Effects of Single, Repeated and Massive Mannitol Infusion in the Dog:

Structural and Functional Changes in Kidney and Brain

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MANNITOL is a six carbon alcohol of molecular weight 182 which, when given intravenously, equilibrates rapidly in the extracellular fluid space with virtually no entry into the body cell mass. It is not metabolized; recovery from the urine is almost complete after approximately 24 hours.[1, 2, 9] The use of mannitol to prevent acute renal tubular necrosis,[1, 9, 10] to reduce intracranial pressure[13] and to correct hypotonic overhydration[6, 9] has focused attention on the possibility of adverse side effects and on the diminished therapeutic effect sometimes observed with continued treatment.

The vacuolization and swelling of renal tubular cells which appear after mannitol injection have been referred to as osmotic nephrosis.[3, 7] Although these changes occur after single injections of mannitol and apparently are not associated with loss of renal function[3] it is not known how long they persist or whether renal impairment follows repeated parenteral administration of mannitol.

Although the immediate effect on plasma sodium concentration is a sharp reduction as new water is withdrawn from sources outside the extracellular phase, repeated or chronic mannitol infusion produces hypernatremia; severe fatal hypernatremia has been reported in patients whose water intake was sharply restricted. Some of these patients were comatose and were also receiving nasogastric tube feedings of large solute loads with a limited water intake[5]; prolonged solute diuresis from all these sources was ultimately lethal.

The diuresis which follows mannitol infusion involves the excretion of a hypertonic urine (600–700 mOsm./l.), and negative free water clearance is demonstrated throughout. Most of the urine solute is the

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mannitol itself, however, and the concentration of electrolyte and urea in this urine is low. Sodium concentration is frequently less than 10 mEq./l. Thus, the ratio of water to electrolyte in such urine is far in excess of that in plasma even though the urine is concentrated far above plasma osmolality; serum sodium concentration must thus rise inevitably with chronic administration of mannitol, just as it does in any other prolonged osmotic diuresis as, for example, that seen in prolonged untreated diabetes mellitus or in the tube feeding syndrome alone. This hypernatremia is presumably accompanied by intracellular hypertonicity as well, and is due to global dehydration. It is assumed but not certain that administration of water to match the urinary loss would prevent hypernatremia.

The nearly complete exclusion of mannitol from brain water * is assumed to account for its ability to lower cerebrospinal fluid pressure,[14] and to reduce total brain mass. In response to the mannitol-induced extracellular hypertonicity, water passes from the cerebrospinal fluid, the brain interstitium, and brain cells into the bloodstream. After repeated mannitol infusions, it is sometimes no longer possible clinically to reduce cerebrospinal fluid pressure with mannitol **; this failure further to reduce brain mass and cerebrospinal fluid pressure may result from eventual entry of mannitol into brain water upon repeated infusion, thus gradually obliterating the mannitol-induced osmolality gradient. Although very little mannitol enters the cerebrospinal fluid after single infusions, this effective diffusion-limitation at the blood-brain barrier may not be able to exclude mannitol completely as large concentrations are built up in the bloodstreams upon repeated or prolonged massive infusion.

These studies were designed to evaluate functional and microscopic changes in the kidney after short- and long-term mannitol infusion; to determine the effect of long-term mannitol infusion on serum electrolyte concentration in the presence of unrestricted oral water intake; to determine whether mannitol might indeed enter brain water in the face of sustained high serum concentrations of mannitol; and to characterize the mechanism of death in massive sustained mannitol infusion in dogs.

Materials and Methods

Experiments were performed on three groups of dogs. In Group I, shortterm functional and microscopic renal changes were evaluated in five dogs, each of which received a single infusion of mannitol. In Group II, changes in renal function and microscopic appearance and alterations in serum electrolyte concentration were evaluated in four dogs during and after repeated mannitol infusions. In Group III, each of eight dogs received a single prolonged massive mannitol infusion of lethal proportions. Changes in electrolyte and mannitol concentration of serum and cerebrospinal fluid were observed in five dogs. Brain size in comparison to cranial volume at the time of death was determined in six dogs. Interstitial and cellular water content of brain was observed in three dogs.

Group I—Single Mannitol Infusion

Five unanesthetized adult female dogs received single intravenous infusions of 25% mannitol in aqueous solution, 6 Gm./Kg. body weight, during a 3-hour period. Dosage here was at 1,372 mOsm./l. and 33 mOsm./Kg. body weight. The total weight of mannitol corresponding to an adult human subject would be about 420 Gm. in 3 hours or about 4,000 ml. of the usual 10% mannitol solution used clinically.

* The term "brain water" is here used to refer to the total water content of the central nervous system: cerebrospinal fluid, cerebral interstitial fluid, and cerebral cell fluid.

** Personal communication from Dr. R. A. Moody.

In two dogs serial renal function studies were performed prior to infusion and at 7, 28, and 145 days after infusion. The remaining three dogs underwent open renal biopsy within 1 hour after infusion, 24 hours after infusion and at intervals up to 150 days after infusion.

Group II—Repeated Mannitol Infusions

Four unanesthetized adult female dogs received daily intravenous infusions of 25% mannitol in aqueous solution, 2 Gm./Kg. body weight, for 18–23 days. The daily weight of mannitol would correspond to 140 Gm. or 1,400 ml. of the usual 10% mannitol solution in an adult man. In two dogs renal function studies were performed prior to the first infusion and at 28, 88, and 140 days after the first infusion. The remaining two dogs underwent open renal biopsy at intervals up to 140 days after the first infusion.

Group III—Single Massive Mannitol Infusion

Eight adult female dogs were anesthetized by intravenous injection of sodium pentobarbital, 30 mg./Kg. body weight. A small polyethylene catheter was introduced percutaneously into the subarachnoid basal cistern to allow sampling of cerebrospinal fluid. Systemic blood pressure was monitored by means of a cannula in the right common carotid artery. The urinary bladder was drained by an indwelling catheter. Each dog received a constant intravenous infusion of 25% mannitol in aqueous solution, 4 Gm./Kg. body weight per hour, for a period of 4 to 6 hours. Here, the dosage would correspond to that of a 5-hour infusion of 1,400 Gm. in an adult human subject, or 14 liters of 10% solution; this is about five times the usual maximum used during neurosurgical procedures.

Samples of serum, cerebrospinal fluid and urine were analyzed at frequent intervals for osmolality and for the concentration of sodium and mannitol in five dogs.

In six dogs after death the cranium was bivalved in a saggital plane and the brain removed. Cranial capacity was determined by reassembling the two halves after sealing all foramina except the foramen magnum; the volume of water needed to fill the cranium through the foramen magnum was then measured three times for each dog. Volume of the removed brain was determined by displacement of water in a graduated cylinder.

In three dogs portions of the cerebrum, cerebellum and brain stem were weighed before and after desiccation. The water content was expressed in grams per 100 grams of dry brain weight.

Renal Function Evaluation—(Groups I and II)

Dogs were confined in metabolic cages and allowed food and water ad libitum. Urine was collected over periods of 24 to 48 hours. Micturition was induced by subcutaneous injection of urecholine, 5 mg. Samples of urine and serum from peripheral blood obtained before and after periods of urine collection were analyzed by conventional methods for osmolality and concentrations of sodium, potassium and creatinine. The data were used to calculate urinary excretion of sodium and potassium as well as creatinine clearance, osmolar clearance and free water clearance using the following formulations:

Symbols	Definitions	Units
C_{cr}	Creatinine clearance	ml./min.
U_{cr}	Urine creatinine concentration	mg./100 ml.
P_{cr}	Plasma creatinine concentration	mg./100 ml.
V	Urine volume	ml./min.
C_{Osm}	Osmolar clearance	ml./min.
U_{Osm}	Urine osmolality	mOsm./l.
P_{Osm}	Plasma osmolality	mOsm./l.
C_{H_2O}	Free water clearance	ml./min.
U_{Na}	Urine sodium concentration	mEq./l.
U_K	Urine potassium concentration	mEq./l.

Formulae

$$C_{cr} = (U_{cr}) \times (V/P_{cr})$$
$$C_{Osm} = (U_{sm}) \times (V/P_{Osm})$$
$$C_{H_2O} = V - C_{Osm}$$

Microscopic Tissue Studies—Groups I and II

Renal biopsies and tissues obtained at autopsy were fixed in 10% neutral formalin. Tissue sections were stained with hemotoxylin and eosin and examined by light microscopy. Renal vascuolar changes were graded as follows:

0 = no vacuoles
1+ = small intracytoplasmic vacuoles in some deep cortical tubules, i.e., medullary rays*
2+ = many small vacuoles in cytoplasm of cells in all deep cortical tubules, and protrusion of vacuoles into lumens of deep cortical tubules
3+ = many small and large confluent vacuoles in cytoplasm of cells in all deep cortical tubules, and protrusion of vacuoles into lumens of deep cortical tubules
4+ = diffuse cytoplasmic vacuolization of cells in deep cortical tubules and mild vacuolization of cells in superficial cortical tubules
5+ = diffuse vacuolization of cells in all cortical tubules

Results

Group I—Single Mannitol Infusion

The degree and persistence of renal tubular vacuolization are summarized in Table 1. Renal biopsy within one hour after infusion revealed a response with 4+ to 5+ vacuolization (Figs. 1A, 1B). Vacuolization diminished during the following 3 weeks (Fig. 2) and was absent by day 21 in all three of the dogs which were subjected to multiple sequential biopsies.

Measurements of serum sodium and potassium concentration, plasma osmolality and renal function (urine volume per minute, osmolar clearance, free water clearance, creatinine clearance and sodium and potassium excretion) are shown in Table 2. Serum sodium and potassium concentrations and plasma osmolality remained within normal limits throughout the study period. Most of the measurements of renal function were also normal; creatinine clearance remained at pre-infusion values. Free water clearance remained negative which is consistent with the observed high urine osmolality. The only change in renal function noted during the 145-day test period was a steady decline in urinary sodium and potassium excretion to amounts only 5 to 20 per cent of pre-infusion values. Serum sodium and potassium concentrations remained normal.

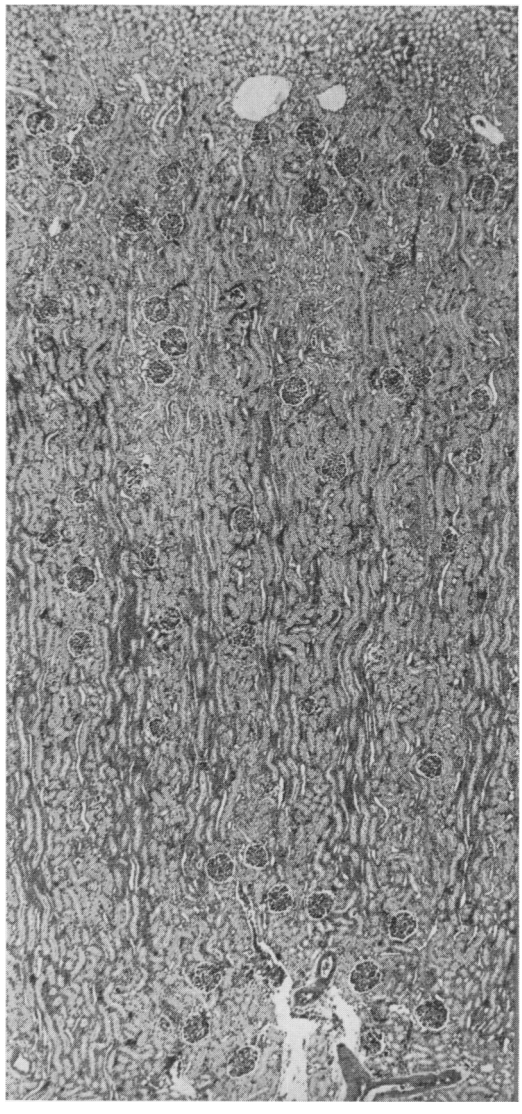

FIG. 1A. The renal cortex from a dog one hour after an intravenous infusion of mannitol, 6 Gm./Kg. body weight, shows diffuse cytoplasmic vacuolization of all cortical tubular cells (graded 5+). Mag. ×40.

* Medullary rays are here defined as the straight tubular columns in the deep portion of the cortex which link the distal convoluted tubules, which are in the renal cortex, with the collecting tubules which are in the renal medulla.

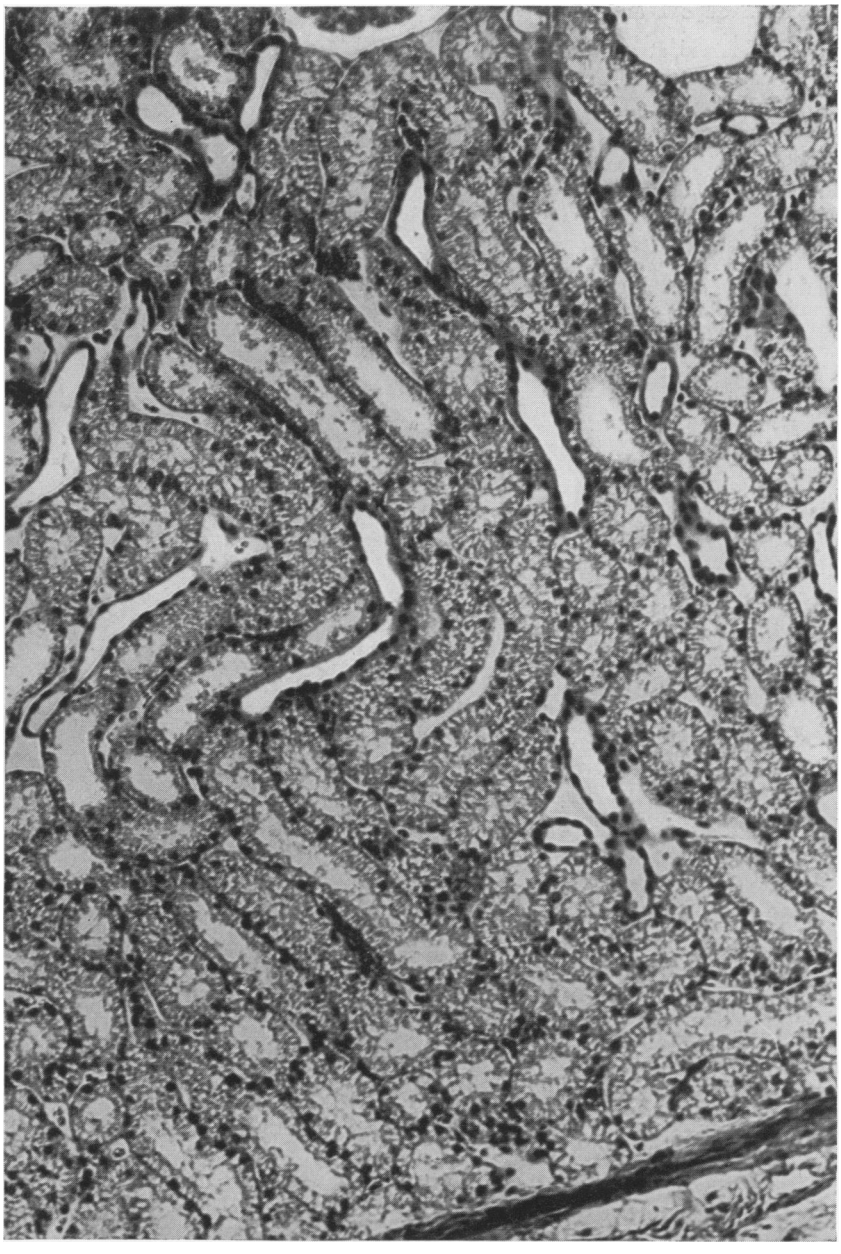

FIG. 1B. Higher magnification of Figure 1 showing diffuse 5+ vacuolization. Mag. × 100.

Group II—Repeated Mannitol Infusions

Tubular vacuolization reached a peak graded at 3+ on the first day of infusion and persisted throughout the 18 to 23 day period of infusion. Vacuolization persisted at a grade of 2+ in all subsequent biopsies and in tissue obtained at autopsy from all four dogs 140 days after the initial infusions (Fig. 3A, 3B).

Early renal function studies and those at 28, 88 and 140 days after the first infusion are shown in Table 3. By these criteria renal function was unaffected in spite of long-lasting cytoplasmic vacuolization in cells of deep cortical tubules.

Serum creatinine concentration and creatinine clearance rates were normal. Free water clearance was consistently negative,

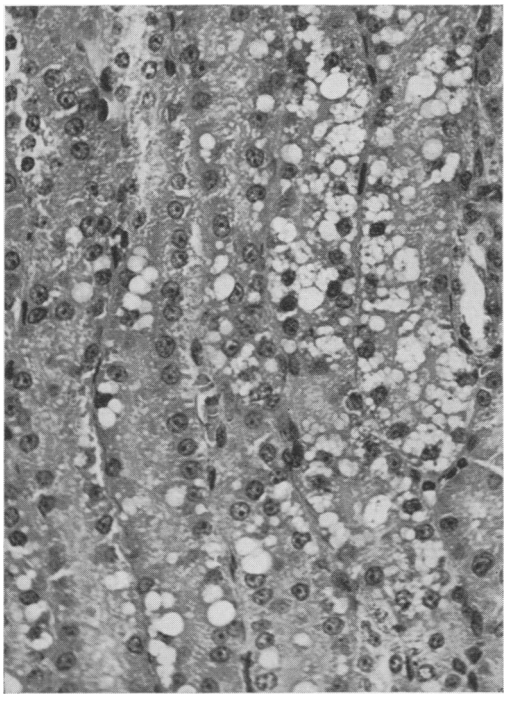

FIG. 2. A highly magnified view of the renal cortex from a dog two weeks after a single intravenous infusion of mannitol, 6 Gm./Kg. body weight, shows persistent minimal focal vacuolization (graded 1+). Mag. ×400.

consistent with renal tubular re-absorption of water presumably in response to antidiuretic hormone; osmolality of random urine samples was never less than 1,500 mOsm./l. Serum concentrations and urinary excretion of sodium and potassium were normal throughout the 140-day test period.

TABLE 1. *Group I—Single Mannitol Infusion—Renal Biopsy Data*

Time After Mannitol Infusion	Tubular Vacuolization Dog No.		
	1	2	3
1 hour	++++	+++++	++++
24 hours	++++	++++	----
2 days	----	+++	----
7 days	0	++	----
14 days	0	0	+
21 days	0	0	0
150 days	0	0	0

TABLE 2. *Group I—Single Mannitol Infusion—Renal Function Data and Electrolyte Concentrations*

Time after Mannitol Infusion	V (ml./min.)	U_{Osm} (mOsm./l.)	P_{Osm} (mOsm./l.)	C_{Osm} (ml./min.)	C_{H_2O} (ml./min.)	$(U_{Na})(V)$ (uEq./min.)	$(U_K)(V)$ (uEq./min.)	P_{cr} (mg./100 ml.)	C_{cr} (ml./min.)	Serum Sodium (mEq./l.)	Serum Potassium (mEq./l.)
Dog No. 1											
Control	0.12	2,032	309	0.79	−0.67	20.0	15.2	1.28	42.0	145	5.5
7 days	0.20	2,372	304	1.56	−1.36	37.2	29.2	1.35	40.0	147	4.8
28 days	0.33	1,708	305	1.85	−1.52	18.0	28.6	1.25	45.0	146	4.5
145 days	0.09	2,024	308	0.59	−0.50	1.4	3.4	1.14	37.6	140	4.8
Dog No. 2											
Control	0.20	1,992	296	1.30	−1.10	53.5	41.6	1.03	50.0	148	5.0
7 days	0.24	2,074	286	1.74	−1.50	44.0	41.4	1.05	47.0	147	5.2
28 days	0.10	2,050	293	0.70	−0.60	9.2	13.7	.96	47.0	146	4.6
145 days	0.08	2,208	316	0.56	−0.48	4.9	6.2	.90	47.5	152	4.7

TABLE 3. *Group II—Repeated Mannitol Infusions—Renal Function Data and Serum Electrolyte Concentrations*

Time after Mannitol Infusion	V (ml./min.)	U_{Osm} (mOsm./l.)	P_{Osm} (mOsm./l.)	C_{Osm} (ml./min.)	C_{H2O} (ml./min.)	(U_{Na}) (V) (uEq./ min.)	(U_K) (V) (uEq./ min.)	P_{cr} (mg./ 100 ml.)	C_{cr} (ml./min)	Serum Sodium (mEq./l.)	Serum Potassium (mEq./l.)
Dog No. 1											
Control	0.14	2,188	322	0.95	−0.81	3.8	16.3	1.34	41.5	147	4.5
28 days	0.18	1,708	295	1.04	−0.86	3.7	10.6	0.76	55.0	146	4.3
88 days	0.16	1,996	307	1.04	−0.88	- - -	- - -	1.17	42.0	145	4.5
140 days	0.17	1,968	300	1.12	−0.95	16.6	15.2	0.80	51.0	146	4.7
Dog No. 2											
Control	0.12	2,132	310	0.83	−0.64	10.3	10.0	1.56	40.0	144	4.4
28 days	0.19	1,772	298	1.13	−0.94	11.8	15.5	1.15	- - -	143	4.1
88 days	0.24	1,536	291	1.27	−1.03	- - -	- - -	1.25	- - -	146	4.3
140 days	0.22	1,586	310	1.12	−0.83	19.6	19.6	0.65	49.0	146	4.9

FIG. 3A. The renal cortex from a dog after 20 daily intravenous infusions of mannitol, 2 Gm./Kg. body weight, shows 2+ focal cytoplasmic vacuolization confined mainly to medullary rays (indicated by arrows) of the deep cortical tubules. Mag. ×40.

Group III—Single Massive Mannitol Infusion

Changes in the mean values of systemic arterial blood pressure, hematocrit and concentration of sodium, mannitol and total solute (osmolality) in plasma, urine and

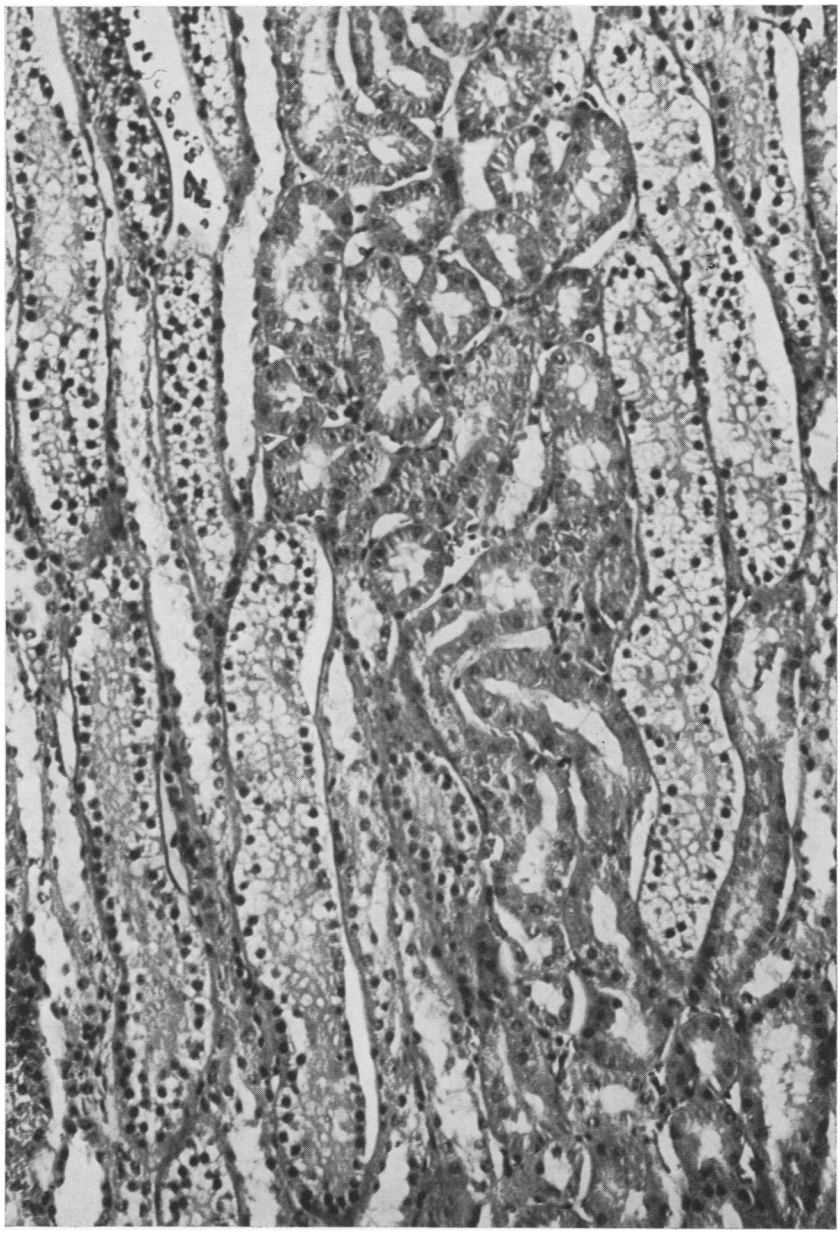

FIG. 3B. Higher magnification of Figure 3 demonstrates the focal nature of the vacuolization. Mag. × 100.

cerebrospinal fluid during massive mannitol infusion are shown in Table 4. Systemic arterial pressure was well maintained until the last hour before death which occurred at 240, 245, 285, 330 and 390 minutes after onset of infusion for the five dogs. The modest drop in pressure at 240 minutes represents a mean of low pressures in three terminal dogs and normal pressures in two dogs which survived for 330 and 390 minutes.

The hematocrit fell from 55 per cent before infusion to 50 per cent after one hour, rose to 53 per cent at 2 hours and then fell steadily until the time of death. There was a steady fall in plasma sodium concentration from 148 mEq./l. to 120 mEq./l. and a continuous rise in plasma osmolality from

TABLE 4. *Group III—Single*

Time, Minutes After Onset of Infusion	Mean Systemic Arterial Pressure (mm. Hg.)	Hematocrit	PLASMA				
			Na. Conc. (mEq./l.)	Osmolality (mOsm./l.)	Mannitol Conc. (mg./100 ml.)	Mannitol Conc.** (mM./l.)	"Crystalloid Osmolality" [($P_{Na} \times 2$) + 10] − P_{Osm} (mOsm./l.)
					****		
0	156	52	148	299	---	---	(−7)
60	158	45	130	326	979	54	56
120	161	40	130	346	1,356	75	76
180	155	50	127	374	1,701	94	110
240	133	43	121	415	2,314	127	163
300***	84	37	120	437	2,885	158	187

* Data in table are means from 3–5 experiments.
** Derived values.
*** Only two animals alive at 300 min.
**** The open dashes indicate that mannitol concentrations were not measured for these time periods. In the

299 mOsm./l. to 437 mOsm./l. Mannitol concentration in plasma increased steadily to a peak of 2,885 mg./100 ml. (158 mM./l.) at the time of death.

The crystalloid osmolality of plasma or of cerebrospinal fluid may be approximated by subtracting an estimate of total electrolyte osmolality from the observed total osmolality of the fluid. The electrolyte osmolality is approximated as twice the sodium concentration in mEq./l. plus 10. When this number is subtracted from the observed total osmolality, the resulting figure is an approximation of osmolality due to other molecular species. In normal dogs and in normal man this figure for crystalloid osmolality is close to zero. In liver failure, renal failure, diabetes, and other situations in which there is a pathological accumulation of crystalloids in the blood, this figure rises. When a solute such as mannitol is being administered, the figure for crystalloid osmolality will closely approximate the osmolar concentration of the mannitol itself. This figure for crystalloid osmolality of plasma and cerebrospinal fluid is shown in Table 4. It is apparent that most of the increase in total plasma osmolality during the first 180 minutes could be attributed to mannitol, and the figure for crystalloid osmolality almost exactly equals the osmolar concentration of mannitol. After 180 minutes, however, the figure for crystalloid osmolality exceeds the mannitol osmolality by approximately 30 mOsm./l. This suggests that under such severe and acute cellular dehydration there is an appearance in the extracellular fluid of some new solute, presumably arising from cells, and which we have not attempted to identify.

Renal excretion of mannitol was maximal between the second and third hour (3.04 Gm./Kg. Body weight/hr.) but failed to match the rate of hourly infusion. Except for a transient drop during the second hour, mannitol concentration in the urine rose throughout the infusion period. The appearance of a peak in total hourly mannitol excretion in the face of steadily rising urine mannitol concentration is the result of a decrease in hourly urine volume after the second hour.

As expected, osmolality of the cerebrospinal fluid increased at a somewhat slower rate than in plasma so that at the end of

Massive Mannitol Infusion, 4 Gm./Kg./Hr.*

URINE				CEREBROSPINAL FLUID				
Na Conc. (mEq./l.)	Osmolality (mOsm./l.)	Mannitol Conc. (mg./ 100 ml.)	Mannitol Excretion** (Gm./Kg. Body Weight/ Hr.)	Na Conc. (mEq./l.)	Osmolality (mOsm./l.)	Mannitol Conc. (mg./ 100 ml.)	Mannitol Conc.** (mM./l.)	"Crystalloid Osmolality" [(P_{Na} × 2) + 10] − P_{Osm} (mOsm./l.)
		****				****		
175	1547	---	---	151	299	---	---	(−14)
53	477	5,632	1.57	157	310	---	---	(−14)
49	469	5,319	2.98	159	317	---	---	(−11)
31	507	6,951	3.04	149	368	---	---	60
13	536	9,353	2.75	142	391	1,538	85	96
5	544	9,036	.36	155	416	1,808	100	96

cerebrospinal fluid the mannitol concentrations at the first 3 periods (0, 60 and 120 mins.) would approximate zero as indicated by the low values for crystalloid osmolality on those occasions. At 180 minutes, the mannitol could be estimated as between 15 and 75 mM./l. (270–1,350 mg./100 ml.) as based on crystalloid osmolality.

the second hour there was still an uncorrected gradient between plasma and cerebrospinal fluid of about 30 mOsm./l. This strong dehydrating gradient had already, at that time, withdrawn so much water from the brain that the cerebrospinal fluid sodium concentration had risen to 159 mEq./l. During this first 2 hours the increased osmolality of cerebrospinal fluid was entirely due to the increased electrolyte concentration as indicated by sodium. Thereafter, new crystalloid osmolality began to appear in the cerebrospinal fluid and by the fourth hour very large concentrations of mannitol were evident. Mannitol appeared to enter the cerebrospinal fluid rather suddenly between the third and fourth hour, and then in large concentrations. At no time did the cerebrospinal fluid show evidence of crystalloid osmolality above that accountable by mannitol.

Changes in extracellular fluid volume were calculated from changes in the plasma sodium concentration, after making allowance for sodium losses in the urine. These calculations were in turn based upon data for body composition in the normal dog, previously published from these laboratories.[8]

In Table 5 are shown calculations of the balance of mannitol and sodium, together with changes in extracellular fluid volume and other pertinent data for a single animal (M-9) that died at 285 minutes after the onset of a single massive mannitol infusion. The initial decreases in hematocrit and plasma sodium concentration were accountable by the addition of 350 ml. to the extracellular fluid volume. During that first hour 594 ml. of urine was excreted and 288 ml. of mannitol solution infused; there was a net loss of 306 ml. from the total body water. A shift of 656 ml. of water from the intracellular water compartment would be required to replace the 306 ml. net loss and supply the 350 ml. incremental volume of the extracellular fluid indicated by the drop in hematocrit and sodium concentration. This represents the osmotic withdrawal of fluid from body cells or other non-extracellular sources, in an amount approximately equal to the volume of mannitol infused.

During the second hour, this same process was maintained but now at a peak rate.

TABLE 5. *Effects of Single Massive*

Time After Onset of Infusion Minutes	Hematocrit	Interval Urine Vol. (ml.)	Interval Mannitol Infusion Vol. (ml.)	Interval Water Balance Urine-Infusion (ml.)	MANNITOL	
					Cumulative Infusion (Gm.)	Urine Mannitol Conc. (mg./100 ml.)
0	55	---	---	---	---	---
60	50	594	288	306	72	5,305
120	53	1,020	288	732	144	5,448
180	45	720	288	432	216	6,903
240	37	477	288	189	288	9,093
285 (death)	23	45	288	(−243)	360	9,310

*Initial weight of dog, 18.2 Kg.
** See text for details on derivation of these figures.

Urine formation and mannitol excretion were maximal. Despite a net fluid loss of 732 ml. (urine volume minus infusion volume) the volume of extracellular fluid still remained slightly expanded (200 ml. larger than the baseline value) but was smaller than had been observed at one hour. As a result, the hematocrit rose during the second hour; sodium loss in the urine (71 mEq./l.) was sufficient to prevent an increase in sodium concentration which remained low at about 130 mEq./l.

After the second hour, renal failure began to become manifest with a falling urine volume and a decreased ability to excrete the continuously presented load of mannitol. In the face of continued mannitol infusion, plasma mannitol concentration now rose sharply, and the volume of extracellular fluid increased further until, at the time of death, with a plasma sodium concentration of only 103 mEq./l., only 50 per cent of the infused mannitol had been excreted. The volume of extracellular fluid had expanded to an estimated value of 5,560 ml., and with a plasma osmolality in the vicinity of 400 mOsm./l., an extreme state of cellular dehydration had been produced.

The extent of cerebral dehydration is evident from Table 6. Brain volume has decreased from 98 per cent of cranial capacity in three control animals, to 77 per cent of cranial capacity in an animal that lived for 390 minutes; and to approximately 82 per cent of cranial capacity in the others. Decrease in the water content of brain tissue after 4 hours of mannitol infusion ranged from 17 per cent for the cerebrum to 24.5 per cent for the brain stem, as shown in Table 7. Although mannitol had entered the cerebrospinal fluid by the end of the experiment in all dogs, the value was still far below that in plasma.

Discussion

Mannitol in 25% aqueous solution was administered to dogs according to three different dosage schedules ranging from therapeutic (clinical) to lethal proportions. In Groups I and II, single and repeated non-lethal infusions, renal function and microscopic renal structure were observed repeatedly in an attempt to determine whether or not renal toxicity was associated with the cytoplasmic vacuolization of mannitol osmotic nephrosis. The single infusion of mannitol, 6 Gm./Kg. body weight in Group I, was an exaggerated dose which is not used frequently in clinical situations. However, the smaller dose, 2 Gm./Kg. body weight, which was infused daily in Group II more nearly approaches the usual clinical dose.

The cytoplasmic vacuoles following mannitol infusion were confined mainly to cells of the deep cortical tubules (medullary

Infusion on Dog No. M-9, Group III

Balance		Sodium Balance				
Cumulative Excretion (Gm.)	Excretion* (Gm./Kg. Body Weight/ Hr.)	Plasma Sodium Conc. (mEq./l.)	Urine Sodium Conc. (mEq./l.)	Cumulative Sodium Excretion (mEq.)	Extracellular Water Vol.** (ECW) (ml.)	Change In ECW From Onset of Infusion (ml.)
---	---	147	97	---	4,410	---
30	1.66	131	46	26	4,760	+350
86	3.05	130	44	71	4,610	+200
136	2.73	126	21	86	4,650	+240
180	2.38	117	9	90	4,900	+490
184	0.23	103	2	---	5,560	+1,150

rays). The degree of vacuolization appeared to be related to the amount of mannitol in a single infusion whereas the duration of vacuolization was related to the number of infusions. Single infusions of 6 Gm./Kg. body weight (Group I) were associated with 4+ to 5+ vacuolization throughout the renal cortex which disappeared within 3 weeks.

By contrast, daily infusions of 2 Gm./Kg. body weight for 18–23 days (Group II) were associated with less striking vacuolization (2+ to 3+) which was limited to deep cortical tubules, but persisted throughout the 140-day period of observation. The slow disappearance of vacuoles may reflect an impaired breakdown of the vacuole content by cells of the deep cortical tubules. Whether or not these vacuoles contained mannitol is not known.

In general, measured renal function of the dogs in Group I was not affected adversely by the mannitol infusion. Osmolar, free water and creatinine clearances were unchanged. The dogs retained the ability to concentrate urine normally. The only suggestion of functional change was the decrease in urinary excretion of sodium and potassium noted 28 and 145 days after infusion (Table 2); the vacuoles had already disappeared. Despite the decrease in sodium and potassium excretion, the serum concentration of both ions remained normal.

Despite marked and prolonged histologic changes, renal function was not affected adversely even in the dogs of Group II which received daily mannitol infusions over a period of almost 3 weeks. Creatinine clearance was unaltered. There were no

TABLE 6. *Group III—Comparison of Brain and Cranial Volume after Sustained Infusion of Mannitol, 4 Gm./Kg. Body Weight/Hr.*

Dog No.	Duration of Infusion (Hours)	Brain Volume* (ml.)	Cranial Volume* (ml.)	Brain Vol. × 100 Cranial Vol.
Control	0	84	85	99%
Control	0	91	93	98%
Control	0	95	98	97%
M-13	2	67	76	88%
M-14	4	64	76	84%
M-9	4½	71	86	82%
M-8	5½	75	91	82%
M-10	6½	68	88	77%

* Mean of 3 measurements.

TABLE 7. *Effect of Mannitol Infusion, 4 Gm./Kg. Body Weight/Hr. on Brain Water Content*

Dog No.	Duration of Infusion (Hours)	Water Content in Grams per 100 Gm. Dry Brain*			Per Cent Decrease in Water Content (Referred to Control Dog)		
		Cerebrum	Cerebellum	Brai Stem	Cerebrum	Cerebellum	Brain Stem
Control	0	329	351	246	0	0	0
M-13	2	306	334	225	7	5	8.5
M-15	3½	294	300	206	10.5	14.5	16
M-14	4	273	277	186	17	21	24.5

* Mean of 2–6 measurements.

significant alterations in electrolyte excretion. A continuing normal response to antidiuretic hormone was suggested by the continuing negative free water clearance despite the maintenance of a continued solute diuresis; this "water saving" property of the kidneys, together with the unlimited oral water intake allowed the animals, combined to prevent any hypernatremia. In patients with some degree of renal functional impairment, and an inability to achieve a maximum urine concentration despite solute diuresis, the dehydrating effect of a solute load is much more marked than it is in patients with good renal function. In none of these animals was there any initial renal functional impairment.

The vacuolization which occurred with mannitol infusion and which has been reported for other solute diuretics[7] bears some resemblance to the microscopic renal changes associated with potassium deficiency.[5, 11, 12] But there are several functional and microscopic points which differentiate the two conditions. In the nephropathy of potassium deficiency, vacuoles are smaller and are confined to the renal papillae; there is polyuria and loss of concentrating ability; free water clearance is usually positive or only slightly negative and there is little or no renal response to antidiuretic hormone.[4]

The massive fatal mannitol infusions in Group III also represent exaggerated doses, larger than those used clinically. This dose was selected to determine whether or not mannitol would enter the cerebrospinal fluid under conditions of a truly massive dose, and to observe alterations in brain water osmolality and brain volume at the lethal termination of such a dose.

Initially, many of the changes observed were the same as those associated with smaller infusions.[2, 9] There was an initial decrease in hematocrit and sodium concentration produced by the sudden presentation of a large mannitol infusion, and the sudden shift of water from a non-extracellular source (presumably body cells in general) to the extracellular space. Despite this, serum osmolality continued to rise at the rate of approximately 30 mOsm./l. each hour. Massive diuresis was quickly initiated, and the hematocrit returned transiently towards normal as large volumes of water were excreted from the expanding extracellular fluid volume. Plasma sodium concentration did not rise at this time, however, because of continued significant sodium losses in the urine; dog M-9 lost approximately 10 per cent of its estimated total exchangeable sodium during the diuresis.

Finally, after 3 hours of continued infusion, hematocrit and plasma sodium concentration fell again as renal compensation started to fail and infusion volumes were continued. Over all, the expansion of extracellular fluid volume was far more the result of water movement out of the body cell mass into the extracellular water, than

it was due to the effect of the retained infusion volume itself.

Although death evidently resulted from severe cellular dehydration with a plasma osmolality of 440 mOsm./l., and severe cerebral dehydration (as indicated by decrease in brain volume), the kidneys were evidently able to maintain water and salt conservation despite decreasing urine volumes. This was evidenced by the fact that up to the final samples, the osmolality of urine far exceeded that of plasma, and the urine sodium concentration was only 5 mEq./l. The new and unaccounted crystalloid appearing in plasma amounted to about 30 mOsm./l. at the end of the experiment, but none of this was evident in the cerebrospinal fluid where the mannitol concentration almost perfectly checked with the value for crystalloid osmolality.

During the first 2 hours, very little mannitol entered the cerebrospinal fluid despite very high concentrations in plasma. Although the osmolality in cerebrospinal fluid increased rapidly and approached (but never equalled) that of plasma, the increase during the first 2 hours was due almost wholly to the loss of water from brain water, with a resultant increase in sodium concentration to approximately 160 mEq./l. Then, between the third and fourth hour, significant movement of mannitol from plasma into brain water began rather abruptly, finally approaching a value about two thirds that of plasma. Viewed either as a gradient of total osmolality, of electrolyte osmolality, or of mannitol osmolality, this gradient persists up to the time of death.

After mannitol has finally entered brain water, its subsequent return to the bloodstream would presumably also be a rather slow process. Were the mannitol infusion to have been discontinued after 3 hours, plasma mannitol concentration could be expected to decrease rapidly as urinary excretion was resumed; a lag in return of mannitol concentration in brain water to blood levels would then result in the holding of water in the brain and the establishment of a gradient the reverse of that observed in these experiments. Such an occurrence might explain the refractory state in which mannitol becomes ineffective in reducing cerebrospinal fluid pressure, or, if used to excess and then suddenly discontinued, could be associated with severe or dangerous degrees of cerebral edema.

The decrease in brain volume observed in these experiments must be assumed to be due to a decrease in brain water volume as evidenced by the rising electrolyte osmolality of the cerebrospinal fluid which continued to rise terminally; our data do not indicate the relative participation, in cerebral dehydration, of the three identifiable compartments of brain water: cerebrospinal fluid, cerebral interstitial water, and cerebral cell water.

Summary and Conclusions

1. Serial renal function studies and microscopic observations were obtained during a 6-month period in two groups of dogs infused with a 25% aqueous solution of mannitol. Five dogs received a single infusion of 6 Gm./Kg. body weight. Four dogs received 18 to 23 consecutive daily infusions at a dose of 2 Gm./Kg. body weight. A third group of five dogs received a constant mannitol infusion of 4 Gm./Kg. body weight each hour until the time of death; osmolality as well as the concentrations of mannitol and sodium in serum and cerebrospinal fluid were compared.

2. Cytoplasmic vacuolization was confined to cortical tubules.

3. The extent and duration of vacuolization were directly related to the dose and number of mannitol infusions.

4. Except for a decrease in urinary sodium and potassium excretion after single large infusions, neither single nor repeated infusions of mannitol had any adverse effect on renal function, even in the presence of vacuoles.

5. In a third group of dogs, sustained infusion of mannitol of 4 Gm./Kg. body weight per hour caused severe cellular and cerebral dehydration with death after 4 to 6 hours.

6. During the first 2 hours of such massive mannitol infusion the major cause for increase in plasma osmolality was addition of the new solute, mannitol. In these experiments there was a fall in plasma sodium concentration which was due in part to dilution by the mannitol infusion and intracellular water but also largely a result of the continued significant renal excretion of sodium.

7. The increase in osmolality of cerebrospinal fluid was due initially to loss of water, with a rise in electrolyte osmolality (as indicated by the sodium concentration) which almost perfectly accounted for the observed total osmolality. Mannitol did not enter the cerebrospinal fluid readily until the third hour of infusion when plasma mannitol concentration rapidly rose to a value approximately two thirds that of plasma.

8. New unidentified crystalloid osmolality finally appeared in the plasma, amounting to about 30 mOsm./l. at the end of the experiment, as evidenced by the difference between the mannitol-induced osmolality, and the observed total osmolality corrected for electrolyte. It is presumed that some substance was being withdrawn from cells, in this extreme situation of extracellular hypertonicity; no such new or unaccounted crystalloid osmolality appeared in cerebrospinal fluid where the observed osmolality was totally accountable on the basis of electrolyte plus mannitol.

9. At death these animals showed a shrinkage of the brain amounting to a maximum of 23 per cent of brain volume; studies of water content of brain tissue itself showed the dehydrating effect to be about evenly distributed between cerebrum, cerebellum, and brain stem.

10. Even with this massive lethal infusion, and with terminal inability of renal function to continue to elaborate high urine volumes, the kidney was still evidently able to maintain some conservation of water and salt suggesting maintenance of some components of tubular function.

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