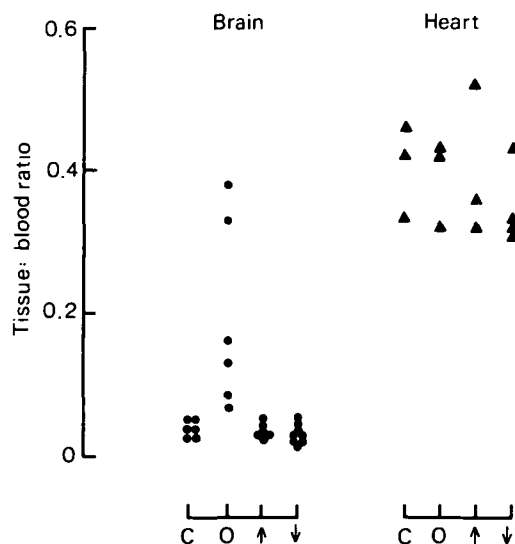


FIG. 2. Pertechnetate tissue: blood partition ratios, corrected for blood content, for brain and heart in the four groups (C = control; O = osmotic opening of BBB; ↑ = hypertension; ↓ = hypotension).

Tissue: blood pertechnetate partition ratios

Blood: tissue partition ratios of $^{99m}\text{TcO}_4^-$ in the myocardium showed no differences in any group (fig. 2).

For brain, each hemisphere was sampled separately, so two values for each animal are shown in figure 2 and table I. Significant differences were found, but these should be interpreted with caution because of the multiple comparisons.

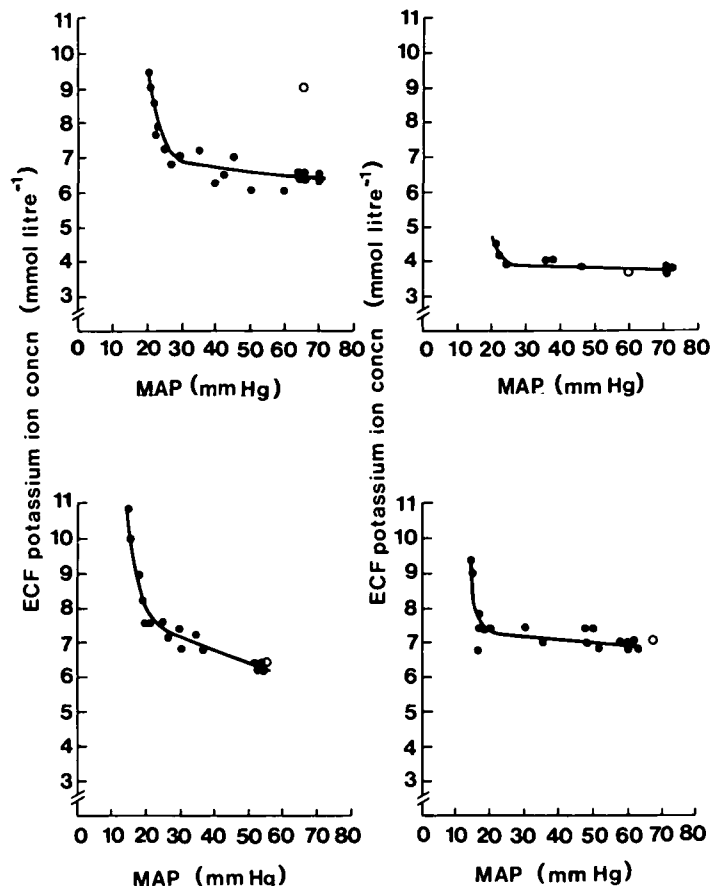


Fig. 3. Cortical extracellular potassium changes plotted against mean arterial pressure (MAP) for four animals. Open circles show values of ECF potassium on returning MAP to normal.

Nevertheless, brain tissue from animals subjected to osmotic damage (group 2) showed a significant five-fold increase in mean brain:blood ratio, each value being greater than control ($P = 0.05$, Randomization test for two independent samples on mean values of left and right brain sample pairs). Compared with control values there was no difference in the partitioning of $^{99m}\text{TcO}_4^-$ between brain and blood in the groups subjected to hypertension (group 3) or to hypotension (group 4). In group 3 there was an increase in mean arterial pressure (MAP) from a mean of 70 to a mean maximum of 168 mm Hg.

Ion flux studies in four animals are shown in figure 3. It was necessary to reduce MAP to < 25 mm Hg before a large flux in cortical extracellular potassium occurred. Even so, when potassium flux indicated brain cell injury, there was no change in the partitioning of $^{99m}\text{TcO}_4^-$

TABLE II. Tissue:blood partition ratios for pertechnetate in the control group. *One sample only

Tissue	Mean	Range
Brain	0.04	0.03–0.05
Myocardium	0.40	0.33–0.46
Liver	0.49	0.43–0.56
Kidney	1.02	0.96–1.07
Quadriceps muscle	0.12	0.11–0.14
CSF	0.03*	—
Submandibular	0.56	0.54–0.58
Parotid	0.32	0.18–0.42
Tongue	0.43	0.42–0.45
Skull	0.14	0.10–0.18
Scalp	0.37	0.29–0.44

across the BBB in the group (4) subjected to profound hypotension.

Other tissues were examined also (table II). The values for liver in the control animals were

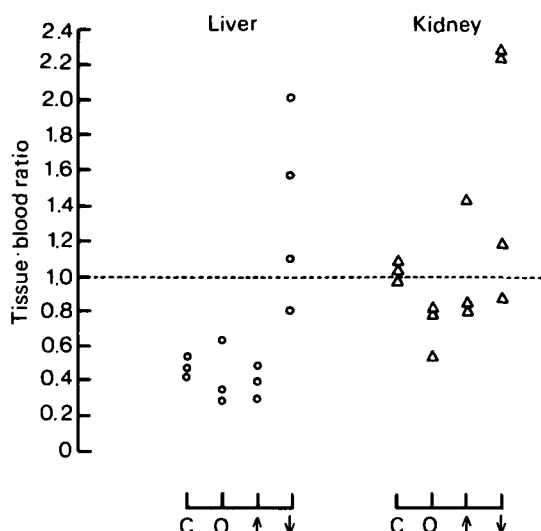


FIG. 4. Perchnetate tissue: blood partition ratios, corrected for blood content, for liver and kidney in the four groups (C = control; O = osmotic opening of BBB; ↑ = hypertension; ↓ = hypotension).

essentially the same as in the groups subjected to osmotic damage or hypertension (fig. 4). However, the values in the group subjected to hypotension were significantly different from those in the control group ($P < 0.03$, Randomization test for two independent samples), with three of the four values greater than unity.

The kidney showed a wider scatter of values, the hypotension and the osmotic groups showing trends to higher and lower values than controls, respectively (fig. 4). The group subjected to hypotension was not significantly different in view of small numbers and the scatter, but three of the four values in that group were greater than unity.

DISCUSSION

The main finding in this study was that, in contrast with the effect of opening the BBB by osmotic insult, profound hypotension sufficient to produce brain injury did not alter BBB function as measured by partitioning of $^{99m}\text{TcO}_4^-$ between brain and blood.

Brain injury was demonstrated at the time of these partitioning measurements by direct measurement of potassium concentrations in cerebral extracellular fluid. This model of cerebral dysfunction has been compared recently with changes in mitochondrial redox activity as an indicator of

litre $^{-1}$ of potassium ion did not occur until reduction of cytochrome a , a_3 reached $> 80\%$. Increases in potassium ion > 1 mmol litre $^{-1}$ were associated with complete reduction of cytochrome and reached a mean change of 3.29 (0.1) mmol litre $^{-1}$ [9], similar to those recorded in this study during hypotension.

The hypotension we achieved also produced no effect on partitioning of $^{99m}\text{TcO}_4^-$ in the myocardium, but was sufficient to influence partitioning in the liver and kidney. The mean values in the latter tissues were greater than unity, implying an interference with an active transport mechanism rather than a change in passive diffusion. This may be explained by the greater susceptibility to hypoxia of active transport mechanisms which remove the tracer from liver and kidney cells, compared with the susceptibility of mechanisms which transport it into these cells.

We used angiotensin II to increase MAP as described by Edvinsson and colleagues [6], but in our study hypertension combined with hypercapnia did not influence the partitioning of $^{99m}\text{TcO}_4^-$. The mean change in MAP in our study was the same as that reported in rats by this group, but their animals had a greater MAP (180 mm Hg) compared with ours (168 mm Hg). In contrast, they showed that angiotensin II produced an increase in protein leakage into the brain, but demonstrated an even greater protein flux with adrenaline, despite a similar increase in MAP. However, it is unclear whether this represents an increase in permeability of the paracellular pathway or an increase in the transcellular pathway resulting from increased pinocytosis [10].

An explanation of the differences between our study and previous reports is the frequent large percentages of tissue blood content recorded, especially following manoeuvres which might have increased vascular engorgement. Without correcting for the blood content in tissue, we would have shown spuriously high $^{99m}\text{TcO}_4^-$ counts resulting entirely from increased blood content, rather than a true increase in partitioning.

In contrast with the effect of hypotension, the osmotic disruption of the BBB produced a striking difference in $^{99m}\text{TcO}_4^-$ partitioning, showing that our model was sensitive to changes in BBB function. Our results are consistent with previously published studies on the effects of hyperosmolar solutions in disrupting the BBB [11–13]. A

greater increase in tissue $^{99m}\text{TcO}_4^-$ in the hemisphere ipsilateral to the intracarotid injection was not a consistent finding, and implies a fairly uniform distribution of arabinose across the circle of Willis. It has been shown that these solutions open the BBB by a non-energy dependent mechanism [13] which is unlikely therefore, to involve pinocytosis. The technique is well established, is reversible and is used in the therapy of human central nervous system malignancies [14].

Our findings conflict with those of Ishikawa and colleagues [2] who showed that hypotension caused either a major breakdown of the BBB with a leak of protein from the intravascular space, or some alteration in a selective active transport system or pinocytosis [10]. The time scale of our study was similar to that in the Ishikawa study, but we found no change in the flux of our much smaller molecule. Furthermore, we also administered Evan's Blue in two preliminary hypotensive experiments, but found no blue staining of the brain in any section. This suggests that there are species differences in addition to different mechanisms of active transport for the various solute molecules crossing the BBB.

$^{99m}\text{TcO}_4^-$ is a complex molecule and may behave as a pseudohalide. There is little information on partitioning of $^{99m}\text{TcO}_4^-$ between brain and blood. The pulmonary epithelium, although not presenting as formidable a barrier as the BBB to penetration of hydrophilic molecules, has been studied [15] in terms of its permeability to $^{99m}\text{TcO}_4^-$ (mol.wt 163 Daltons), chloride and mannitol (mol.wt 160 Daltons). It was found that chloride and $^{99m}\text{TcO}_4^-$ had similar permeabilities and that, in penetrating the epithelia, 50 % of each passed through the cell wall and 50 % through the intercellular junctions (paracellular pathway). In contrast, mannitol has a permeability coefficient less than 50 % that of $^{99m}\text{TcO}_4^-$ and, as a hydrophilic molecule, it is assumed to pass entirely through the paracellular pathway. These results suggest that, if the impermeability of the BBB to $^{99m}\text{TcO}_4^-$ remains intact after hypotension, then so does the impermeability of the BBB for mannitol, explaining its continued efficacy in the management of cerebral oedema under such circumstances. A seven-fold difference in the permeability of the epithelium to $^{99m}\text{TcO}_4^-$ has also been reported [15], depending on the direction of movement; that is, permeation from mucosa to serosa is less than in the opposite direction. This demonstrates an active transport

system for $^{99m}\text{TcO}_4^-$ at least in the pulmonary epithelium.

Sucrose (mol.wt 342 Daltons) and ^{51}Cr -EDTA (mol.wt 341 Daltons), a non-charged hydrophilic molecule, have a low permeation of the BBB similar to that of mannitol [16]. The very low concentration of all these molecules in the brain, even after allowing prolonged periods for diffusion to occur, implies:

- (1) A low penetration of the BBB because of greatly impaired diffusion through the paracellular pathway.
- (2) An active transport system that is eliminating these molecules from the brain to the blood across the BBB.
- (3) Some active transport from the blood to the brain, but a more effective transport system out of the brain at the choroid plexus which removes the tracer.

Active transport mechanisms may be involved in $^{99m}\text{TcO}_4^-$ transport in the liver and kidney, and may be affected by severe hypotension. However, in these experiments, severe hypotension did not affect these mechanisms in the brain.

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REFERENCES

1. Ito U, Go KG, Walker JT, Spatz M, Klatzo I. Experimental cerebral ischaemia in Mongolian Gerbils III. Behaviour of the blood brain barrier. *Acta Neuropathologica* 1976; 34: 1-6.
2. Ishikawa T, Funatsu N, Okamoto K, Takeshita H, McDowall DG. Blood-brain barrier function following drug-induced hypotension in the dog. *Anesthesiology* 1983; 59: 526-531.
3. Petito CK, Pulsinelli WA, Jacobsen G, Plum F. Edema and vascular permeability in cerebral ischaemia. Comparison between ischaemic neuronal damage and infarction. *Journal of Neuropathology and Experimental Neurology* 1982; 41: 423-436.
4. Sage JI, Van Uiter RL, Duffy TE. Early changes in blood brain barrier permeability to small molecules after transient cerebral ischaemia. *Stroke* 1984; 15: 46-50.
5. Chieu CD, Sun CL, Kopin IJ, Fredericks WR, Rapoport SI. Entry of [^3H]-norepinephrine [^{125}I]-albumin and Evan's Blue from blood into brain following unilateral osmotic opening of the blood brain barrier. *Brain Research* 1978; 145: 291-301.
6. Edvinsson L, Johansson BB, Larsson B, Mackenzie ET, Skarby T. Calcium antagonists effects on cerebral blood flow and blood brain barrier permeability in the rat. *British Journal of Clinical Pharmacology* 1983; 79: 141-148.

7. Morris PJ, Heuser D, McDowall DG, Hashiba M, Myers D. Cerebral cortical extracellular fluid H^+ and K^+ activities during hypotension in cats. *Anesthesiology* 1983; 59: 10–18.
8. Seigel S. *Non-Parametric Statistics for the Behavioural Sciences*. Tokyo: McGraw Hill, 1956; 152–154.
9. Milito SJ, Raffin CN, Rosenthal M, Sick TJ. Potassium ion homeostasis and mitochondrial redox activity in brain: relative changes as indicators of hypoxia. *Journal of Cerebral Blood Flow and Metabolism* 1988; 8: 155–162.
10. Johansson B. Pharmacological modification of hypertensive blood–brain barrier opening. *Acta Pharmacologica et Toxicologica* 1981; 48: 242–247.
11. Rapoport SI, Hart M, Klatzo I. Reversible osmotic opening of the blood–brain barrier. *Science* 1971; 173: 1026–1028.
12. Inoue T, Nagara H, Kondon A, Fukui M, Tateishi J. Effects of intracarotid hyperosmolar mannitol in triethyl tin (TET)-induced rat brain edema; preservation of blood–brain barrier (BBB) in TET edema. *Brain Research* 1987; 414: 309–313.
13. Greenwood J, Luthert PJ, Pratt OE, Lantos PL. Hyperosmolar opening of the blood–brain barrier in the energy-depleted Rat brain. Part 1. Permeability studies. *Journal of Cerebral Blood Flow and Metabolism* 1988; 8: 9–15.
14. Williams WT, Lowry RL, Eggers GWN. Anesthetic management during therapeutic disruption of the blood–brain barrier. *Anesthesia and Analgesia* 1986; 65: 188–190.
15. Man SFP, Ahmed IH, Man GCW, Nguyen A. Characteristics of pertechnetate movement across the canine tracheal epithelium. *American Review of Respiratory Diseases* 1985; 131: 90–93.
16. Bradbury M. *The Concept of a Blood–Brain Barrier*. Chichester: John Wiley & Sons, 1979; 101.