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Research Article

To investigate the pathophysiology of cerebral edema occurring during treatment of diabetic coma, the effects of hyperglycemia and rapid lowering of plasma glucose were evaluated in normal rabbits. During 2 h of hyperglycemia (plasma glucose=61 mM), both brain (cerebral cortex) and muscle initially lost about 10% of water content. After 4 h of hyperglycemia, skeletal muscle water content remained low but that of brain was normal. Brain osmolality (Osm) (343 mosmol/kg H₂O) was similar to that of cerebrospinal fluid (CSF) (340 mosmol/kg), but increases in the concentration of Na⁺, K⁺, Cl⁻, glucose, sorbitol, lactate, urea, myoinositol, and amino acids accounted for only about half of this increase. The unidentified solute was designated "idiogenic osmoles". When plasma glucose was rapidly lowered to normal with insulin, there was gross brain edema, increases in brain content of water, Na⁺, K⁺, Cl⁻ and idiogenic osmoles, and a significant osmotic gradient from brain (326 mosmol/kg H₂O) to plasma (287 mosmol/kg). By similarly lowering plasma glucose with peritoneal dialysis, increases in brain Na⁺, K⁺, Cl⁻, and water were significantly less, idiogenic osmoles were not present, and brain and plasma Osm were not different. It is concluded that during sustained hyperglycemia, the cerebral cortex adapts to extracellular hyperosmolality primarily by accumulation of idiogenic osmoles rather than loss of water or gain in solute. When plasma glucose is [...]

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Studies on Mechanisms of Cerebral Edema in Diabetic Comas

EFFECTS OF HYPERGLYCEMIA AND RAPID LOWERING OF PLASMA GLUCOSE IN NORMAL RABBITS

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ABSTRACT To investigate the pathophysiology of cerebral edema occurring during treatment of diabetic coma, the effects of hyperglycemia and rapid lowering of plasma glucose were evaluated in normal rabbits. During 2 h of hyperglycemia (plasma glucose = 61 mM), both brain (cerebral cortex) and muscle initially lost about 10% of water content. After 4 h of hyperglycemia, skeletal muscle water content remained low but that of brain was normal. Brain osmolality (Osm) (343 mosmol/kg H₂O) was similar to that of cerebrospinal fluid (CSF) (340 mosmol/kg), but increases in the concentration of Na⁺, K⁺, Cl⁻, glucose, sorbitol, lactate, urea, myoinositol, and amino acids accounted for only about half of this increase. The unidentified solute was designated "idiogenic osmoles". When plasma glucose was rapidly lowered to normal with insulin, there was gross brain edema, increases in brain content of water, Na⁺, K⁺, Cl⁻ and idiogenic osmoles, and a significant osmotic gradient from brain (326 mosmol/kg H₂O) to plasma (287 mosmol/kg). By similarly lowering plasma glucose with peritoneal dialysis, increases in brain Na⁺, K⁺, Cl⁻, and water were significantly less, idiogenic osmoles were not present, and brain and plasma Osm were not different. It is concluded that during sustained hyperglycemia, the cerebral cortex adapts to extracellular hyperosmolality

primarily by accumulation of idiogenic osmoles rather than loss of water or gain in solute. When plasma glucose is rapidly lowered with insulin, an osmotic gradient develops from brain to plasma. Despite the brain to plasma osmotic gradient, there is no net movement of water into brain until plasma glucose has fallen to at least 14 mM, at which time cerebral edema occurs.

INTRODUCTION

The problem of cerebral edema complicating diabetic coma has been known at least since the studies of Dillon, Riggs, and Dyer (1). These workers reviewed 21 uncomplicated deaths in patients with diabetic ketoacidosis and in eight of these, the clinical picture and postmortem examination (excluding cranial contents) revealed no obvious cause of death. All patients were under 45 years old and had developed a characteristic clinical picture consisting of hyperpyrexia, hypotension, increasing depth of coma, and tachycardia. These signs usually developed when the patients were showing biochemical improvement, and presaged a rapid downhill course which terminated fatally within a few hours. At autopsy, all such patients had gross and/or microscopic evidence of cerebral edema.

The aforementioned findings were largely unappreciated, although there were several sporadic reports of unexplained deaths from diabetic ketoacidosis (2, 3). Recent awareness of the syndrome of cerebral edema complicating treatment for diabetic coma was stimulated by descriptions of the deaths of two young patients apparently recovering from ketoacidotic coma (4).

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There have since been several similar reports (5-7) as well as two cases of cerebral edema associated with nonketotic hyperosmolar coma with hyperglycemia (nonketotic coma) (8, 9). Recent evidence suggests that most patients being treated for diabetic ketoacidosis develop increased intracranial pressure (10) and that rapid lowering of blood glucose in hyperglycemic dogs is often associated with increased cerebrospinal fluid (CSF)¹ pressure (11).

The mortality among patients with such cerebral edema has been close to 100% (3, 4), and treatment has generally been ineffectual. Although there have been some studies on the changes which occur in the CSF during rapid lowering of plasma glucose, alterations in water, electrolyte, and carbohydrate metabolism in brain under these circumstances are largely unknown. Furthermore, in previous experimental work, plasma glucose has been lowered by intravenous hydration rather than with insulin (12), so that any possible effects of insulin on brain have not been studied. It is the purpose of the present investigation to study the changes which occur in brain and CSF during sustained hyperglycemia, and to elucidate the pathophysiology of cerebral edema which may occur during rapid lowering of plasma glucose. Skeletal muscle, representing about 40% of body weight, was also studied in order to compare changes in brain with those occurring in other tissues.

METHODS

Studies were done in New Zealand white rabbits, weight 1.8-2.7 kg, maintained on an *ad lib.* diet of Purina Chow and water. Animals were anesthetized with intravenous sodium pentobarbital. A tracheostomy was performed after which mechanical ventilation was carried out for the duration of each experiment (Harvard Respirator No. 661; Harvard Apparatus Co., Inc., Millis, Mass.) at a respiratory rate of 25 per min and tidal volume based on the weight of the animal (13). Hyperglycemia was induced by infusion of 50% glucose into the inferior vena cava via a polyethylene catheter in the femoral vein. A bolus of 2.4 g/kg was followed by an average sustained infusion of 36 mg/kg per min. The rate of infusion was adjusted to maintain plasma glucose at about 60 mM for periods of 1-4 h. The bolus contained 25 μ Ci of uniformly labeled [¹⁴C]glucose (Nuclear Dynamics, Inc., El Monte, Calif., specific activity 298 mCi/mmol), at an average concentration of 0.9 μ Ci/mmol and the infusion contained 25 μ Ci at a mean concentration of 0.2 μ Ci/mmol.

Seven series of rabbits were studied: (1) control; (2) 1 h of hyperglycemia; (3) 2 h of hyperglycemia; (4) 4 h of hyperglycemia; (5) 4 h of hyperglycemia, then rapid (about 3 h) lowering of plasma glucose to 30 mM by infusion of insulin and 0.45% NaCl; (6) same as (5), except that glucose was rapidly (about 4 h) lowered to normal (less than 14 mM); (7) same as (5) except that plasma

glucose was rapidly lowered to less than 14 mM by glucose-free peritoneal dialysis and infusion of 0.45% NaCl.

Urine was collected via an indwelling Foley catheter and the volume was continuously recorded throughout the duration of the experiment. The 0.45% NaCl infusion in groups 5, 6, and 7 contained 20 meq/liter of potassium acetate and was calculated to restore the animal to its pre-experimental weight over a period of 3-4 h. Crystalline insulin was given intravenously in groups 5 and 6 at a rate of 50 U/kg each 2 hr. Group 5 animals were treated until their plasma glucose had fallen to about 30 mM, at which time the experiment was terminated. The average time of insulin administration was 3 h (range, 2-5 h) and the mean insulin dosage was 200 U. Group 6 animals were treated until their plasma glucose had fallen to less than 17 mM; they received a mean of 200 U insulin over a period of 3-7 h (average = 4 h). During preliminary experiments in three animals, we attempted to lower plasma glucose with smaller quantities of regular insulin (10-25 U/2 h). During the 4 h after cessation of glucose infusion, plasma glucose fell only from 59.0 to 48.8 mM. In group 7 animals, peritoneal dialysis was continued until the plasma glucose had fallen below 14 mM, at which time the experiment was terminated. The 0.45% NaCl infusion and peritoneal dialysis (100 ml per exchange) were regulated so that animals were restored to their pre-experimental weight at the conclusion of the procedure. The average duration of peritoneal dialysis for the series was 4 h (range 2.25-6 h). Measurements were made of ¹⁴C activity, Na⁺, K⁺, Cl⁻, and free glucose in urine, plasma, CSF, brain, and muscle. In addition, the pH, sorbitol, and osmolality (Osm), were measured in plasma; sorbitol and Osm were measured in CSF; Osm, sorbitol, lactate, free amino acids, myoinositol, water content, and extracellular space (ECS) were measured in brain, while ECS, sorbitol, and water content were measured in skeletal muscle. About 10 min before the conclusion of each experiment, specimens were obtained in the following manner.

Skeletal muscle (*sartorius*) was gently dissected free from fascial attachments with blood supply intact. The muscle body was then rapidly cross-clamped with two hemostats, severed at both ends with a scalpel, and plunged into liquid nitrogen. The top of the skull was then removed with the dura intact using a trephine and rongeur. Hemostasis was secured using Gelfoam (The Upjohn Co., Kalamazoo, Mich.) and bone wax, and the dura was then removed using a dural forceps and dural scissors. Arterial blood was obtained via a catheter in the femoral artery and cisternal CSF was removed with a bivalve needle. Then, in rapid succession, the carotid arteries were severed with a scalpel, the animal was decapitated, and the whole brain was scooped out with a spatula. The cerebral hemispheres were rapidly separated from the rest of the brain and immersed in a Dewar flask filled with liquid nitrogen. The elapsed time from interruption of blood supply to immersion of the brain or muscle tissue in liquid nitrogen was less than 8 s in all cases. Such treatment has been shown to prevent autolysis of glucose and glycolytic intermediates (14).

Preparation of tissues was accomplished in the following manner: The cerebral hemispheres (or muscle sample) were transferred to a mortar and pulverized with a stainless steel bush chisel while under liquid nitrogen. Three samples of about 0.5 g were then quickly transferred to tared precooled homogenizer tubes and weighed to the nearest 0.1 mg. 3 g of ice cold 3% trichloroacetic acid was then added to each tube and the samples were homogenized with a glass pestle. The homogenates were then centrifuged at 0°C for 1 h and the deproteinized supernatant was used for deter-

¹ Abbreviations used in this paper: CSF, cerebrospinal fluid; ECS, extracellular space; Osm, osmolality; TR, total radioactivity.

mination of glucose, sorbitol, total amino acid, lactate, myoinositol, urea, and ^{14}C activity.

Three additional 0.5 g samples were transferred to tared conical flasks, weighed to 0.1 mg, oven dried at 105°C for 36 h, and then weighed again; subtraction of the dry weight from the wet weight gave the tissue water content. Each sample was then extracted with 0.75 N HNO_3 for 24 h, after which the supernatant was used for determination of Na^+ , K^+ , and Cl^- (15).

The Na^+ and K^+ were measured with a flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass.) and Cl^- with Cotlove apparatus, all by previously described analytical methods (15, 16). The extracellular space (ECS) of brain and muscle were determined as the chloride space relative to CSF (17) or plasma (18), respectively. Brain tissue Osm was determined on five additional samples (each about 0.3 g) of liquid nitrogen frozen tissue by extraction in boiling distilled water. The analytical method has already been reported in detail from this laboratory and has previously been evaluated during hyper- and hypo-osmolar states in the rabbit (19).

Free glucose was measured by the *o*-toluidine method (20). Myoinositol (21), sorbitol (22), and lactate (23) were all determined by measuring the rate of conversion of NAD to NADH_2 in a Zeiss spectrophotometer (Carl Zeiss, Inc., New York) at 340 nm. The enzymes for each assay were myoinositol dehydrogenase (Sigma Chemical Co., St. Louis, Mo.), sorbitol dehydrogenase (Boehringer Mannheim Corp., New York), and lactic dehydrogenase (Sigma), respectively.

The sorbitol assay was modified from the published method (22) as follows: Each sample was filtered twice through an AGM-60 ion exchange resin, H^+/OH^- form (J. T. Baker Chemical Co., Phillipsburg, N. J.), prior to analysis. Glycine- NaOH buffer, $\text{pH} = 9.5$ was used instead of pyrophosphate buffer and triethanolamine- NaOH buffer, $\text{pH} = 7.4$ was used instead of Tris buffer.

This method for myoinositol determination (21) was modified by substitution of 0.1 M pyrophosphate buffer, $\text{pH} = 9.0$ ($\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$, 74.8 g; semicarbazide HCl , 8.33 g; glycine, 1.67 g; adjust pH to 9.0 with 12 N NaOH); make up to 1 liter with distilled H_2O) for the sodium carbonate buffer. Free amino acids were determined with ninhydrin reagent (24), and urea by Technicon Auto-Analyzer technique (Technicon Co., Inc., Tarrytown, N. Y.).

The ninhydrin reaction measures total alpha amino nitrogen which includes urea. Thus, the urea concentration was subtracted from the total alpha amino nitrogen to give the free amino acid concentration. The accuracy of each method was substantiated by recovery of standards from brain tissue extracts. Recovery of a 5.55 mmol/liter glucose standard was 90–102% for five samples. For sorbitol, both 100 and 200 $\mu\text{mol/liter}$ standards were used for each assay; recovery was 80–110% for eight of each of the two standards. Recovery of a 4.4 mmol/liter lactic acid standard was 98–102% for eight determinations. Both 5 mmol/liter aspartic acid and 5 mmol/liter glutamic acid were used as standards for each amino acid assay. The recovery was 85–120% for 12 determinations for each standard. Both 0.2 and 0.3 mM myoinositol standards were used for the myoinositol determination; recovery was not attempted. For all recovery experiments, standards were added to the tissue extract in a concentration which was similar to that of the unknown in each extract.

Activity of ^{14}C was determined by adding 10 ml of Aquasol (New England Nuclear Corp., Boston, Mass.) to 0.5

ml of either tissue extract, plasma, or CSF in a counting vial (Packard Instrument Co., Inc., Downers Grove, Ill.). Plasma and CSF samples were treated with trichloroacetic acid in a similar manner to the tissue samples and were processed in the same manner, to assure similar quenching. The samples were counted with 70% efficiency in a Nuclear-Chicago liquid scintillation spectrometer (Nuclear-Chicago Corp., Des Plaines, Ill.). The total radioactivity (TR) for glucose (dpm/mmol glucose) in brain, and CSF was calculated by dividing the total radioactivity (dpm/g fresh wt) by the glucose concentration (mmol/kg H_2O) (25). After determining the total radioactivity for plasma, glucose was removed by treatment with CuSO_4 and $\text{Ca}(\text{OH})_2$ (26) and the supernatant was counted again. Subtraction of the value so obtained removed ^{14}C activity due to glucose metabolites in plasma and gave the actual specific activity (SA) for glucose (dpm/mmol).

Metabolites of glucose in brain were determined by column chromatography in the following manner: After total counts per minute were determined on tissue, the extract was filtered through a Bio-Rad ag 1-xS, 100×200 mesh, acetate form column (column 1) (Bio-Rad Labs, Richmond, Calif.). The extract which had been filtered through the column was then passed through a Mallinckrodt c g 120, H^+ form, 100×200 mesh column (column 2) (Mallinckrodt Chemical Works, St. Louis, Mo.). The first column retains lactate and pyruvate primarily, while the second column retains amino acids primarily. The eluate which passes through both columns is the so-called neutral fraction, which is mainly glucose but also contains fructose and sorbitol. Column 1 was then treated with 1 M formic acid to remove lactate, and with 4 M formic acid to remove pyruvate. Column 2 was treated with 2 M NH_4OH to remove amino acids. By then counting the eluates from columns 1 and 2, the glucose metabolites in tissue could be determined as a percent of the total ^{14}C activity present in the tissue (minus the ^{14}C which has been lost as CO_2). Counting of the eluate which had passed through both columns gave the amount of ^{14}C activity which remained as neutral fraction (primarily glucose).

Four additional animals were treated with glucose infusion, insulin, and hypotonic NaCl as in group 5. In these animals and in four normal rabbits, the cortex was frozen in liquid nitrogen as previously described. Triplicate samples of about 0.5 g were extracted with hot 30% KOH and ethanol, then hydrolyzed with H_2SO_4 (25). This procedure serves to hydrolyze glycogen to glucose, which was then determined in the supernatant (20).

The albumin space was measured in tissue samples as follows: 15 min prior to the conclusion of the experiment, the animal was given intravenously 10 μCi of ^{125}I -labeled albumin (E. R. Squibb & Sons, New York). Weighed samples of plasma and brain were counted in an automatic gamma counter for 1 min. The albumin space was then calculated as the ratio:

$$\frac{\text{dpm/g brain tissue}}{\text{dpm/g plasma}} \times 100.$$

The pH was measured in arterial blood on a Radiometer-Copenhagen pH meter.

RESULTS

Acute hyperglycemia

Brain. In Tables I, II, and III are shown the control values and the changes in plasma, CSF, brain, and

TABLE I
Plasma and CSF Electrolytes

	Plasma					CSF			
	Osm	Na	K	Cl	pH	Osm	Na	K	Cl
	<i>mosmol/kg</i>		<i>meq/liter</i>			<i>mosmol/kg</i>		<i>meq/liter</i>	
Control (14)									
Mean	295	140	3.54	100	7.33	301	152	3.23	128
±SE	1	1	0.10	2	1.7×10^{-9} mM H ⁺	3	2	0.10	1
Hyperglycemia—1 h (7)									
Mean	321	130	3.11	92.5	7.32	318	159	3.29	132
±SE	4	1	0.20	1	2.8×10^{-9} mM H ⁺	4	4	0.20	1
Hyperglycemia—2 h (10)									
Mean	339	128	3.00	96.1	7.31	338	171	3.59	135
±SE	8	2	0.10	2	1.8×10^{-9} mM H ⁺				3
Hyperglycemia—4 h (8)									
Mean	338	128	2.90	101	7.29	340	169	3.92	136
±SE	5	3	0.20	5	3.0×10^{-9} mM H ⁺	4	7	0.31	2
Insulin, plasma glucose 63 → 29.6 mM (6)									
Mean	310	135	4.19	108	7.21	316	143	2.80	128
±SE	10	2	0.30	3	4.0×10^{-9} mM H ⁺	12	6	0.11	8
Insulin, plasma glucose 54 → 10.0 mM (6)									
Mean	287	141	3.46	116	7.19	301	153	2.91	133
±SE	4	4	0.20	4	1.0×10^{-8} mM H ⁺	4	2	0.20	2
Peritoneal dialysis, plasma glucose 56 → 13.6 mM (5)									
Mean	291	136	3.20	107	—	297	152	2.92	130
±SE	2	2	0.26	2		4	6	0.15	5

() = number of animals.

skeletal muscle which occurred during 1, 2, and 4 h of sustained hyperglycemia. While the plasma glucose was maintained at about 60 mM, the free glucose concentration in the brain rose from 3 to 13 mmol/kg H₂O after 2 h and did not increase further (Fig. 1). Similarly, the CSF glucose, after increasing from 5 to 20 mM at 2 h, then also plateaued. There was a highly significant correlation between the glucose concentration in CSF with that in brain water ($r = 0.98$, $P < 0.001$), with the concentration of the latter almost exactly paralleling that of the former (Fig. 1). Assuming that the glucose concentration in cisternal CSF is similar to that of the brain extracellular fluid, the brain intracellular glucose rose from 1.9 mmol/kg H₂O in the control state to a maximum of 10 mmol/kg H₂O after 4 h of hyperglycemia (using Cl space as the brain ECS).

In the control animal, the sum of the concentrations in brain of Na⁺, K⁺, Cl⁻, glucose, urea, myoinositol,

sorbitol, lactate, and amino acid was 269 mmol/kg brain H₂O, while the measured brain Osm was 299 mosmol/kg brain H₂O (Tables I and II). The unidentified solute was thus equivalent to 30 mmol/kg H₂O. For purposes of comparison, it was assumed that this quantity of solute did not change during experimental procedures. The 30 mmol of solute/kg brain H₂O has been designated as "other" in Fig. 2. Any additional unidentified solute which was present following experimental procedures was presumed to have either arisen de novo in brain or to have been transported into brain from the circulation. Such unidentified solute has been designated as "idiogenic osmoles".

After 1 h of hyperglycemia, the brain had not reached osmotic equilibrium with the plasma and CSF (Tables I and II), and there was no loss of brain water content. The increase in brain Osm was almost entirely accounted for by the increase in brain glucose; there

was no significant change in the concentration of Na⁺, K⁺, or Cl⁻. Cerebrospinal fluid Osm apparently had increased secondary to both a loss of water (evidenced by an increase in the concentrations of Na⁺ and Cl⁻) and a gain in glucose (Tables I and IV).

After 2 h of hyperglycemia, there was osmotic equilibrium between plasma, CSF, and brain (Tables I and II), with a 10.9% loss of brain water. Although there were slight falls in the brain content of Na⁺, K⁺, Cl⁻, and osmoles, none of the changes were significant ($P > 0.1$). There were significant increases in the concentrations of Na⁺, K⁺, osmoles ($P < 0.01$), and Cl⁻ ($P < 0.05$), suggesting that the increase in brain Osm was due primarily to the loss of brain water (Fig. 3). Brain glucose increased significantly ($P < 0.01$) but the total of sorbitol, lactate, amino acids, urea, and myoinositol did not, so that after 2 h, there were 14 mmol/kg H₂O of undetermined solute present in the brain (Fig. 2).

After 4 h of hyperglycemia, the brain water content had returned to the same level as in the control state

(Fig. 3). The osmolalities of plasma, CSF, and brain were not significantly different. Brain content and concentration of Na⁺, K⁺, and Cl⁻ were not significantly different from the control animal, and there were no significant increases in brain glucose, lactate, sorbitol, amino acid, urea, or myoinositol. The brain osmole content was significantly higher than control (Table II), ($P < 0.01$) but only 43% of this increase could be accounted for by changes in Na⁺, K⁺, Cl⁻, and glucose (Fig. 2). Column chromatography of three brain trichloroacetic acid extracts, with 96% recovery, revealed the following distribution of radioactivity: glucose, 51%; amino acid, 23%; lactate, 12%. The brain glucose total radioactivity was unchanged from the value at 2 h (Table III).

Muscle. During 4 h of hyperglycemia, skeletal muscle lost about 10% of its water content after 2 h and then plateaued (Fig. 3). There was no significant change in the content of Na⁺, K⁺, or Cl⁻ (Table III). Skeletal muscle glucose rose to a high of 8.0 mmol/kg H₂O during 4 h of hyperglycemia, with most of the glucose

TABLE II
Brain Water and Electrolytes

	g H ₂ O/100 g wet wt	Osm	Na	K	Cl	g H ₂ O/100 g dry wt	Osm	Na	K	Cl	ECS
		mosmol/kg H ₂ O	meq/kg H ₂ O				mosmol/kg dry wt	meq/kg dry wt			%
Control (14)											
Mean	78.96	299	64.6	109	46.0	376	1147	238	406	173	27.7
±SE	0.23	6	1	1	1	4	28	4		2	0.
Hyperglycemia—1 h (7)											
Mean	79.00	312	64.3	111	46.3	373	1197	240	413	174	28.1
±SE	0.22	8	3	2	1	4	39	6	7	4	0.8
Hyperglycemia—2 h (10)											
Mean	76.85	339	70.2	115	48.9	335	1136	236	390	170	28.6
±SE	0.66	8	2	2	1	13	37	7	11	6	0.2
Hyperglycemia—4 h (8)											
Mean	79.00	343	65.9	112	51.2	376	1278	237	410	181	29.9
±SE	0.80	7	1	1	1	8	18	10	12	7	0.7
Insulin, plasma glucose 63 → 29.6 mM (6)											
Mean	78.57	338	65.8	111	49.8	372	1278	241	407	182	30.0
±SE	0.40	8	2	4	2	9	23	6	8	5	0.9
Insulin, plasma glucose 54 → 10.0 mM (6)											
Mean	81.69	326	60.7	109	44.2	450	1465	272	488	200	27.0
±SE	0.67	4	2	2	1	22	38	8	19	12	0.8
Peritoneal dialysis, plasma glucose 56 → 13.6 mM (5)											
Mean	80.40	294	61.2	107	47.5	411	1211	249	439	195	31.3
±SE	0.18	8	1	2	1	5	26	6	10	7	2.0

() = number of animals.

TABLE III
Muscle Water and Electrolytes

	g H ₂ O/100 g wet wt	Na	K	Cl	g H ₂ O/100 g dry wt	Na	K	Cl	ECS
	meq/kg H ₂ O				meq/kg dry wt				%
Control (14)									
Mean	76.40	28.6	134	11.0	324	89.7	438	40.4	8.7
±SE	0.4	1	1	1	7	6	10	3	0.4
Hyperglycemia—1 h (7)									
Mean	75.60	27.0	139	13.6	310	82.0	421	41.0	11.1
±SE	0.3	1	2	1	4	6	8	4	0.8
Hyperglycemia—2 h (10)									
Mean	74.28	26.2	147	11.2	291	74.6	428	31.9	8.1
±SE	0.41	1	2	1	4	3	7	1	0.3
Hyperglycemia—4 h (8)									
Mean	74.04	27.1	147	14.3	290	78.4	424	41.4	9.9
±SE	0.42	1	1	1	7	3	10	1	0.5
Insulin, plasma glucose 63 → 29.6 mM (6)									
Mean	76.68	27.4	129	13.9	332	86.7	424	44.2	10.8
±SE	0.84	1	6	1	15	1	7	2	1.5
Insulin, plasma glucose 54 → 10.0 mM (6)									
Mean	77.90	26.2	133	14.9	355	92.9	471	53.6	9.3
±SE	0.67	1	4	1	14	4	7	6	0.5
Peritoneal dialysis, plasma glucose 56 → 13.6 mM (5)									
Mean	77.08	31.0	130	18.6	337	104	424	62.3	12.1
±SE	0.3	2	5	1	6	7	13	2	0.3

() = number of animals.

being present in the extracellular (Cl⁻) space. The initial intracellular glucose was 2.6 mmol/kg H₂O and during 4 h of hyperglycemia, there was no increase (Fig. 1).

Treatment within insulin

Brain. After 4 h of hyperglycemia, the plasma glucose in group 5 animals was lowered from 63 mM to 30 mM in 3 h by infusion of insulin and 0.45% NaCl. When the plasma glucose was about 30 mM the experiment was terminated in each case. In all these animals, there was no visible bulging of the brain through a trephine opening in the skull. The brain content of water, Na⁺, K⁺, and Cl⁻ was not significantly different from that of either the control animal or the animals which had been hyperglycemic for 4 h. However, there was a significant ($P < 0.05$) osmotic gradient between the brain (338 mosmol/kg H₂O) and plasma (310 mosmol/kg H₂O) (Tables I and II). Despite a fall in plasma Osm from 351 to 310 mosmol/kg H₂O, there was no change in brain osmole content, while

brain water content and ECS were both normal. The brain glucose total radioactivity rose significantly but there was no change in lactate or amino acid concentration.

When the plasma glucose was rapidly lowered to 10 mM, in all cases there was visible bulging of the brain through a trephine opening in the skull. Highly significant increases in the brain content of Na⁺, K⁺, Cl⁻, water, and osmoles ($P < 0.01$) were present, although the concentrations of Na⁺, K⁺, and Cl⁻ were normal or low (Table II, Fig. 2). Brain free glucose was slightly elevated, while the concentrations of lactate, urea, sorbitol, amino acids, and myoinositol were not significantly increased (Table IV). The increase in brain osmole content, when compared with the hyperglycemic state, was less than could be accounted for by the increase in brain content of Na⁺, K⁺, and Cl⁻ (Table II). Thus, additional idiogenic osmoles apparently appeared in brain when the plasma glucose was lowered to normal levels with insulin (Fig. 2). In four additional such animals, the mean brain glycogen (±SEM) was 60±2 mg (glucose)/100 g fresh tissue.

Brain glycogen in four normal rabbits was 55 ± 2 mg (glucose)/100 g fresh tissue.

Muscle. In skeletal muscle, the water content increased back to control values when the plasma glucose was lowered to 30 mM. There was no significant increase in the muscle content of Na^+ , K^+ , or Cl^- . When the plasma glucose was lowered to 10 mM, muscle water content increased significantly to 9.6% above the control value ($P < 0.05$). There was a highly significant increase in the K^+ content of muscle when compared with either the control ($P < 0.05$) or 4-h hyperglycemic animals ($P < 0.01$), but Na^+ and Cl^- content did not change significantly. These data are shown in Table III.

Despite the significant increases in K^+ content of both brain and muscle, there was no significant change in the K^+ concentration of either CSF or plasma (Table I). The plasma K^+ did not decrease during the time tissue K^+ was increasing because potassium acetate was being continuously infused intravenously.

The albumin space (\pm SEM) was 2.1 ± 0.2 g/100 g wet wt in brain and 0.8 ± 0.3 g/100 g wt in muscle (four animals). In four normal rabbits, the albumin space was 1.9 ± 0.4 g/100 g wet wt in brain, and 0.7 ± 0.2 g/100 g wet wt in skeletal muscle.

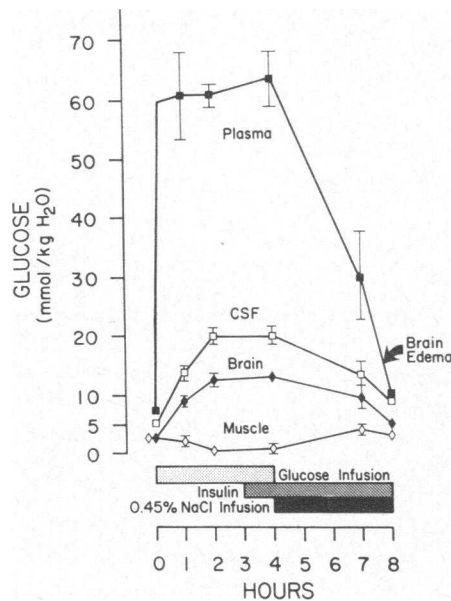


FIGURE 1 Free glucose concentrations. Comparison of the glucose concentration in plasma, CSF, brain, and muscle during glucose infusion (1-4 h), and cessation of glucose followed by insulin and 0.45% NaCl infusion (4-8 h). The muscle glucose is given in millimoles per kilogram intracellular water; brain glucose is in millimoles per kilogram tissue water. Cerebral edema did not occur until plasma glucose was lowered to less than 14 mM with insulin.

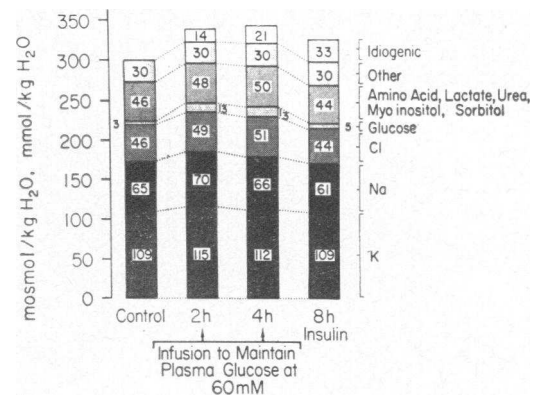


FIGURE 2 Brain solute concentrations. The concentrations in brain water of Na^+ , K^+ , Cl^- , glucose, amino acids, lactate, sorbitol, urea, and myoinositol. In the control animal, the measured Osm is 30 mosmol/kg H_2O less than the sum of all measured solute. After 4 h of hyperglycemia, an additional 21 mosmol/kg H_2O of unmeasured solute (idiogenic osmoles) is present. After cessation of glucose infusion (4 h) and administration of insulin (4-8 h), 33 mosmol/kg H_2O of idiogenic osmoles are present.

Treatment with peritoneal dialysis

Brain. The plasma glucose was again rapidly lowered, from 56 mM to 14 mM, but with 0.45% NaCl infusion and glucose-free peritoneal dialysis; no insulin was given. There was no visible evidence of brain swelling observed through a trephine opening in the skull. The brain water content was 9% higher than in the control animal ($P < 0.01$), but was significantly less than in the insulin-treated group ($P < 0.05$). The brain content of both K^+ and Na^+ was higher than the control values, but neither was significantly different. Osmolalities of brain (294 mosmol/kg H_2O) and plasma (290 mosmol/kg) were not significantly different in these animals and there was no evidence of idiogenic osmole accumulation (Tables II and IV). There was a highly significant difference ($P < 0.01$) in the brain osmole content (1,211 mosmol/kg dry wt) when compared with the insulin-treated group (1,465 mosmol/kg dry wt). Concentrations in brain of amino acid, urea, and myoinositol were not increased above normal but brain lactate was significantly higher than normal.

Muscle. In skeletal muscle, the water content was significantly ($P < 0.01$) higher than in the hyperglycemic animals, but no different than in the insulin-treated group. The muscle K^+ content was somewhat higher, although not significantly different, than either the control or hyperglycemic animals, but was significantly lower ($P < 0.01$) than in the insulin-treated animals (Table III).

TABLE IV
Brain and CSF Carbohydrate Metabolism

	Brain							CSF		
	Glucose	Sorbitol	Lactate	Amino acid	Urea	Myoinositol	TR	Glucose	Sorbitol	TR
	mmol/kg H ₂ O							dpm/mmol	mmol/kg	
Control (8)										
Mean	2.8	0.05	5.4	27.8	6.3	6.3	—	5.2	0.11	—
±SE	0.2	0.02	0.5	2.5	0.1	1.3	—	0.4	0.02	—
Hyperglycemia—1 h (4)										
Mean	9.0	0.18	—	—	—	—	—	13.7	0.10	—
±SE	0.9	0.06	—	—	—	—	—	1.2	0.02	—
Hyperglycemia—2 h (4)										
Mean	12.6	0.30	6.1	29.0	6.1	6.6	649	19.7	0.17	373
±SE	1.4	0.09	0.8	4.2	0.6	0	83	1.5	0.07	20
Hyperglycemia—4 h (4)										
Mean	13.0	0.26	5.9	29.3	5.4	8.9	622	19.6	0.14	429
±SE	0.6	0.04	0.6	4.4	0.9	1.0	39	1.4	0.04	57
Insulin, partially treated (plasma glucose 63 → 29.6 mM) (6)										
Mean	9.4	0.23	8.7	26.5	6.0	7.4	1946	13.4	0.12	588
±SE	1.9	0.02	0.6	1.2	0.6	0.8	402	1.9	0.03	87
Insulin, treated (plasma glucose 54 → 10.0 mM) (6)										
Mean	4.9	0.12	6.9	25.1	6.3	5.5	3324	9.0	0.14	594
±SE	1.3	0.04	0.6	0.7	1.3	1.3	157	1.4	0.01	217
Glucose-free peritoneal dialysis (plasma glucose 56 → 13.6 mM) (5)										
Mean	5.2	—	9.9	26.2	5.3	6.1	3054	10.4	—	875
±SE	0.5	—	1.7	3.1	1.0	1.1	572	1.1	—	59

() = number of animals.

TR = glucose total radioactivity (dpm per millimole glucose).

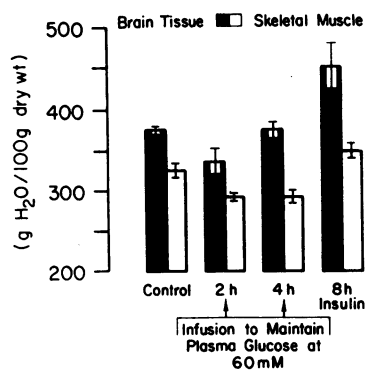


FIGURE 3 Brain and muscle water content. After 2 h of hyperglycemia, both brain and muscle water content are decreased by about 10%. After 4 h of hyperglycemia, muscle water content remains low but brain water content is the same as in the controls. When plasma glucose is rapidly lowered with insulin (4–8 h), both brain and muscle gain significant quantities of water.

DISCUSSION

The results of the present study demonstrate that during sustained hyperglycemia of 4 h duration, the cerebral cortex adapts to extracellular hyperglycemic hyperosmolality largely by accumulation of undetermined solute (idiogenic osmoles), with no significant loss of water or increase in the concentration of electrolytes, lactate, amino acids, or other glucose metabolites (Fig. 2 and 3). By contrast, skeletal muscle under the same conditions loses significant quantities of water.

1 h after the induction of hyperglycemia, there was no loss of water or change in the concentration of electrolytes in cerebral cortex, while the brain Osm was less than that of plasma and CSF. After 2 h of hyperglycemia, brain Osm increased and was the same as that of CSF and plasma. At least four factors appeared to have contributed to this increase in brain Osm (Figs. 2, 3): (a) loss of brain water content; (b) gain of

TABLE V
Muscle and Plasma Carbohydrate Metabolism

	Muscle		Plasma		SA
	Glucose	Sorbitol	Glucose	Sorbitol	
	mmol/kg H ₂ O		mmol/kg		dpm/mmol
Control (8)					
Mean	3.1	0.04	7.6	0.06	—
±SE	0.3	0.01	0.5	0.01	—
Hyperglycemia—1 h (4)					
Mean	7.1	0.11	60.4	0.17	—
±SE	1.2	0.05	7.3	0.04	—
Hyperglycemia—2 h (4)					
Mean	5.8	0.11	60.3	0.11	295
±SE	0.4	0.04	2.3	0.06	83
Hyperglycemia—4 h (4)					
Mean	8.0	0.17	63.2	0.27	290
±SE	0.7	0.04	1.1	0.02	77
Insulin (plasma glucose 63 → 29.6 mM) (6)					
Mean	9.0	0.14	29.6	0.16	506
±SE	1.4	0.03	7.4	0.03	106
Insulin (plasma glucose 54 → 10.0 mM) (6)					
Mean	5.0	< 0.01	10.0	0.09	287
±SE	0.4	—	0.4	0.03	50
Peritoneal Dialysis (plasma glucose 56 → 13.6 mM) (5)					
Mean	7.7	—	13.6	—	514
±SE	0.1	—	1.1	—	58

() = number of animals.

SA = glucose specific activity (dpm/millimole glucose).

glucose in brain; (c) loss of electrolyte from brain; (d) gain in undetermined solute (idiogenic osmoles) by brain.

When hyperglycemia was sustained for 4 hours, brain water content increased back to the normal value, but there was no further change in brain Osm, which was not different from that of plasma and CSF (Tables I, II). At this time, the changes in concentration of electrolytes, glucose, and other solute from normal values could account for about half of the increase in brain Osm (Tables II, IV); it appeared, therefore, that 21 mmol/kg H₂O of idiogenic osmoles were present in the brain (Fig. 2). Thus, the appearance of idiogenic osmoles in brain seems to play an important role in the prevention of a change in water content of the cerebral cortex.

By contrast, when plasma Osm is elevated by substances other than glucose, idiogenic osmoles do not appear to be present in brain. In acute or chronic hyperosmolar states induced by high Na⁺ peritoneal dialysis, or intravenous infusion of hypertonic NaCl,

urea, or sucrose, changes in brain water and electrolyte concentration are adequate to account for changes in brain Osm (16, 27, 28) (assuming brain and plasma Osm change by similar amounts). Thus, the hyperosmolality induced by hyperglycemia appears to be distinctly different than that caused by several other osmotically active substances.

The increase in brain Osm during hyperglycemia, without significant alteration in brain water content or the concentration of electrolytes and glucose metabolites (Tables II, IV), strongly suggests the formation of new osmotically active solute by brain. Evidence for the formation of such solute has been presented by other workers (28–30) as has the converse, osmotic inactivation of solute (31, 32). Although it is possible that the idiogenic osmoles might be transported into brain from the circulation rather than generated in brain, the former possibility appears remote; the sum of all osmotically active constituents of both plasma and CSF was similar to the measured Osm (Tables I, IV, V).

The identity of the idiogenic solute is only speculative at the present time, although many investigators have suggested that it might consist of glucose metabolites (10-12, 33). Our data does not support such a contention, since after 4 h of hyperglycemia, there was no change in the brain glucose total radioactivity (dpm per millimole glucose) (Table IV). As all radioactivity in brain was originally present as glucose, a significant accumulation in brain of any glucose metabolite(s) would result in an increase of the glucose total radioactivity (34). This observation strongly suggests that these idiogenic osmoles are probably not metabolites of glucose. Furthermore, the concentrations of the principal metabolites of glucose in brain—lactate and amino acid—did not increase, either during hyperglycemia or when insulin was given (Fig. 2, Table IV). The fact that lactate and amino acid concentration in brain did not increase following insulin administration, despite the significant increase in glucose total radioactivity, probably reflects increased glucose uptake and augmented turnover of lactate and amino acid (34, 35). There are no other glucose metabolites (excluding CO₂) which have been shown to accumulate in brain in osmotically significant quantities (34, 36) and 90% of the total radioactivity which was recovered by column chromatography in our brain extracts consisted of glucose, lactate, and amino acid. Nonetheless, the possibility exists that the increase in glucose total radioactivity following insulin administration reflects accumulation in brain of some as yet unidentified metabolite of glucose other than those shown in Fig. 2.

Another possibility for the genesis of the idiogenic solute is a change in the binding of Na⁺ or K⁺ to intracellular protein anions. Under these circumstances, total content of Na⁺ and K⁺ in tissue is unchanged, but that which is available to exert an osmotic effect has increased (30, 37).

The concept of retention of idiogenic solute in cerebral cortex is largely dependent upon the validity of our method for determination of brain Osm. It may be that limitations in the methodology which are imposed by experimental conditions could account for some of the discrepancy noted in comparisons of brain versus plasma Osm (Tables I, II). The accuracy of this method, however, appears documented by the findings that: (a) brain Osm was altered in a manner similar to that of plasma and CSF in response to changes induced in plasma Osm (19); and (b) the Osm of brain was similar to that of plasma and CSF in a steady state (19) (Tables I, II).

When plasma glucose in the hyperglycemic animal is rapidly lowered by infusion of insulin and hypotonic saline, an osmotic gradient is induced between brain and plasma (Tables I, II) but there is no change in the cortical content of Na⁺, K⁺, Cl⁻, water, or osmoles as

long as the plasma glucose remains above 14 mM (Fig. 1). When plasma glucose is further lowered below 14 mM, cerebral edema occurs which is characterized by a significant augmentation in the cerebral cortex content of Na⁺, K⁺, Cl⁻, and water. Idiogenic osmoles are not only retained in the brain under such circumstances, but the quantity which are present during hyperglycemia increases still further as the plasma glucose is lowered towards normal with insulin (Fig. 2).

By contrast, when the plasma glucose and Osm are lowered by means of peritoneal dialysis and hypotonic saline infusion, an osmotic gradient between brain and plasma does not develop. The brain content of Na⁺ and K⁺ is significantly less than when insulin is given ($P < 0.05$), there is no significant gain in brain osmole content, and idiogenic osmoles are not present. Some cerebral edema is present, as indicated by a significant ($P < 0.01$) increase in brain water content. The brain water content in the insulin-treated animals was significantly greater than that of the peritoneal dialysis group ($P < 0.05$). Thus, although brain swelling may occur when plasma glucose is rapidly lowered without insulin (11, 12), there is no increase in brain solute activity and the swelling is of a lesser magnitude.

Although the aforementioned changes were found in the cerebral cortex, our data may not be representative of changes occurring in other parts of the brain. In the normal rabbit, there are significant variations in water and K⁺ content among different areas of the brain (15), and compensatory changes might have occurred in regions which were not investigated in the present study. In the clinical syndrome of cerebral edema complicating the treatment of diabetic coma, however, autopsy studies have shown the cerebral cortex to be most prominently involved (1, 2, 7).

Several possible mechanisms for the production of cerebral edema during rapid lowering of plasma glucose have been suggested, and these include: (a) Increased formation of fructose and/or sorbitol via an increase in glucose metabolism by the polyol pathway during hyperglycemia. If these sugar alcohols are generated in the brain during hyperglycemia and are unable to leave the central nervous system when the blood glucose is rapidly lowered, they might act as osmotically active particles, attracting water into the brain and leading to cerebral edema (11). During hyperglycemia, the concentration of sorbitol has been shown to increase in aorta (38), peripheral nerve (39, 40), ocular lens (41), and in the CSF (11). When the plasma glucose in hyperglycemic dogs is rapidly lowered by intravenous infusion of 0.9% NaCl, the CSF pressure increases concomitant with a further increase in CSF sorbitol (11). It has been suggested that the elevated CSF sorbitol might be secondary to accumulation of osmotically significant quantities of

sorbitol in brain (11, 12, 10). The present study does not support these contentions. The levels of CSF sorbitol present in our hyperglycemic rabbits, 0.1–0.2 mmol/kg H₂O, are similar to those previously reported in the CSF of hyperglycemic dogs (11, 12). However, brain sorbitol levels attained a maximum value of only 0.3 mmol/kg H₂O; such quantities, although higher than normal, are of virtually no osmotic significance. Although fructose was not measured in the present study, previous studies show that the elevations in brain fructose are accompanied by corresponding elevations in brain sorbitol (12). Thus, it is unlikely that fructose, which is produced by oxidation of sorbitol, would be significantly increased, particularly since there was no change in the brain glucose total radioactivity.

(b) Cerebral anoxia secondary to impaired cerebral circulation (42) with breakdown of the "Na pump," causing a loss of K⁺ and a gain of Na⁺ and water in brain (4, 43). In the present study, brain content of both Na⁺ and K⁺ increased; these alterations should not occur with anoxia (43).

(c) Disequilibrium between the pH of the blood and CSF (4). Rapid increases in systemic pH may be important in the genesis of cerebral edema associated with ketoacidosis. In the present study, blood pH was not significantly acidotic prior to therapy and did not increase during treatment (Table I).

(d) Retention of glucose in brain when plasma glucose and Osm is rapidly lowered, by a mechanism similar to (a) above (33). Suggestions that free glucose might be retained in brain are based on the findings in a few reported instances that the lumbar CSF glucose in man may exceed the plasma glucose when the latter has fallen rapidly (4, 6–9). In the present study, however, glucose was not retained in the brain as the plasma glucose was lowered, although the CSF glucose did fall at a slower rate than that of the plasma.

(e) Increased production of myoinositol in brain during hyperglycemia may occur, with subsequent retention of this cyclic hexose in the brain when plasma glucose is rapidly lowered. Water might then enter the brain by a mechanism similar to (a) above (12, 44). Brain myoinositol has been reported to increase during hyperglycemia in dogs (12). In one of these dogs, when plasma glucose was rapidly lowered by 0.45% NaCl infusion, brain myoinositol was shown to increase by 5.5 mM over the hyperglycemic value. We were unable to confirm this latter observation. Although brain myoinositol did increase by 2.6 mmol/kg H₂O after 4 h of hyperglycemia, its concentration in the brain quickly fell to control values when plasma glucose was rapidly lowered to normal (Table IV).

(f) A breakdown in the blood-brain barrier mechanisms, with abnormal entry into brain of substance(s)

which are normally excluded by the blood-brain barrier (4). Such a mechanism might include alterations in capillary or brain cell membrane permeability, with leakage of intravascular or interstitial contents into brain substance (45). In the present investigation, the albumin space, representing the per cent plasma in tissue, was normal in both cerebral cortex and muscle. A normal albumin space strongly suggests that there was no significant alteration in capillary permeability, although it does not rule out this possibility. The fact that insulin resulted in an apparent nonspecific increase in Na⁺, K⁺, and Cl[−] content might be secondary to cortical cell damage resulting in an increase in cortical cell membrane permeability, rather than a direct effect of insulin on brain.

Although insulin causes a net movement of K⁺ into several different types of cells (46–48) and has been shown to effect cellular efflux of Na⁺ (49, 50), a direct effect of insulin on transport of Na⁺ and K⁺ in brain has not previously been demonstrated. In vitro studies have, in fact, suggested that insulin might not affect K⁺ transport in brain (51). However, it is difficult to extrapolate the results of in vitro studies to an in vivo system, where other effects of insulin, such as increased uptake and turnover of lactate and amino acids (35, 36) might secondarily result in a net increase of Na⁺ and K⁺ entering brain cells. In addition, elimination of the blood-brain barrier, as well as the damage to brain tissue created by its removal and slicing, may well result in metabolic changes that would further complicate the comparison of in vitro with in vivo studies.

Data from the present study (Table II) demonstrate a significant increase in brain content of Na⁺ and K⁺ after the administration of insulin. Whether by a direct effect on ion transport or as a secondary phenomenon, these data strongly suggest that insulin may increase transport of Na⁺ and K⁺ into brain cells. Such a suggestion is augmented by the contrasting effects of insulin versus peritoneal dialysis on brain content of Na⁺, K⁺ and osmoles (Table II). It may be that these phenomena, when observed in conjunction with rapid lowering of plasma glucose to normal, represent a unique effect of insulin on brain. The fact that there is a small increase in brain K⁺ when plasma glucose is lowered by means of peritoneal dialysis might be attributed to the presence of augmented quantities of endogenous plasma insulin during extracellular hyperglycemia. Hyperglycemia, however, may in some manner interfere with the action of insulin on cellular uptake of K⁺. It has been demonstrated that insulin increases uptake of K⁺ by skeletal muscle (46). In the present study, however, despite the administration of over 200 U of regular insulin, no increase in the K⁺ content of

skeletal muscle or brain was observed as long as the plasma glucose was elevated (Table II, III).

The increase of both water and K⁺ content in brain might be postulated to occur in conjunction with an increase in brain glycogen, as both water and K⁺ may be incorporated into other tissues when glycogen is deposited (52, 53). Brain glycogen, however, was not significantly different from normal when the increase in brain water and K⁺ content was maximal. The failure of water to initially move against an osmotic gradient into the brain was unexpected (Tables I, II) and differs from other situations where there is an acute osmotic imbalance between plasma and brain. In acute water intoxication, we have shown that there is a rapid net movement of water into brain (19) and after 2 h of acute hyperglycemia, there is a net loss of brain water (Table II, Fig. 3). Stern and Coxon (54) have shown that an acute change in plasma Osm of at least 35 mosm/kg H₂O is probably necessary for a net movement of water into brain. Such a change did not occur until the plasma glucose had been lowered to less than 14 mM (Table II).

In the clinical setting, brain edema complicating non-ketotic coma has not been shown to occur until the plasma glucose has reached normal levels (8, 9, 55). These clinical observations and the data of the present study suggest that during the treatment of non-ketotic coma, insulin administration probably should be stopped when the plasma glucose has fallen to 14 mM, at which time a glucose containing solution should be administered.

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