Intravenous Mannitol Therapy in a Vasogenic Cerebral Edema Model: Quantitative Measurements of Tissue Concentrations and Effect

A thesis presented to the University of Manitoba

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Degree of Master of Science in Surgery

Anthony Michael Kaufmann M.D., B.Sc.(Medicine), F.R.C.S.(C.)

Assistant Professor, Department of Neurological Surgery

University of Pittsburgh

Suite B-400

Presbyterian University Hospital

200 Lothrop Street

Pittsburgh, Pennsylvania, 15213-2582

Phone:(412) 647-6358

Fax:(412) 647-5996

E-mail: kaufmann@neuronet.pitt.edu

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INTRAVENOUS MANNITOL THERAPY IN A VASOGENIC CEREBRAL EDEMA MODEL: QUANTITATIVE MEASUREMENTS OF TISSUE CONCENTRATIONS AND EFFECT

BY

ANTHONY MICHAEL KAUFMANN

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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ABSTRACT

Intravenous administration of mannitol is a universally accepted cornerstone in the treatment of cerebral herniation and acute elevations of raised intracranial pressure. Its clinical efficacy in these emergent situations is well established. Prolonged elevations of intracranial pressure are also commonly treated with repeated doses of mannitol, although the benefit has never been proven. It has been proposed that a potential adverse consequence of this treatment is rebound cerebral edema, due to cerebral accumulation of mannitol. This has been difficult to substantiate or refute based upon clinical observations, due to the heterogeneity of the brain injured patient population, and the unpredictable nature of cerebral swelling and edema.

The purpose of the present investigation was to study the pharmacokinetics of mannitol administered for treatment of vasogenic cerebral edema. A brain injury was produced with a cortical cold-injury in 23 cats, maintained under general anaesthesia for five or twenty-one hours. Control animals received no mannitol, while treatment groups received either a single dose or five four-hourly doses of 1/3 g/kg radio-labeled mannitol. Liquid scintillation counting was employed to determine the concentrations of mannitol in the cerebral tissue, cerebrospinal fluid, plasma, and urine. Cerebral water content and linear progression of edema were also measured.

Rapid plasma clearance prevented accumulation of mannitol after multiple intravenous injections, as 84 ± 2% of the infused mannitol was excreted through the urine. However,

there was progressive accumulation of mannitol within cerebral tissue, especially in the edematous white matter, where it reached 0.33 ± 0.03 mg/g after five doses, exceeding the trough plasma concentrations by a ratio of 2.69 to 1. Water content measurements showed that single-dose mannitol failed to reduce cerebral water content and edema progression at four hours post-injection, while multiple-dose mannitol treatment produced a 3% increase of water content in edematous regions ($p=0.003$). These results demonstrated a reversal of the osmotic concentration gradient between edematous brain and plasma following multiple mannitol doses, associated with exacerbation of vasogenic cerebral edema.

KEY WORDS: mannitol, vasogenic cerebral edema, cerebral water content, cold-injury, radio-label

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I dedicate this to my family, who have shaped my past and present, and inspire my vision of the future.

A.M.K.

INTRODUCTION

BACKGROUND

The osmotic diuretics were introduced into neurosurgical practice more than 30 years ago. Mannitol has become the most widely employed of these agents, and it is a cornerstone of treatment for patients with suspected or measured intracranial pressure elevations. A single dose has proven effective in transiently reducing cerebral bulk, and thereby reducing intracranial pressure and displacement of brain tissue by intracranial masses [2,17,28,46,55,56,57,61,63,70,71]. These observations have led to the common clinical practice of administering repeated doses of mannitol to patients with brain injury and sustained intracranial pressure elevations, since cerebral edema and raised intracranial pressure are important factors related to poor neurological outcome following brain injury [8,9,35,36,50,51,73,76]. However, no placebo controlled clinical trials have been performed to validate the use of repeated doses of mannitol in the setting of brain injury. Furthermore, it has been proposed that mannitol may extravasate across damaged and leaky cerebral capillaries and accumulate in cerebral tissue, resulting in a reversed osmotic gradient which may increase the cerebral water content and cause rebound cerebral edema [40]. The subacute effects of single and multiple dose mannitol administration have not been well established. The purpose of the present study was to investigate the use of single and multiple doses of mannitol in a brain injury model. The concentrations of mannitol were measured and the effect upon extent of cerebral edema was assessed.

CEREBRAL EDEMA

Blood-brain barrier The cerebral capillaries differ significantly from those elsewhere in the body. They are relatively impermeable by virtue of tight junctions between endothelial cells, which block intercellular passage of solutes. Furthermore, the cells have few pores or endocytotic vesicles, abundant mitochondria to facilitate active transport processes, and are surrounded by a thick extraluminal basement membrane and astrocyte foot processes. This combined structure constitutes the blood-brain barrier [13]. While highly lipid soluble molecules may diffuse through the endothelial cells, the flux of fluids and solutes is largely controlled by active processes. The brain tissue is thereby protected from potentially toxic substances, and its water content remains unaltered despite typical fluctuations of systemic hydration and osmolarity. The cerebral water content is normally very stable, at approximately 70% in white matter and 80% in grey matter (by weight). Only under extreme conditions will significant amounts of water pass across the blood brain barrier.

Water Flux The flux of water (Jv) across the blood-brain barrier is influenced by the hydrostatic and electrochemical forces according to the equation:

$$Jv = K (\Delta P - \Sigma\sigma \cdot \Delta\pi) \qquad [1]$$

where K is the filtration coefficient of the membrane, ΔP the difference in hydrostatic pressures, and $\Sigma\sigma \cdot \Delta\pi$ is the sum of products of reflection coefficient, σ, with total

possible osmotic pressure gradients for each solute, $\Delta\pi$ [12]. Since the normal blood-brain barrier has a very low permeability to ions and polar compounds of even low molecular weight, σ is close to unity. Any initial filtration of water due to hydrostatic pressures, ΔP, will be offset by the resultant osmotic gradient in the opposite direction. However, osmotic pressure gradients may significantly promote the flux of water. Under conditions of increased permeability of the blood-brain barrier, the filtration of plasma fluids and constituents into tissue becomes possible. This is the mechanism thought to account for the formation of vasogenic cerebral edema.

Classification Cerebral edema is defined as an increase of brain tissue volume due to an increase of its water content [38]. The most common pathophysiological mechanism leading to this cerebral edema is a vasogenic process, in which cerebral capillaries become leaky [40,41]. The increased hydraulic conductance of the disrupted blood-brain barrier allows extravasation of plasma fluids, solutes and small molecular weight proteins into the cerebral extracellular space. The edema fluid and constituents are driven by hydrostatic and oncotic pressure forces [21,23], and advance through the cerebral white matter where axons are oriented in a parallel fashion. This allows expansion of the extracellular space, rather than in grey matter where cellular processes are intertwined. Substances of diverse molecular weight spread from the site of injury at the same speed, indicating bulk flow movement of the edema fluid [10,15,69]. Any type of injury may create disruption of the blood brain barrier and result in vasogenic cerebral edema [38,40]. This typically progresses for up to 72 hours following the inciting injury, and results in a 7 to 8% increase of cerebral

intracranial volume, while edematous white matter volume increases 30 to 40% [62]. The rate and degree of vasogenic edema formation depend upon the size of the injury, and the hydraulic pressures which are directly related to systemic arterial blood pressure [21]. Substances extravasating with the edema fluid will advance over an equal area in a given time, regardless of the time of administration following the creation of the cerebral injury [10,41].

Clinically, vasogenic edema is most commonly seen as a result of head injury, cerebral infarction, or hemorrhage. The most widely employed experimental model of vasogenic edema is created with a cortical freezing lesion [38]. This results in a core area of injury with hemorrhagic necrosis, from which plasma derived edema fluid extravasates from disrupted cerebral capillaries.

Other types of cerebral edema include cellular edema, interstitial edema, and osmotic edema. In cellular edema, intracellular swelling is produced either by poisoning or by an ischemic insult. Interstitial edema results from hydrocephalus as cerebrospinal fluid enters the periventricular white matter. Osmotic edema is created when osmotic gradients are established between plasma and cerebral tissue, as may develop in conditions of water intoxication or rapid hemodialysis [38,64]. It has also been postulated that repeated doses of mannitol may lead to the cerebral accumulation of mannitol, with the resulting osmotic gradients promoting flux of water into the brain, creating rebound cerebral edema.

Cerebral edema from any cause may result in local increase of tissue pressure and intracranial pressure [48,69]. This potentially leads to reduced perfusion or ischemia, as well as displacement of cerebral tissue. No effective treatments to halt or reverse the formation of cerebral edema have been devised. Therefore, therapeutic endeavors are directed at reduction of brain volume and intracranial pressure.

INTRACRANIAL PRESSURE

Definition The mature cranium is a relatively closed and rigid space, containing brain tissue and its extracellular fluid, cerebrospinal fluid, and blood. The Monroe-Kellie doctrine indicates that increased volume of any intracranial component must be accompanied by an equal decrease in another, otherwise intracranial pressure increases. The normal intracranial pressure is between eight and eighteen torr. It rises as a result of intracranial space occupying lesions (e.g. hemorrhage, tumor), increases of cerebrospinal fluid volume (i.e. hydrocephalus), cerebral blood volume (i.e. swelling), or water content (i.e. edema). This pressure-volume relationship is exponential, such that following initial compensation for added volume, intracranial pressure rises steeply with further addition of volume.

The pressure volume index was established as a clinical means to assess the intracranial compliance, and is defined as the volume of fluid that would theoretically have to be added into the intracranial space to produce a tenfold increase of intracranial pressure [52]. It is

calculated by adding measurement of the initial intracranial pressure (P_1), and intracranial pressure following injection of 1 ml of fluid (P_2), into the equation:

$$PVI = \Delta \text{ Volume} / \log P_1 P_2 \qquad [2]$$

The normal pressure volume index is 26 ± 4 ml, and values of less than 13 ml are indicative of markedly compromised intracranial compliance, corresponding to the steep slope of the pressure volume curve. This highlights the significance of even small increases of intracranial blood volume or cerebral edema.

Elevations of intracranial pressure are common following brain injury from any cause, and provide a powerful prognostic sign of a poor outcome. The resultant neuropathological changes are seen in more than 80% of fatal traumatic injuries. While intravenous mannitol therapy transiently reduces intracranial pressure, its efficacy in outcome benefit has never been proven. Furthermore, effect of repeated doses has not been well defined, and the potential of paradoxically increasing intracranial mass effect, by way of rebound cerebral edema, remains largely unexplored.

Cerebral ischemia The normal cerebral blood flow (CBF) for mixed cortical gray and white matter is 50 ± 20 ml/100g/minute, which is closely coupled to cerebral metabolic demands, and precisely regulated by reflex alterations of cerebrovascular resistance (CVR). These are mediated by constriction and dilation of cerebral arterioles, in response to systemic arterial

blood pressure (ABP), arterial carbon dioxide and oxygen contents, pH, and cerebral metabolic rate [65]. Cerebral perfusion is also influenced by regional tissue pressure and global intracranial pressure (ICP) and cerebral perfusion pressure (CPP) according to the following equations:

$$CBF = CPP / CVR \quad [3]$$

&

$$CPP = ABP - ICP \quad [4]$$

Brain injury may cause loss of normal cerebrovascular reactivity and/or elevations of global or regional intracranial pressure. Higher cerebral blood flow caused by vasodilatation results in increased cerebral blood volume, and this hyperemia or swelling may produce intracranial pressure elevations. Conversely, flow less than 20 ml/100g/minute may be inadequate to sustain cerebral energy requirements, leading to loss of cellular function, and ultimately cellular injury and ischemic death.

Cerebral shift & herniation Focal mass effect within the intracranial space may result from space occupying lesions (e.g. hematoma, neoplasm, abscess), or localized cerebral edema or swelling. These elements are often simultaneously present and interrelated. The resulting

displacement and mechanical distortion of cerebral tissue, referred to as shift, may cause further neural and vascular injury by compression or traction forces. Herniation refers to the condition when cerebral tissue shifts into an opening of the rigid intracranial space, such as the temporal uncus through tentorial insicura, or cerebellar tonsils through the foramen magnum. This may produce immediately life threatening brain stem compression. Any process promoting formation or progression of cerebral edema may potentiate cerebral shift and herniation. Single dose mannitol has certainly been extremely useful in the emergent reduction of intracranial bulk, and thereby temporarily may reduce cerebral shift. However, cerebral edema typically progresses over several days, and the effects of multiple doses of mannitol are not well established.

Treatment A self perpetuating cycle of neural injury may progress following brain injury, in which the pathological changes of edema, swelling, intracranial pressure elevations, ischemia and cerebral displacement all contribute to further dysfunction and damage (Figure 1). This further injury in turn results in progression of the pathophysiological state. There are no effective treatments for cerebral edema associated with common forms of brain injury, including traumatic head injury and cerebral hemorrhage or infarction. Therefore, therapeutic interventions have been directed toward reducing the adverse consequences of cerebral edema such as raised intracranial pressure, cerebral shifts and herniation. However, the efficacy of some commonly employed measures have been questioned. For example, hyperventilation has long been recognized as an effective means to reduce intracranial blood volume transiently, by virtue of the reflex cerebral arteriolar vasoconstriction in response to

hypocapnia. The resultant reduction of intracranial pressure has been extrapolated to be beneficial, since there is a strong correlation between intracranial hypertension and poor outcome following brain injury. However, the effect of hyperventilation is transient, and more importantly, recent direct CBF measurements have demonstrated potentially dangerous degrees of cerebral ischemia associated with its use. This has led to a reevaluation of the unreserved use of this treatment modality in patients with brain injury [24].

The widespread use of intravenous mannitol administration to patients with brain injury may also have adverse potential consequences. In fact, no study has specifically demonstrated beneficial effect on outcome related to the use of repeated doses of mannitol. Furthermore, while several studies have shown mannitol to reduce intracranial pressure and increase cerebral perfusion pressure and cerebral blood flow transiently [14,32,53-56,60,67,71], the mechanism of action remains the subject of considerable debate.

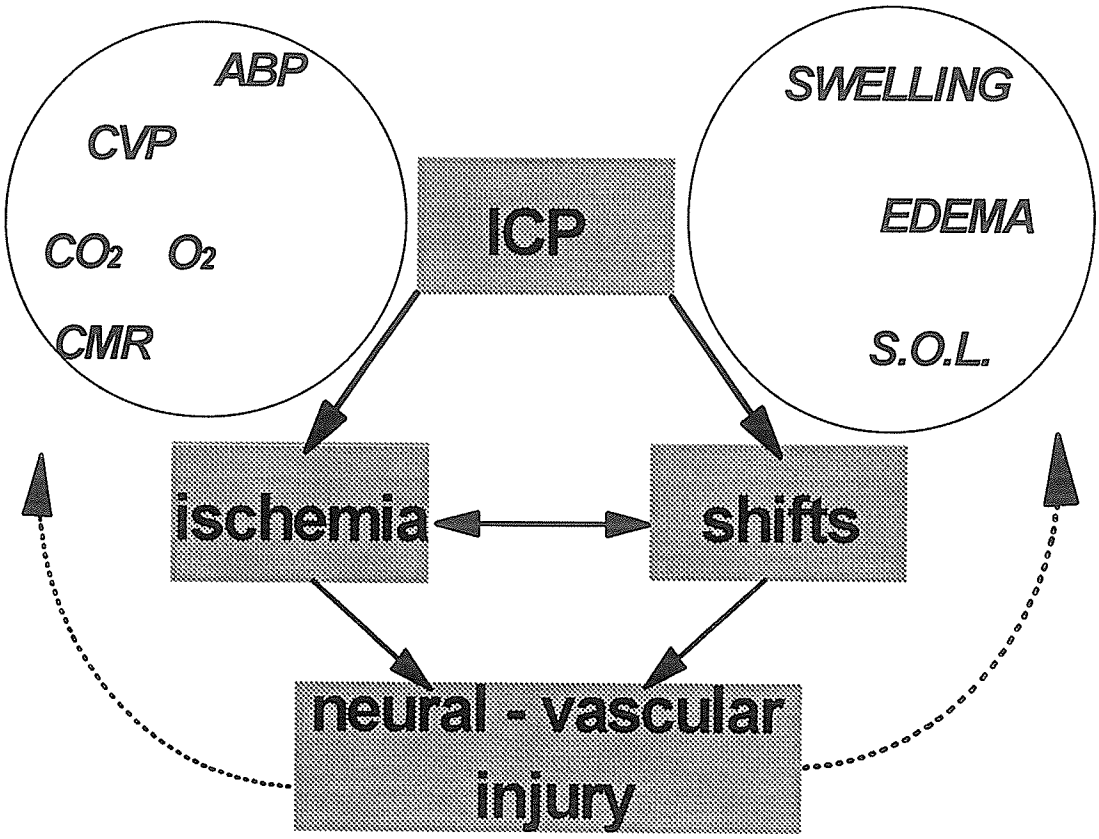


Figure 1 **Brain Injury**: Multiple factors influence the destructive processes of cerebral ischemia and shift. These include vascular factors such as arterial blood pressure (ABP), central venous pressure (CVP), arterial carbon dioxide and oxygen (CO_2 and O_2), and cerebral metabolic rate (CMR). Intracranial pressure (ICP) also exerts a major influence upon this ongoing injury, and is dependent on additional factors including cerebral swelling, edema, and any intracranial space occupying lesions (SOL).

MANNITOL: MECHANISMS OF ACTION

Osmotic diuresis Mannitol is a simple sugar with a molecular weight of 182 Daltons. It is typically supplied as a 25% solution, with a calculated osmolarity of 1372 mOsm/l. It is administered intravenously, as gastrointestinal absorption is minimal. It is confined to the extracellular space, and not significantly metabolized. Mannitol is freely filtered across the glomerulus, and rapidly excreted in urine. Diuresis is induced by elevated osmolarity of the glomerular filtrate, thereby hindering tubular reabsorption of water. Mannitol also promotes a tremendous shift of fluids from the intracellular to extracellular space, in the order of 1 liter per 50g mannitol administered [78].

These actions have led to the administration of mannitol for the purposes of promoting diuresis, as may be indicated for the prophylaxis of renal failure, oliguria, and promotion of renal excretion of toxic substances. Mannitol is also employed in the treatment of elevated intraocular pressure, and "reduction of intracranial pressure and treatment of cerebral edema by reducing brain mass"[20].

Caution must be employed in the use of mannitol, as excessive losses of water and solutes may produce dangerous electrolyte imbalances. Excessive free water losses may cause hypernatremia, and elevations of serum osmolarity greater than 320 mOsm are associated with a significant risk of renal failure. The intracellular to extracellular shifts of relatively hypotonic fluid may aggravate preexisting hyponatremia. The rapid increase of intravascular volume may also aggravate conditions of pulmonary edema and congestive heart failure.

Intracranial hypertension Osmotic dehydration of the brain with intravenous diuretics was described more than 30 years ago, and this proposed mechanism for the action of mannitol remains widely accepted today [33,34,57,69]. Mannitol is relatively impermeable to the normal cerebral capillaries, which comprise the anatomical substrate of the blood-brain barrier [3,59,66,75]. Following a single intravenous administration, an osmotic gradient across the blood-brain barrier between high plasma and low cerebral concentrations of mannitol is established. The gradient promotes the efflux of water across the intact blood-brain barrier, and thereby reduces cerebral water content. The reduction of cerebral volume by dehydration results in a drop of intracranial pressure, as predicted from the Monroe-Kellie doctrine and the intracranial pressure-to-volume relationship. Reductions of cerebral water content in only non-edematous brain have been demonstrated in the first hour following mannitol administration, suggesting that an intact blood-brain barrier is required for the osmotic gradient to become established and effective [17,28,46,61,63,81]. This osmotic effect has been measured at 15 to 30 minutes following bolus infusion of 0.25 to 2.0 g/kg. The optimal dosage and administration technique have not been established, with proponents of dosages that range from 0.25 to 2.0 g/kg. Reduction of water content in areas of edematous cerebral tissue have also been demonstrated in a few studies [2,57]. It has been suggested that the dehydration of edematous and non-edematous brain is dependent upon the timing of mannitol infusion following formation of the cerebral injury [68]. However, some studies have failed to demonstrate cerebral water content reduction, or a poor correlation between changes in cerebral water content and the course of intracranial pressure reduction following intravenous mannitol administration [28,29,31,77]. Alternative theories have been

proposed to account for the observed effects of mannitol therapy.

It has been proposed that mannitol may act by means of a reflex cerebral vasoconstriction, leading to a reduction of intracranial blood volume and therefore intracranial pressure. Rapid intravenous infusion results in a transient increase of plasma volume. There is also a direct effect on blood rheology, with reduction of viscosity related to red blood cell dehydration and increased deformability. These processes leading to plasma expansion and reduced blood viscosity may result in increased cerebral blood flow. In response, an autoregulatory vasoconstriction of cerebral arterioles has been observed, which in turn reduces cerebral blood volume, bulk, and intracranial pressure [55,56,71]. The time course of these events may better correlate with rapid reduction of intracranial pressure observed following mannitol infusion [29,55]. However, not all agree that such a vasoconstriction response occurs [5].

The third mechanism by which mannitol may reduce intracranial pressure is by promoting cerebrospinal fluid absorption or reducing its formation [20,70,72,77]. While this mechanism may account for some of the persisting intracranial pressure changes following mannitol administration, it is not a likely explanation of early intracranial pressure reduction.

Rebound Cerebral Edema Brain injury may result in disruption of the blood-brain barrier, with a resultant increase in its permeability. In such an injury, mannitol extravasates across the blood-brain barrier and enters the cerebral extracellular space, where it remains extracellular, is not metabolized [15,25,59], and advances through the white matter by bulk flow with edema fluid [10,15,69]. There is limited data regarding the concentration of

mannitol in cerebral tissue following a standard therapeutic dose. A single study in 1964 by Wise *et al* measured the relative concentrations in brain and plasma following a 3 g/kg mannitol dose in non-lesioned dogs [81]. After 6 hours the plasma concentrations still exceeded those of brain and cerebrospinal fluid, with the implication that the osmotic gradient favored cerebral dehydration. However, studies of brain injury have demonstrated increased flux of mannitol into cerebral tissue when the blood-brain barrier is disrupted, suggesting that in this situation, mannitol may accumulate in cerebral tissue [15,19,74]. Since plasma mannitol concentrations decline in an exponential manner following intravenous infusion [4,11,18], cerebral accumulation of mannitol would result in a reversed osmotic gradient across the blood-brain barrier. This would promote the influx of water across the cerebral capillaries, and increase cerebral water content and edema. Permeability data and equations indicate that very small osmotic gradients may exert strong forces upon the flux of water across the blood-brain barrier. A 1 mOsm gradient is equivalent to 19 mm Hg hydrostatic pressure [22]. Therefore, small concentration gradients of mannitol across the blood-brain barrier may potentiate influx of water, and result in rebound cerebral edema.

There is evidence suggesting that reversed osmotic gradients may be established across the blood-brain barrier following mannitol administrations, and that there may be associated flux of water into cerebral tissue. Solutes may accumulate in cerebral tissue following intravenous infusions, especially in regions with cerebral edema associated with blood-brain barrier damage [15,79]. Cerebral edema may be produced by rapid alterations of plasma

osmolarity, as demonstrated in experiments of renal dialysis in nephrectomized dogs [64]. This shift of brain fluids has also been implicated in the "disequilibrium syndrome" seen in some dialysis patients. A delayed increase of cerebral mass and intracranial pressure has also been observed following repeated doses of mannitol in goats with induced vasogenic cerebral edema, supporting the concept of rebound cerebral edema [27]. However, there have been no previous studies which quantified cerebral mannitol concentrations, and the effects upon cerebral water content following multiple mannitol doses, in the setting of brain injury.

Whether rebound cerebral edema actually does occur in brain injured patients who receive repeated mannitol doses is difficult to determine, because of the unpredictable progression of cerebral edema, and the variations of intracranial pressure over the course of hours and days [26]. Critically ill patients often also suffer from concurrent multiple organ problems, which can influence their intracranial pressure and neurological status. It is therefore difficult to assess the long-term effects of multiple doses of mannitol. Even if transient reductions of intracranial pressure follow each administration, there is no assurance that cerebral edema is favorably affected, and that outcome is improved.

SUMMARY AND STUDY GOALS

The many studies performed in animals and humans have established that single dose mannitol has a transient beneficial effect on intracranial pressure, cerebral perfusion pressure, cerebral blood flow, and short-term neurological outcome. However, none has assessed the

efficacy of multiple doses of mannitol, especially as related to the cerebral accumulation and postulated rebound cerebral edema in the setting of brain injury. These issues must be further studied, in order to optimally make use of this agent and compare its efficacy to any new therapeutic alternatives.

In the present experimental study, single and repeated doses of mannitol were administered in a feline model of brain injury, in order to obtain quantitative measurements of plasma and cerebral mannitol concentrations and evaluate the effects upon cerebral edema. It was hoped that this information would better elucidate the potential benefits and adverse effects of this osmotic diuretic agent commonly employed in the treatment of patients with cerebral edema and brain injury.

EXPERIMENTS

STUDY DESIGN

The present study was designed to assess the influence of mannitol treatment in the setting of brain injury with vasogenic cerebral edema. The twenty-three animals all received a cortical cold injury and were divided into four study groups. In the two treatment groups, mannitol was administered intravenously 1 hour following a cortical cold injury, as either a single dose, or 5 repeated doses at 4 hour intervals. The doses and interval of mannitol administration were selected to parallel those typically used in the clinical setting. Each dose consisted of 1/3 g/kg of 20% mannitol solution, intravenously administered by infusion pump over 15 minutes. Animals were then sacrificed 4 hours following the last dose (i.e. 5 or 21 hours following cortical cold injury). The concentrations of mannitol in cerebral tissue, cerebrospinal fluid, plasma, and urine were determined. The other 2 study group animals served as time matched controls. These animals were also maintained under general anaesthesia for 5 or 21 hours following a cortical cold injury. However no mannitol was administered. In all 23 animals, the progression of cerebral edema and cerebral water content were measured after either 5 or 21 hours.

HYPOTHESES

The hypotheses to be addressed include:

1. Mannitol has no sustained effect upon cerebral edema as assessed by edema progression and cerebral water content measurements.

2. Mannitol accumulates in normal and edematous brain, following single and repeated doses.

3. The plasma-to-brain concentration gradient of mannitol produces a paradoxical aggravation of cerebral edema following multiple doses.

METHODS

ANIMAL PREPARATION

Animals were treated according to CCAC guidelines, with approval from the University of Manitoba, School of Medicine, Animal Care Committee. A cortical cold-injury was employed to produce vasogenic cerebral edema in this feline brain injury model. Cats weighing between 2.5 and 3.1 kg were surgically prepared after anaesthetic induction with intraperitoneal injection of 30 mg/kg body weight of pentobarbital sodium (Nembutal)[1]. A tracheostomy was performed and continuous mechanical ventilation initiated. General anaesthetic was thereafter maintained with supplemental intravenous injections of 10 mg of pentobarbital and 1 mg of pancuronium (Pavulon)[2]. Intermittent arterial blood gases and continuous end-tidal carbon dioxide were monitored[3], and ventilation was adjusted to maintain (arterial carbon dioxide) $PaCO_2$ between 35 to 40 torr. Supplemental oxygen was added to maintain the arterial oxygen PaO_2 above 100 torr. An esophageal temperature probe was placed, and normothermia maintained by means of an adjustable heating blanket. Femoral arterial and venous lines were established for continuous arterial blood pressure monitoring, intermittent blood sampling, and administration of drugs. A continuous infusion of 0.9% normal saline at 3 ml/kg/hr was established, for fluid maintenance. A bladder catheter was placed, and urine output was collected.

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[1] Abbott Laboratories, Ltd., Toronto, Ontario, Canada

[2] Organon, Toronto, Ontario, Canada

[3] Hewlett-Packard model 78353A capnometer, Mississauga, Ontario, Canada

Vasogenic cerebral edema was produced by a cortical cold-injury in all animals. With the cat positioned right lateral decubitus, a linear parasagittal scalp incision was made. A 10 x 5 mm left parietal craniectomy was then fashioned with a high speed electric bur, under cool saline irrigation. An operative microscope was employed, and the underlying dura was carefully preserved to avoid mechanical injury of the underlying brain. The cortical cold injury was produced with a freezing probe, which consisted of a glass housing surrounding a metal rod. The apparatus was filled with a dry ice-acetone mixture that bathed the metal rod, which projected through the glass. Once homogenous cooling of the probe was achieved, the tip was applied to the intact dura for two minutes. The scalp incision was then closed, and the cat repositioned supine. Thirty minutes later, all animals received 1 ml/kg of 1% Evan's blue dye, intravenously infused over 30 minutes. This agent served as a marker of cerebral edema, as discussed below.

The anaesthetized animals were then sacrificed either 5 or 21 hours following the cortical cold injury, by intravenous injection of potassium chloride. Cerebrovascular washout was then performed by transcardiac perfusion for 30 seconds, with 75 to 100 ml of normal saline under 100 cm H_2O mean pressure. The brain was rapidly removed and transferred to a -70° C freezer for 10 minutes to facilitate handling and sectioning [39]. The brain was sliced parasagittally through the center of the cortical cold injury, and photographed with an accompanying scale. Edema progression was measured on these images. Multiple edematous and non-edematous cerebral samples were then rapidly collected with a 50 μL scoop, for measurements of regional cerebral water content. The brains for treatment group animals

were further sampled for measurement of regional cerebral mannitol concentrations.

CEREBRAL EDEMA PROGRESSION

Evan's blue dye served as a marker of the edema fluid, as this dye binds to plasma proteins. These labeled proteins, especially albumin, extravasate with plasma fluids and solutes across the disrupted blood-brain barrier, and advance through white matter with the edema fluid. The edematous cerebral tissue thereby becomes discolored blue, and is readily identifiable [41]. The progression of cerebral edema was directly measured from enlarged photographs of the parasagittally sectioned brains (Figure 2). The line of maximum distance between the inner edge of the cortical cold injury, and advancing edge of Evan's blue stained edema was measured against the accompanying scale. Comparisons were made between edema progression 5 and 21 hours following the cortical cold injury, as well as between treatment and control study groups.

CEREBRAL WATER CONTENT

Cerebral water content of regional samples were calculated from measurements of specific gravity. This simple and accurate gravimetric technique was first described by Nelson et al.[58], and has been modified by others [37,47,49]. It has been widely employed to study experimental cerebral edema, and in some human studies as well. Water content is determined from specific gravity of small tissue samples, based on the observation that specific gravity of tissue solids is constant, even in the presence of edema. Therefore changes

in percent water content may be calculated from alterations of tissues specific gravity, such that:

$$\% \text{ wt } H_2O / \text{ wt TISSUE} = 1 - \{(SG_t - 1) / (1 - 1/SG_s)\, SG_t\} \qquad [5]$$

where {wt H_2O / wt tissue } is the weight or volume of water over the weight of the total tissue sample; Sg_t is the specific gravity of the tissue sample; and SG_s is the specific gravity of the solid component of brain.

Gravimetric columns were prepared according to the technique described by Marmarou, such that a linear relationship between depth and specific gravity was established [47]. Briefly, two mixtures of bromobenzene and kerosene were precisely prepared. The less dense mixture "A" was continuously dripped and stirred into mixture "B", while this second container drained into a graduated cylinder at a rate double that of A to B. This resulted in columns with linear relationship between depth and specific gravity (Figure 3). Column calibration was achieved by measuring depth of immersion of standard K_2SO_4 solutions of known specific gravity, and linearity was confirmed by calculation of linear regression. Columns were maintained on a vibration insulated table for a maximum of three days, and satisfactory linearity confirmed prior to each use (r^2>0.9998).

Graphical plotting of specific gravity as a function of depth was then performed, with the

slope (m) and the intercept (B) determined by computer. The specific gravity of each specimen (Sg_t) was then calculated with the formula:

$$SG_t = B - m\,(div) \qquad [6]$$

where (div) represents the equilibration depth of cerebral specimens measured at one minute. Cerebral water content was then calculated with constants determined for feline brain, using the modified formula initially described by Marmarou et al [49]:

$$\text{wt } H_20/\text{wt tissue} = \{1597.3 / SG_t\} - 1464.9 \qquad [7]$$

Regional cerebral water content was measured in samples of non-edematous cortex and white matter, obtained from both hemispheres. Samples (50 μL) from the Evan's blue stained edematous white matter were collected from three regions: directly beneath the cortical cold injury; the central area of edema; and the advancing edge of edema (Figure 2). The fresh samples were then immediately immersed in the graduated density column, and the equilibration depth recorded at 1 minute. The modified Nelson's equation was employed to determine the cerebral water content, with constants determined for feline brain, as outlined above.

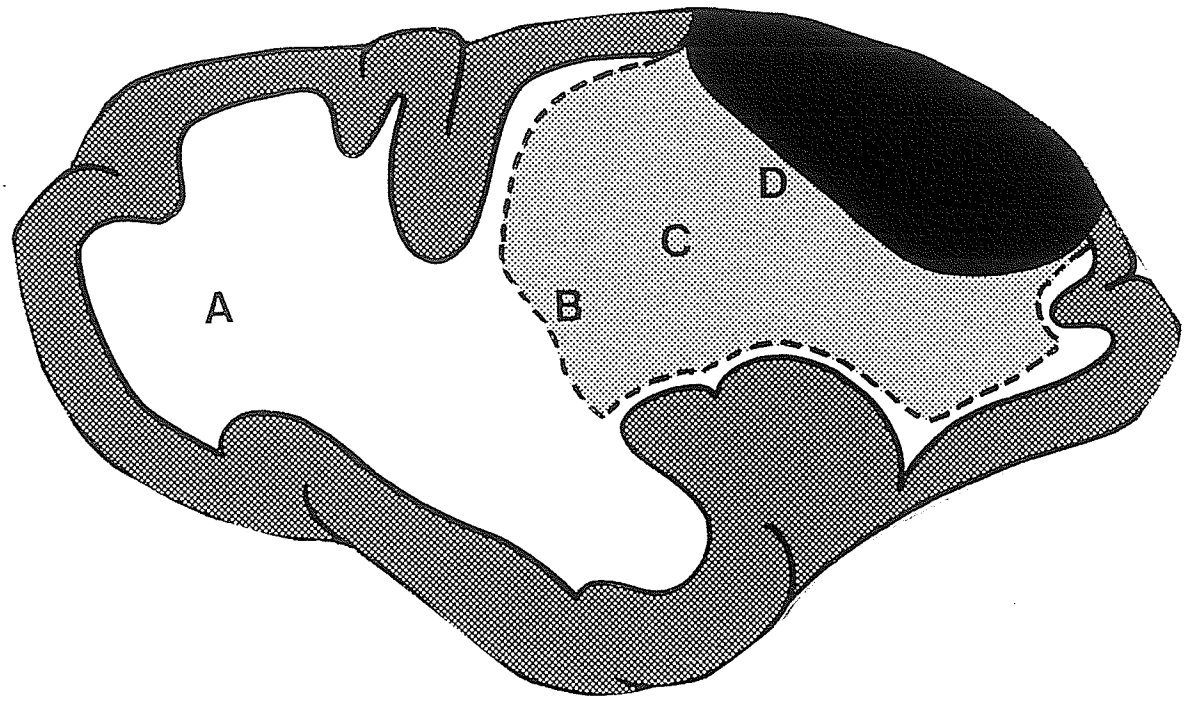


Figure 2 **Parasaggital Brain Section**: This diagram illustrates the sectioned brain, including the parietal cortical cold injury (black), and the underlying edematous white matter (light shaded). Edema progression was measured from the inner edge of the cortical cold injury, to the most distant edge of the Evan's blue discolored edematous white matter. Cerebral samples were collected thereafter for cerebral water content measurement in all animals, and also for cerebral mannitol measurement in the treatment groups. Samples were taken from normal cortex and white matter (dark shaded and white) and also from three regions of edema: (D) immediately beneath the cortical cold injury (*sub-lesion*), (C) in the middle zone of edema (*mid-edema*), and (B) from the advancing edge of edema. These italicized labels are referred to in subsequent figures.

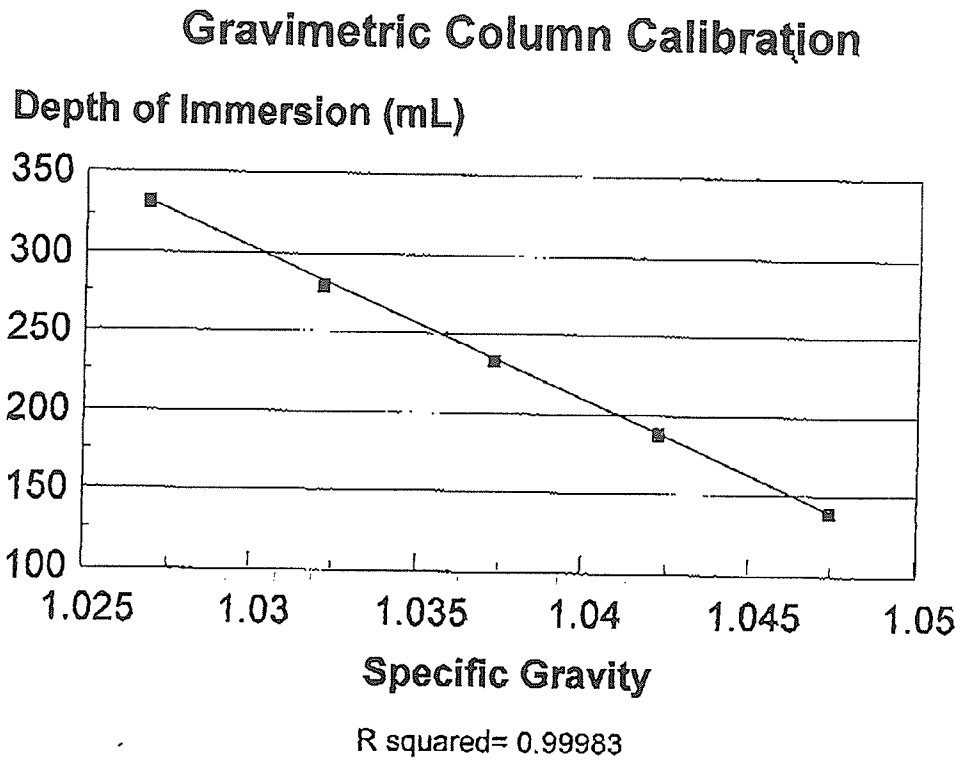


Figure 3 Gravimetric Column Calibration: Graduated density columns of bromobenzene and kerosene were created such that a linear relationship between depth in the column (mL) and specific gravity was established. This was confirmed by determining the depth of immersion of standard solutions, and calculating the correlation coefficient, r^2.

MEASUREMENT OF MANNITOL CONCENTRATIONS

Only animals in the treatment group received intravenous mannitol beginning one hour following the cortical cold injury as either a single dose or five doses given at four hour intervals. Each dose consisted of 1/3 g/kg body weight of 20% mannitol solution, infused over 15 minutes. The total dosage of mannitol was determined for each animal, and mixed with radio-labelled D-[1-^{14}C]-mannitol[4]. A total of 50 μC was combined with the single dose and 125 μC with the five repeated doses. Small volumes of these radio-labelled mannitol mixtures were collected, and later used to determine the mannitol concentrations in tissue and fluid samples, as described below.

The concentrations of mannitol in plasma, brain, cerebrospinal fluid and urine were determined in the treatment group animals that received the 20% radio-labelled mannitol. The appropriate volume of all fluids and tissue required were selected to provide adequate counts of radio-labelled mannitol, as predicted from previously published data [4,11,15,18,19,59,74,81]. Blood samples of 500 to 1000 μL were collected at timed intervals following each dose of mannitol, and totalled approximately 5 ml per each four hours. The blood was centrifuged to provide 200 μL plasma samples. In addition, 500 to 750 μL of cerebrospinal fluid was obtained by cisterna magna puncture immediately prior to animal sacrifice. A sample of the total urine output was collected (50μL) at the end of the experiments, four hours after the last dose of mannitol. Cerebral tissue blocks of 25 to 100 mg were dissected from the sectioned brain, from regions comparable to those used for

[4] D-[1-^{14}C] mannitol, 50 μCi/ml, Amersham, Oakville, Ontario

specific gravity measurements. These samples from normal and edematous brain regions were rapidly weighed and then solubilized for 12 to 18 hours in 1.0 ml Scinti-gest[5] and 0.2 ml water at 50°C.

The concentration of radio-labelled mannitol was determined by means of liquid scintillation counting. All samples were combined with 15 ml of Cytoscint[6] scintillation fluid. The count of depolarizations per minute of each sample (DPM_{sample}) was measured on a Rackbeta automatic scintillation spectrometer[7], including background and quench corrections. Similarly, the depolarizations per minute of a precisely diluted sample of the radio-labelled 20% mannitol, which had been administered, were also determined (DPM_{dose}). The concentration of mannitol in each sample (mg / ml or g) was calculated with the equation:

$$[\text{mannitol}] = DPM_{sample} / DPM_{dose} \qquad [8]$$

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[5] Scinti-gest supplied by ICN Biomedicals, Inc., Irvine, California

[6] Cytoscint scintillation fluid: ICN Biomedicals, Inc., Irvine, California

[7] Rackbeta automatic scintillation spectrometer model 1219 (LKB Wallac), LKB Wallac, Turku, Finland

STATISTICAL ANALYSIS

Independent Student's t-test was used to compare results of cerebral water content, progression of edema, mannitol concentrations and various physiological measurements in mannitol treatment groups with their respective time-matched control groups. The paired Student's t-test was used to compare cerebral water content and mannitol concentration differences between ipsilateral and contralateral non-edematous cerebral regions. Analysis of variance was employed to compare cerebral mannitol concentrations between tissue regions. Differences were considered significant for p less than 0.05. Variations from the mean are given as standard errors.

RESULTS

PHYSIOLOGICAL VARIABLES

Serum osmolality, sodium, and net fluid balance measured at the end of each study period did not differ significantly between study and control groups. There was a greater reduction of osmolality and sodium in the 21 hour control and treatment groups, compared to the 5 hour groups, which may have resulted from the positive fluid balance in the 21 hour groups. The baseline mean arterial blood pressures were similar in the paired 21 hour study groups ($p=0.173$). There were no major or persisting deviations of blood pressure (BP) throughout the experiments.

PHYSIOLOGICAL VARIABLES					
	Net Fluid Balance (mL)	Sodium (mM/L)	Osmolality (mM/kg)	Baseline BP (mm/Hg)	Average BP (mm/Hg)
5 Hr Control	-36±21	-1±1	-3±3	---	---
5 Hr Mannitol	-16±16	-6±2	-1±8	---	---
21 Hr Control	21±20	-10±1	-21±7	120±10	125±6
21 Hr Mannitol	17±11	-11±3	-21±8	110±6	109±4

During 21 hour experiments, mean arterial BP was recorded every 30 minutes and an average BP determined. There was no difference between the control and treatment groups ($p=0.173$), nor any difference between baseline and average BP in the 21 hour control or treatment groups ($p=0.56$ and 0.74 respectively).

CEREBRAL EDEMA PROGRESSION

The cortical cold injury was consistently produced, and well delineated with Evan's blue dye. The cerebral surface clearly showed the distinct darkly blue stained cortical freezing injury, corresponding in size to the probe tip which induced the lesion. There were also visible signs of cerebral hemispheric swelling, with narrowing of sulci and widening of nearby gyri. The sectioned ipsilateral hemisphere showed the dark blue of the cortical cold injury extending through the full cortical surface. A homogeneous staining of light blue extended from the cortical cold injury out through the white matter.

The linear measurements of maximal edema progression demonstrated a significant advance of this edema between 5 and 21 hours post freezing injury (8.3 ± 1.0 mm and 12.5 ± 0.7 mm, respectively, $p \leq 0.05$). These measurements were not significantly altered by single or five repeated doses of mannitol ($p=0.70$ and 0.73, respectively) (Figure 4).

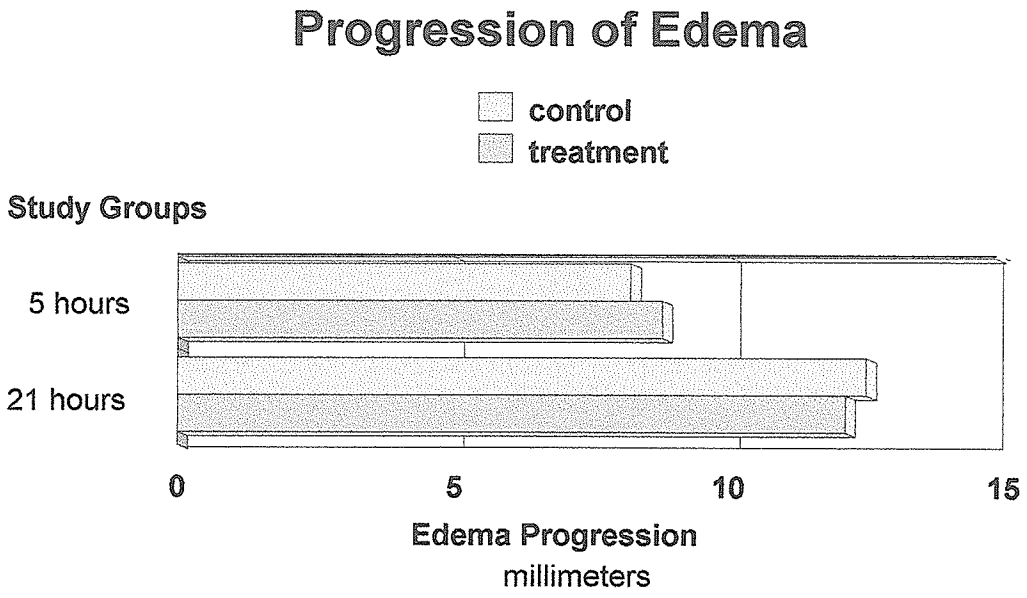


Figure 4 **Progression of Edema:** The distance of edema progresion was measured at 5 or 21 hours following the cortical cold injury, in animals recieving mannitol as well as controls. The progression was measured from beneath the cortical cold injury to the advancing edge of edematous white matter. No statistically significant diference was found between mannitol treatment and control animals, although edema progressed between 5 and 21 hours.

CEREBRAL WATER CONTENT

The regional cerebral water content in control animals five hours following the cortical cold injury was 69.2 ± 0.1% and 80.4 ± 0.1% in non-edematous white matter and cortex respectively (Figure 5). There was no difference between ipsilateral and contralateral samples of white matter (p=0.44) or cortex (p=0.66). The water content of edematous white matter was much higher, and greatest immediately beneath the cortical cold injury. The water content in the central zone of edema was 84.2 ± 1.0%. There was no statistically significant change following a single dose of mannitol, as measured four hours after the injection, in white matter (p=0.89), cortex (p=0.05) or edematous white matter (p=0.55). In cortex the cerebral water content was 80.6 ± 0.03 and 80.1 ± 0.04 in controls and single-dose mannitol groups (p=0.05).

The cerebral water content remained unaltered in control group animals, 21 hours after the cortical cold injury (Figure 6). No alteration of water content following the five doses of mannitol was demonstrated in normal white matter and cortex (p=0.96 and 0.50 respectively). However, there was a statistically significant 3% increase of cerebral water content in the central region of edematous white matter, to 86.2 ± 0.5% associated with multiple dose mannitol administration (p=0.003).

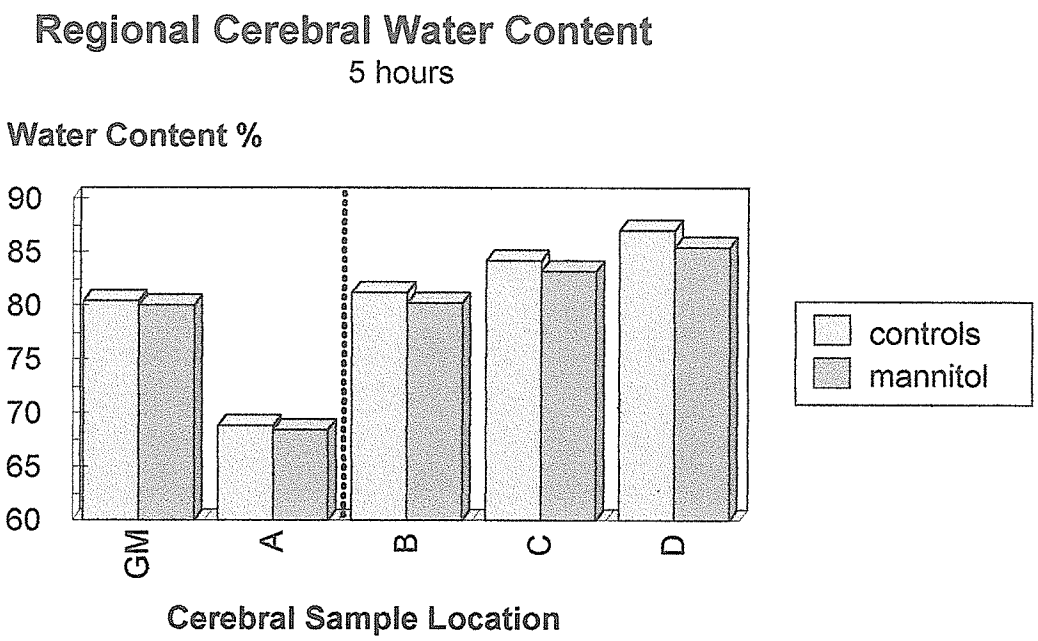


Figure 5 **Regional Cerebral Water Content at 5 Hours**: Water content was determined in non-edematous cortex (GM) and white matter (A), and in the three regions of edematous white matter: directly beneath the cortical cold injury (D), in the middle zone of edema (C), and at the advancing edge of edema(B). No statistically significant differences were seen between mannitol and control group animals.

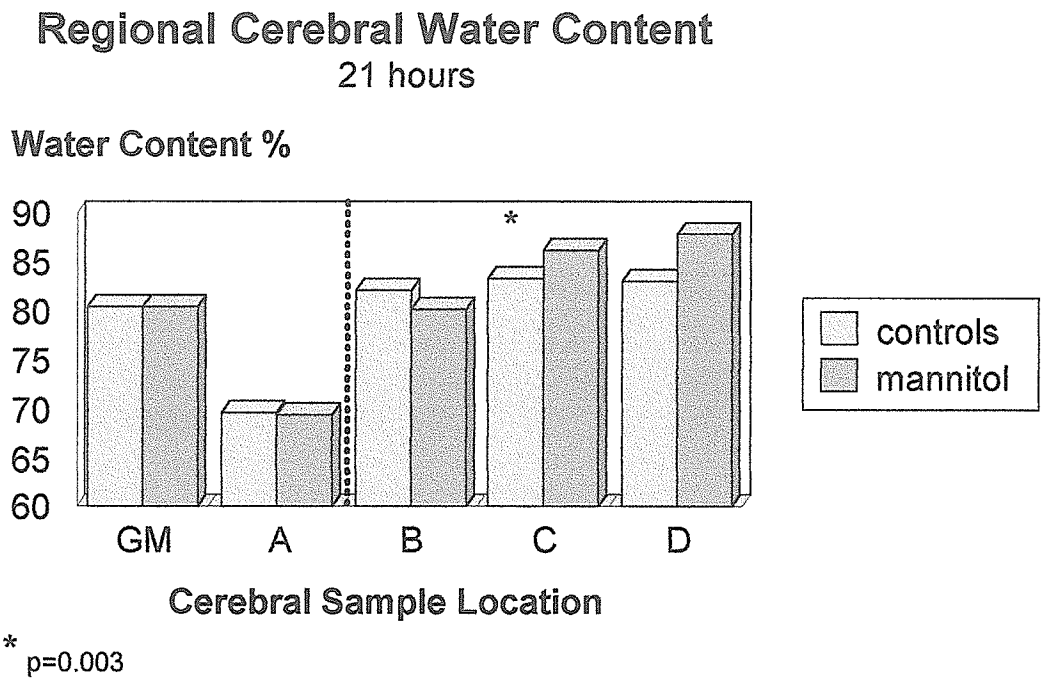


Figure 6 **Regional Cerebral Water Content at 21 Hours** Water content was determined in non-edematous cortex (GM) and white matter (A), and in the three regions of edematous white matter: directly beneath the cortical cold injury (D), in the middle zone of edema (C), and at the advancing edge of edema (B). There is a significant increase in water content of the middle zone of edema associated with repeated doses of mannitol, compared to controls.

MANNITOL CONCENTRATIONS

Each intravenous dose of mannitol resulted in high peak plasma concentrations, which were similar following single and repeated administrations (Figure 7). The mannitol was then rapidly cleared from plasma, such that trough levels at four hour intervals were the same following a single or five repeated doses (0.122 ± 0.018 mg/ml and 0.123 ± 0.016 mg/ml respectively; p=0.448). Collections of total urine output indicated that of the infused mannitol, 73 ± 3% and 84 ± 2% was excreted through the urine, following single and five repeated doses, respectively.

The concentrations of mannitol in cerebral tissue four hours following a single dose were 0.028 ± 0.002 mg/g and 0.046 ± 0.003 mg/g, in non-edematous white matter and cortex respectively. These concentrations increased more than threefold after five doses, to 0.102 ± 0.010 mg/g and 0.142 ± 0.010 mg/g (p=0.0005 and 0.0001), respectively.

Mannitol concentrations in edematous white matter were significantly greater than in normal brain as compared by ANOVA ($p<0.01$)(Figure 8). Directly beneath the cold injury the concentration was 0.193 ± 0.016 mg/g following a single dose, and 0.331 ± 0.032 mg/g after five doses (p=0.004). These values were 7-fold and 3-fold greater than those for normal white matter, following single and repeated doses, respectively. Mannitol concentrations within the edematous white matter decreased progressively toward the advancing edge of edema, with significantly lower concentrations in each region towards non-edematous white matter ($p<0.01$). After five doses, concentrations were greater than the trough plasma concentrations. Mannitol concentrations in the cerebrospinal fluid parallelled the cerebral

concentrations, increasing from 0.032 ± 0.008 mg/ml after a single dose, to 0.075 ± 0.020 mg/ml after five doses of mannitol (p=0.018).

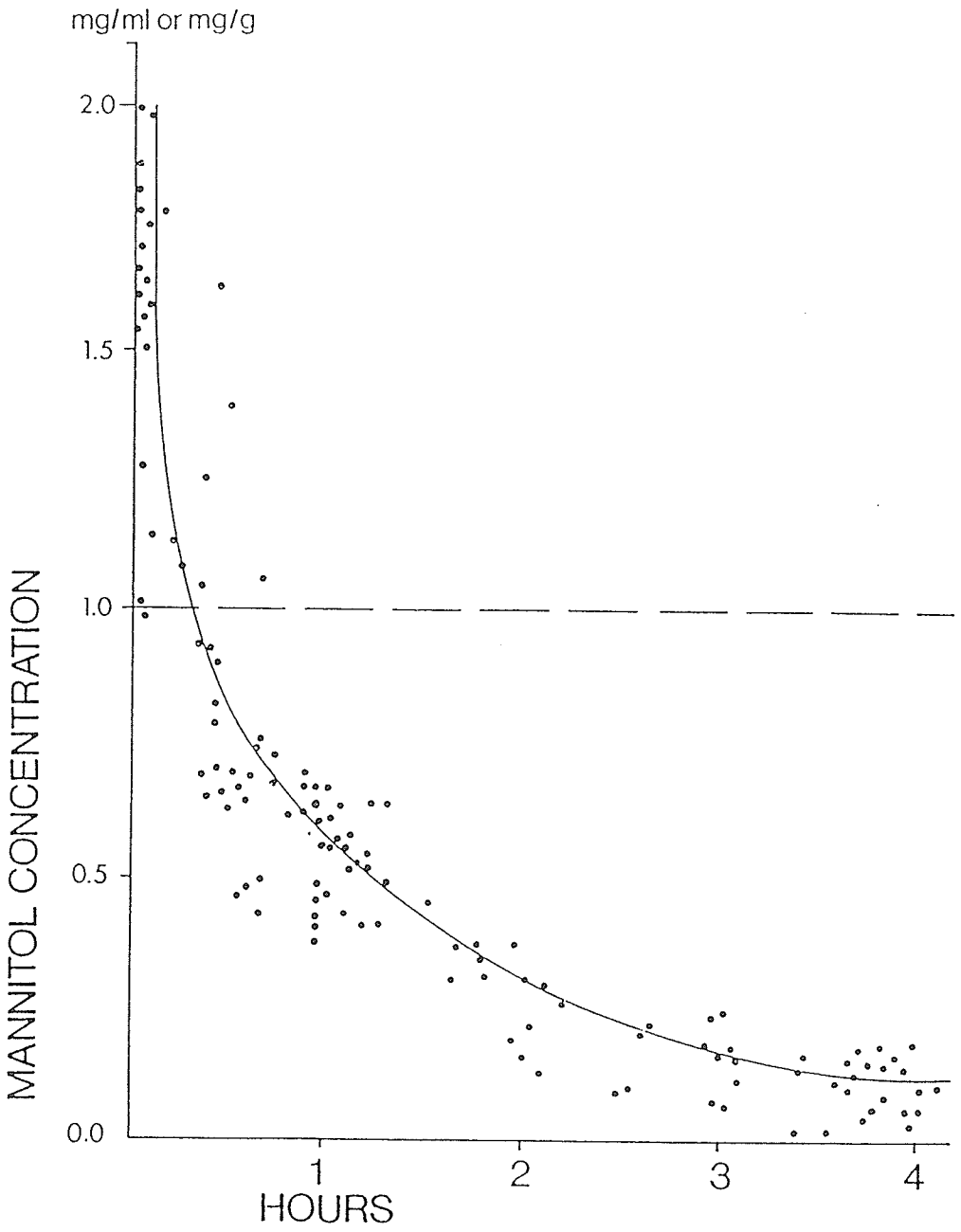


Figure 7 **Plasma Mannitol Concentrations**: Repeated doses of mannitol administered at four hour intervals produced no progressive increase of plasma mannitol concentrations after five doses. Rapid clearence from plasma is demonstrated after each intravenous bolus. Data points are from all five animals that received the multiple doses of mannitol.

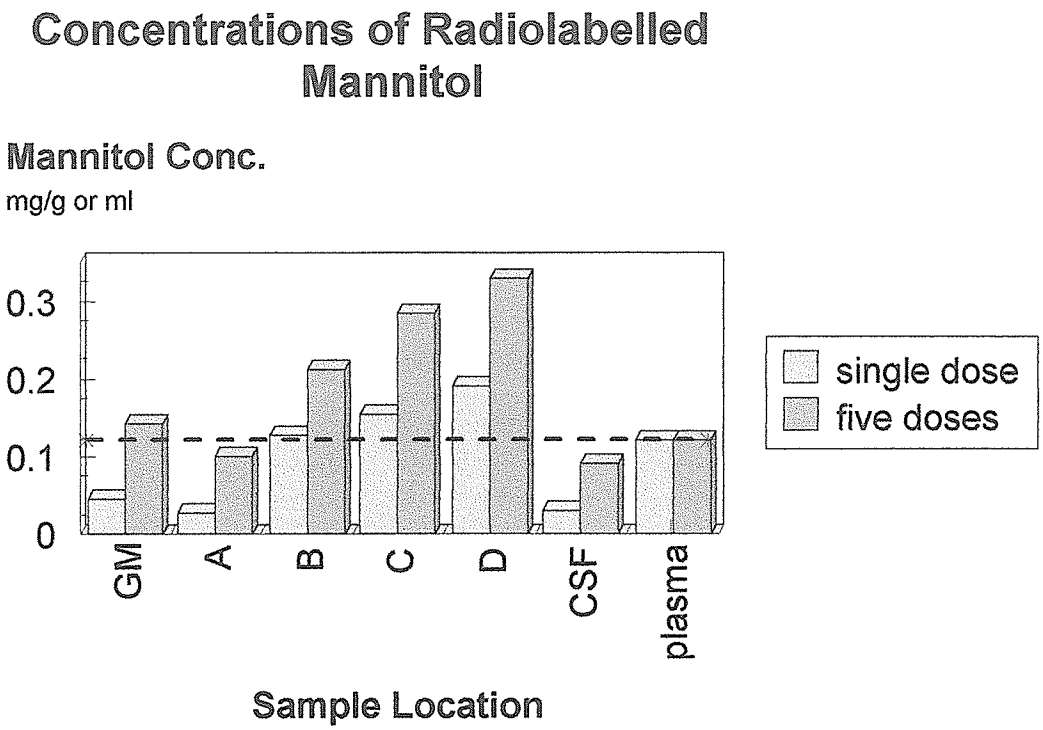


Figure 8 **Mannitol Concentrations**: Samples of brain tissue, as well as blood and cerebrospinal fluid (CSF) collected from animals that received one or five doses of mannitol are displayed. Non-edematous cerebral samples were obtained from cortex (GM) and white matter (A), while edematous samples were again collected from direcetly beneath the cortical cold injury (D), the middle zone of edema (C), and the advancing edge of edema (B). The broken line demonstrates the trough plasma concentrations, relative to cerebral concentrations measured four hours following the last dose.

CEREBRAL MANNITOL CONCENTRATIONS*

Mannitol Doses (1/3 g/kg)	Edematous			Non-edematous	
	Sub-lesion	Mid-edema	Advancing Edge	White Matter	Cortex
Single dose [mean]	0.19	0.16	0.13	0.03	0.05
Standard deviation	0.04	0.02	0.02	0.006	0.001
n	7	7	7	6	7
Five doses	0.33	0.28	0.21	0.12	0.16
Standard deviation	0.08	0.07	0.03	0.02	0.02
n	5	5	5	5	5

*mannitol concentrations in mg/g with brain tissue

DISCUSSION

CEREBRAL EDEMA

Experimental alteration of brain bulk with osmotic diuretics was first reported in 1919 [80]. Osmotic diuretics were then introduced for the purpose of cerebral dehydration in 1956 [33,34], and the effect of mannitol upon cerebral water content has been a topic of interest and debate since then. Mannitol has been shown to transiently reduce the cerebral water content by 2% [20]. However, the efficacy of repeated doses has not been established, and a risk of rebound or paradoxical aggravation of cerebral edema has been suggested [27,40].

There is extensive literature to document that transient cerebral dehydration is produced by osmotic diuretics, and especially mannitol [2,7,28,46]. The osmotic theory states that mannitol produces reduction of cerebral bulk by osmotic dehydration, acting at regions of normal blood-brain barrier [63]. The majority of these measurements were made in the first hour following intravenous administration, and demonstrated reduced cerebral water content only in non-lesioned cerebral hemispheres [17,28,46,61,63,81]. There are also studies that have shown reduced cerebral water content in regions of vasogenic edema following mannitol infusion [2,57]. While mannitol permeability increases in response to blood-brain barrier disruption [15,19,74], it must be recalled that although the area of cerebral edema has an expanded extracellular space, the integrity of its capillaries remains intact [10,41]. Therefore, osmotic gradients may become established and promote flux of water in edematous regions as well as in non edematous brain. This mechanism likely accounts for the reduction of cerebral water content and bulk following a single dose. However, if multiple doses of

mannitol result in cerebral concentrations exceeding those in blood, then rebound cerebral edema may develop.

The long term effects of mannitol upon cerebral water content have not been well characterized, and no placebo controlled randomized clinical studies have been conducted. Controlled clinical studies are difficult to conduct, because of the heterogeneous nature of the brain injured patients, as well as the unpredictable rate and extent of cerebral edema formation. Furthermore, brain tissue is not readily available for repeated measurements of cerebral water content. While computerized tomographic and magnetic resonance imaging may identify edematous tissue, they do not provide a means to readily quantify the cerebral water content. Intracranial pressure measurements have also been employed as an indirect estimation of brain swelling. However, intracranial pressure values are highly variable and unpredictable and may be a poor reflection of cerebral water content and edema progression [26]. Therefore, these were measured directly in the present study.

In the present study, cerebral water content in non-edematous cortex and white matter remained unchanged at 5 and 21 hours. This is an indication of the efficacy of cerebral homeostatic mechanisms and normal blood-brain barrier function. Beneath the cortical cold injury water content increased in the white matter. There was however no change in the water content of this edematous brain between 5 and 21 hours, although the volume of edema increased, as assessed by the edema progression measurements. Previous studies have identified a relatively constant rate of edema formation over this time course [1,41].

Mannitol had no significant effect upon linear progression of edema, after a single or 5 repeated doses. This was to be expected, as the formation and advance of edema fluid is thought to be primarily related to hydrostatic forces originating at the region of blood-brain barrier disruption. There was also no significant reduction of cerebral water content following single or multiple doses of mannitol. However a trend towards reduced cerebral water content in normal cortex and edematous white matter was seen following a single mannitol dose. While the previously reported observations of cerebral dehydration in the first minutes and hour following intravenous mannitol may have occurred in these animals as well, however, no persisting change was seen four hours later.

The cerebral water content of edematous white matter increased significantly 21 hours following the cortical cold injury. This observation does not preclude a transient reduction of cerebral bulk initially following the intravenous bolus, although a rebound of cerebral edema was measured four hours later. The 3% increase in regional cerebral water content indicates a major increase in cerebral water volume, which may produce significant mass effect, and local or global elevations of intracranial pressure.

MANNITOL PHARMACOKINETICS

Pharmacokinetic studies of mannitol following single-dose administration have demonstrated the same pattern of plasma clearance as observed in the present study [4,11,18]. Plasma concentration peaks at the end of the rapid intravenous injection, and then declines exponentially as a result of renal excretion and systemic redistribution. Further

pharmacokinetic analysis of single dose data from the present study confirmed the exponential decline of plasma mannitol concentrations, which were best fitted to a one-compartment model with elimination from the central compartment, with a plasma half life of 0.7 ± 0.3 hours (personal communication: Robert Ariano). The rapid plasma clearance prevented plasma accumulation of mannitol after multiple doses at four hour intervals. This is in agreement with the clinical observation that hyperosmolality after multiple doses of mannitol in patients is not caused by plasma accumulation of mannitol, but rather by hypernatremia secondary to fluid restriction and dehydration. If normal fluid balance is maintained, as in our animals, the serum osmolality and sodium levels are unaffected by multiple doses of mannitol [25].

Mannitol concentrations in normal brain tissue following a single dose were comparable to those reported by Wise et.al [81]. In those experiments, a 3 g/kg dose of radiolabeled mannitol was administered to non-lesioned dogs. The brain-to-plasma concentration ratio measured 6 hours thereafter was 0.61:1. We observed lower ratios of 0.23:1 for white matter, and 0.37:1 for cortex, measured only 4 hours after a lower intravenous dose 1/3 g/kg. These differences in dosage and time likely explain the lower ratios we obtained in the present study.

Although mannitol has low permeability across normal cerebral capillaries [3,59,66,75], an intravenous bolus produces transiently high plasma concentrations which allows mannitol to enter the cerebral extracellular space. Mannitol entry into brain has been previously reported.

Normal brain will initially accumulate 1% of the plasma level [75] when subjected to steady state plasma mannitol concentrations. The concentration will increase to 3 to 7% by four hours [3,75]. It is also known that mannitol remains within the cerebral extracellular space and is not significantly metabolized [15,59].

The present study found that five repeated doses of mannitol resulted in a threefold increase of concentration in non-edematous brain, indicating accumulation of mannitol in normal brain tissue. Therefore mannitol entering the cerebral extracellular space at times of high plasma concentration may become "trapped", relying on the slow clearance mechanisms of diffusion into the cerebrospinal fluid or back diffusion through capillaries. The 3 fold increase of cerebral mannitol in non-edematous brain was not likely related to alteration of blood-brain barrier function after 21 hours, as no associated alterations of cerebral water content were observed.

Mannitol concentrations in edematous white matter were markedly higher than in normal brain after a single dose, because of increased permeability across the disrupted blood-brain barrier. Five repeated doses resulted in a further nearly one and a half fold increase of mannitol concentrations throughout the edematous white matter. Several factors may account for this lesser increase compared to the 3 fold increase in normal brain. First, mannitol extravasating from the cortical cold injury was distributed throughout the expanding volume of edematous white matter by the process of bulk flow [10,15,69], and the mannitol concentrations decreased towards the advancing edge of edema. The clearance of edema

fluid into cerebrospinal fluid may also have limited the accumulation of mannitol in edematous brain [15,69]. Some amount of back diffusion of mannitol from the cerebral extracellular space to plasma may also have occurred at the cortical cold injury although would be limited in areas of normal blood-brain barrier function. Another reason for a lesser increase of mannitol concentrations in edematous white matter relates to the high cerebral concentrations following a single dose which would limit the trans-capillary gradient of mannitol during later doses.

It appears that mannitol accumulates in normal and edematous brain following the first repeated dose. An alternative explanation to account for this increase is a possible increase of blood-brain barrier permeability itself. There was, however, no evidence of this in normal brain regions, as cerebral water content values remained remarkably constant over the 21 hour period. Mannitol concentrations also increased in edematous brain. The stability of even the disrupted blood-brain barrier over time is suggested by observations that the vasogenic edema is produced at a constant rate [1,41]. Furthermore, the blood-brain barrier remains intact throughout most of the edematous white matter.

REBOUND EDEMA

In vasogenic edema, the flux of water at the cortical cold injury is dependent upon hydraulic forces. The permeability of this disrupted blood-brain barrier is high, and therefore, osmotic gradients will not become established. However, the integrity of the blood-brain barrier in the immediately adjacent edematous brain is partially intact [6,16], and the surrounding

edematous white matter has normal blood-brain barrier function [7,42]. Osmotic forces may therefore exert a considerable influence on the flux of water in the edematous brain, as an osmolar gradient of 1 mOsm/L is equivalent to a hydrostatic force of 19 torr [22]. Therefore, mannitol entering the edematous brain from the region of the cortical cold injury may influence changes of cerebral water content.

Multiple doses of mannitol resulted in osmotic concentration gradients between edematous cerebral tissue and plasma which potentially favored the influx of water into brain. The ratio was 1.58:1 for tissue samples obtained immediately beneath the cortical cold injury following a single dose, and 2.69:1 following five repeated doses. Indeed, cerebral water content was significantly higher in edematous white matter following multiple doses of mannitol, compared to control animals without mannitol administration. Mannitol concentrations in normal brain were much lower than in edematous regions, and no increase of cerebral water content occurred following multiple doses of mannitol.

The magnitude of the reversed concentration gradient favouring net flux of water into brain is underestimated by our measurements of cerebral mannitol concentration. The cerebral extracellular space has been estimated to equal around 15% of the total cerebral volume but rises to an estimated 33% in vasogenic edema models [23,44,62]. The measured cerebral mannitol concentrations are based on total mass of each sample, while mannitol which enters cerebral tissue remains in the extracellular space, and is not metabolized [15,59]. Therefore, the concentration within the extracellular space volume is approximately three times higher

than that directly measured in each cerebral sample. This higher concentration in the extracellular space influences the flux of water relative to plasma concentrations across the blood-brain barrier (Figure 9).

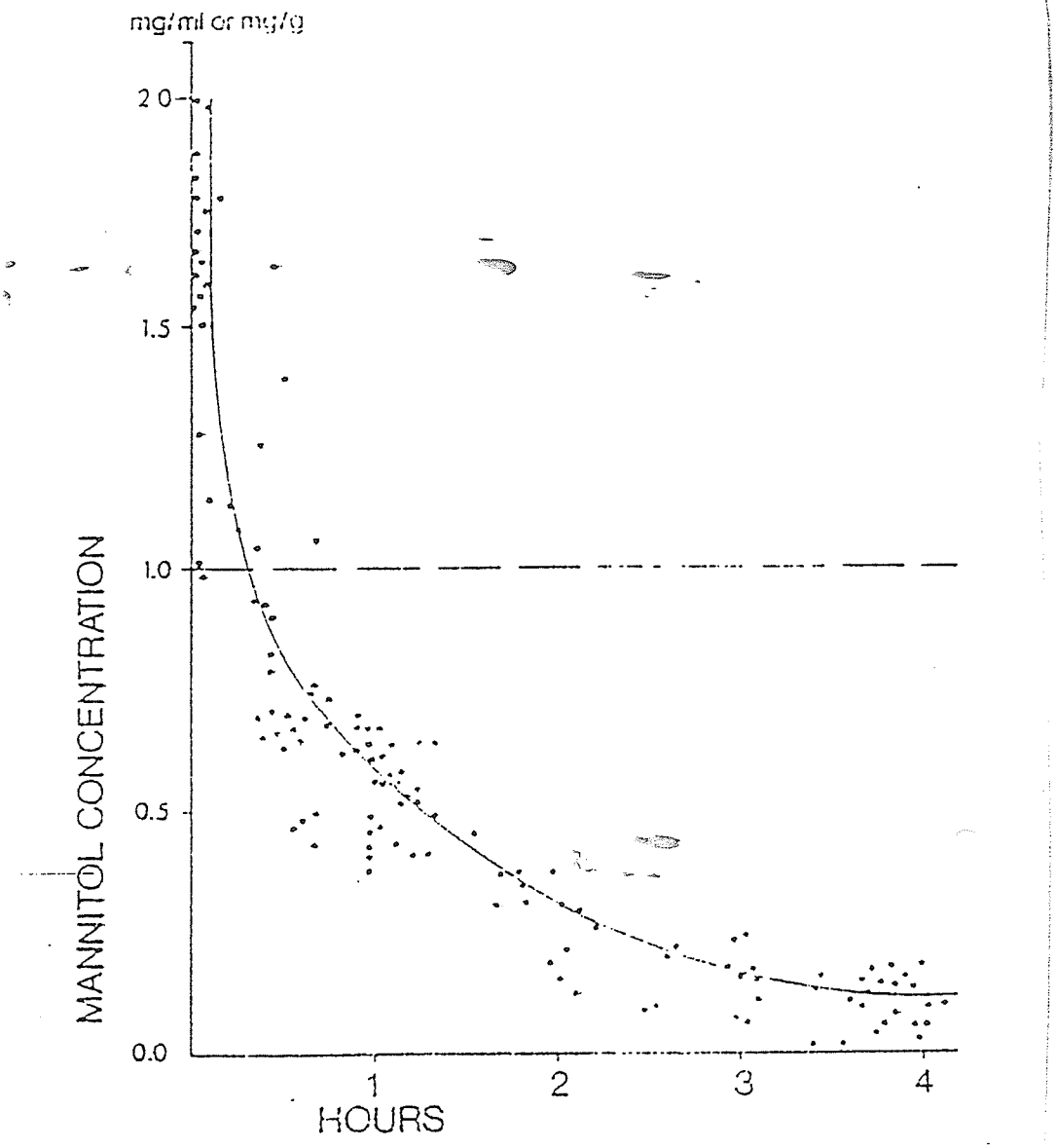


Figure 9 Graph showing the measured mannitol concentration in plasma after repeated injections of mannitol. The *horizontal interrupted line* represents the estimated concentration of mannitol in the cerebral extracellular space after five injections at 4-hour intervals. This assumes mannitol is confined to the extracellular space, which comprises 33% of the cerebral mass. This demonstrates a reversal of the mannitol concentration between plasma and cerebral extracellular space, which favored an influx of water into the tissue.

CONCLUSIONS

It is in the setting of acute intracranial hypertension that mannitol has proven most effective. Although the mechanism of its action remains debated, administration of a single intravenous bolus has become a cornerstone in the emergent treatment of patients with measured or suspected elevations of intracranial pressure or cerebral herniation. Repeated doses of mannitol are often employed in the treatment of brain injured patients, especially those with head trauma. It is not uncommon for mannitol to be administered empirically to these patients, although a beneficial influence upon recovery from brain injury has also not been demonstrated [35,76]. Furthermore, the actual risk of rebound cerebral edema related to mannitol accumulation has not been determined, and is difficult to monitor in the clinical setting.

The present study demonstrated no alteration of cerebral water content or edema progression four hours following a single 1/3 g/kg dose. Nevertheless, mannitol did enter cerebral tissue, with concentrations in edematous regions exceeding plasma concentrations after four hours. Administration of multiple doses of mannitol was associated with further elevations of mannitol concentrations in the brain. The 3% increase of cerebral water content in the region of vasogenic cerebral edema may have resulted from a reversal of mannitol gradients across the blood-brain barrier. In the clinical setting, these potentially adverse affects of repeated use of mannitol may be difficult to distinguish from the progressive nature of cerebral edema and swelling. Nevertheless, in the absence of evidence for beneficial effect, the use of repeated doses of mannitol should be reassessed.

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