

The Mechanism of Acidosis Produced by Hyperosmotic Infusions *

ROBERT W. WINTERS, PETER R. SCAGLIONE, GABRIEL G. NAHAS, AND
MARYAGNES VEROSKY

(From the Departments of Pediatrics and Anesthesiology, College of Physicians & Surgeons,
Columbia University, New York, N. Y.)

It has been known for many years that the administration of large loads of sodium chloride, either orally (2) or by infusion of isotonic (3, 4) or hypertonic (5-10) saline produces a disturbance in acid-base equilibrium of the blood as evidenced by a fall in total carbon dioxide content of the plasma or implied by a fall in the difference between the concentrations of sodium and chloride in the plasma. In the relatively few instances in which blood pH has been recorded, acid values have been found (4, 8, 10).

The mechanism of the acidosis produced by a load of sodium chloride has received only scant attention. Peters and Van Slyke (11) commented that the acidosis must in part be due to dilution of the blood. Shires and Holman (4) adopted a similar viewpoint and coined the term "dilution acidosis." They pointed out that whereas dilution of a bicarbonate-buffered solution *in vitro* with saline would not produce a fall in pH, dilution of the extracellular fluid *in vivo* with isotonic saline would, for with continuing production of carbon dioxide, the dilution would affect only the bicarbonate concentration. Presumably after administration of hypertonic saline, dilution of extracellular bicarbonate occurs because water is osmotically shifted from the intracellular compartment. If there were no change in PCO_2 , extracellular pH would fall providing the

diluting fluid is poor in bicarbonate. Such a conclusion is clearly implied in the work of Wolf, McDowell, and Steer (7, 8) on the osmometric behavior of the body fluids. Indeed in several of their experiments (8) an acidosis was documented but was not further commented upon.

Recently Sotos, Dodge, and Talbot (10) have shown the possibility of additional factors in the pathogenesis of the acidosis. They infused intact rabbits with large loads of hyperosmotic solutions of sodium chloride, sucrose, and urea and observed a severe metabolic acidosis. They concluded that the acidosis was due mainly to a release of acid from the cells into the extracellular fluid presumably indicating a disturbance in cellular metabolism secondary to hyperosmolality of the body fluids. The renal losses of bicarbonate and the preterminal respiratory depression were additional factors in the production of the acidosis.

The purpose of the present experiments was to study quantitatively in the dog the mechanism of the acidosis that follows administration of moderate hyperosmotic loads of sodium chloride and mannitol. The animals were nephrectomized, and arterial PCO_2 was held nearly constant by mechanical ventilation, conditions which avoid the complicating factors of renal and respiratory changes.

Methods

Mongrel dogs were fasted and deprived of water for 24 hours before the experiments. The animals were anesthetized with sodium pentobarbital (30 mg per kg body weight), intubated, given succinylcholine chloride (1 mg per kg body weight initially and additional doses as required), and ventilated with 100% oxygen by a respiratory pump adjusted frequently to keep PCO_2 within a narrow range of control values. The renal and splenic pedicles were ligated through an abdominal incision. Arterial blood samples were collected from an indwelling polyethylene cannula inserted into the femoral artery; cerebrospinal fluid was collected from a needle inserted

* Submitted for publication June 4, 1963; accepted December 3, 1963.

This work was supported by grants from the Health Research Council of the City of New York (U-1127) and from the National Institutes of Health (H-4859 C2 and H-5506 C) and in part by the General Research Support grant from the National Institutes of Health of the U. S. Public Health Service.

A portion of this work was presented at the XXII International Congress of Physiological Sciences, Leiden, September 10-17, 1962 (1).

into the *cisterna magna*. The sample size of cerebrospinal fluid was always less than 0.5 ml.

After a 30- to 60-minute control period, the dogs were infused for 30 minutes with a measured volume of either 0.75 M sodium chloride, 1.5 M mannitol, or 1.5 M urea in 3% glucose. Arterial blood samples were collected anaerobically at intervals before, during, and after completion of the infusion. Each sample was analyzed in duplicate or triplicate for whole blood pH, plasma total CO₂ content, plasma sodium and chloride concentration, total protein, and osmolality. In addition, in some experiments whole blood lactate and glucose were also measured.

Blood pH was determined at 38° C with a Knick 35 pH meter using a Sanz microelectrode. Plasma total CO₂ content was measured with a Natelson microgasometer. Plasma sodium was measured by an internal standard method with a Patwin flame photometer; plasma chloride was determined with a Cotlove chloridometer. Total osmolality was measured by freezing point depression with a Fiske osmometer, blood lactate by the method of Barker and Summerson (12), glucose by the method of Nelson (13), and total protein by the biuret procedure (14). The standard error of an individual determination was ± 0.01 pH unit for blood pH, ± 0.1 mmole per L for total CO₂ content, and ± 1.0 mEq per L for plasma sodium.

Calculations¹

Acid-base data. The values for Pco₂ and [HCO₃]_i of control plasma and cerebrospinal fluid were calculated from total CO₂ content and pH using the Henderson-Hasselbalch equation with values for pK' and S (the CO₂ solubility factor) as given by Severinghaus, Stupfel, and Bradley (15, 16) for blood and those of Alexander, Gelfand, and Lambertsen (17) for cerebrospinal fluid. These constants were modified slightly to take account of the increasing sodium concentration in the samples obtained after saline loading; such modifications were calculated from published data on aqueous solutions (18, 19).

To calculate the effect of dilution upon [HCO₃]_e, it was necessary to take account of the contributions of $\Delta(\text{Buf})_b$ and of $\Delta(\text{HCO}_3)_r$ to the observed change in

[HCO₃]_e. The effect of $\Delta(\text{Buf})_b$ was calculated from the following equation (20):

$$\Delta(\text{Buf})_b = (V_p (0.1 [\text{Pr}]_p) + V_r (2.3) [\text{Hgb}]_r) \Delta\text{pH}. \quad [1]$$

The following values were assumed: $V_p = 0.04$ L per kg body weight; $V_r = 0.03$ L per kg body weight; $[\text{Hgb}]_r = 20$ mmole per L. $[\text{Pr}]_p$ (in grams per liter) and ΔpH were measured. Since the effects of expansion of V_p were probably offset by the fall in $[\text{Pr}]_p$ in any one experiment, the control value for $[\text{Pr}]_p$ was used with a constant V_p for all samples in that experiment. The effect of $\Delta(\text{HCO}_3)_r$ was computed on the assumption that the bicarbonate concentration in red cell water was $0.7 [\text{HCO}_3]_r$ and that the total body red cell water was $0.63 V_r$. Changes in the latter term as a function of changing osmolality were disregarded, since they were too small to be important in the over-all calculation. The amount of "new" bicarbonate derived from the above two sources [i.e., $\Delta(\text{Buf})_b + \Delta(\text{HCO}_3)_r$] was converted to a concentration (i.e., "new" $[\text{HCO}_3]_e$) with an assumed volume distribution of 25% of body weight.

Although the precision of some of the assumptions in the above calculations can be questioned, even major errors in these assumptions are not likely to affect the conclusions since the over-all effect of "new" $[\text{HCO}_3]_e$ is small (e.g., Table I).

The "corrected" $[\text{HCO}_3]_e$ was computed as the difference between postinfusion observed $[\text{HCO}_3]_e$ and "new" $[\text{HCO}_3]_e$. For purposes of Figure 3 and Table IV, "corrected" $[\text{HCO}_3]_e$ was expressed as a dilution ratio computed by dividing it by the respective control value for $[\text{HCO}_3]_e$.

$[\text{U.A.}]_e$ was approximated as the difference between $[\text{Na}]_e$ and the sum of $[\text{Cl}]_e$ and $[\text{HCO}_3]_e$.

Dilution data. The concentration of a univalent ion in the extracellular fluid was calculated from its concentration in plasma water and a Donnan factor of 0.95 for cations and 1.05 for anions. Plasma water was calculated from the $[\text{Pr}]_p$ (21).

In the construction of Figure 3 and Figure 4, the following equation from Wolf and McDowell (7) was used:

$$V_e' = \frac{W'(V_e A + L)}{WA + L}, \quad [2]$$

where W was calculated from the osmotic distribution of infused sodium in the saline experiments (i.e., $W = L_{\text{Na}}/\Delta[\text{Na}]_e$) and from the osmotic distribution of infused solute in the mannitol experiments (i.e., $W = L/\Delta A$). W' was assumed to equal W , since the load of water infused was always less than 5% (and usually less than 3%) of the probable value of W and since insensible water loss was not taken into account. L was derived from the volume and osmolality of the infusate, the latter being determined after dilution of the infusate to a value close to that expected in the plasma.

To calculate the reference lines in Figure 3, a series of absolute values for V_e were substituted into Equation 2, and the corresponding values for V_e' were calculated as

¹ Abbreviations and symbols: V indicates the volume of a specific compartment of the body fluids, the particular compartment being indicated by the subscript; V_e and V_e' are the volumes of the extracellular fluid before and after infusion; W and W' are the volumes of the total body water before and after infusion. A indicates the initial osmolality of the extracellular fluid; L is the load of osmotically active solute administered, and L_{Na} is the amount of sodium infused; Buf indicates the nonbicarbonate buffers of the blood; Pr indicates protein; U.A. indicates undetermined anion; $[\]$ represent a concentration and $()$ indicate a total amount per kilogram body weight. Subscripts p , b , e , r , and csf indicate plasma, whole blood, extracellular fluid, erythrocytes, and cerebrospinal fluid, respectively.

TABLE I
Observed and derived data on dog II*

Time	Observed data							Derived data										Dilution ratio						
	[Osm] _p	[Na] _p	[Cl] _p	pH _b	[CO ₂] _p	[Pr] _p	[Lactate] _b	[Glucose] _p	[U.A.] _p	W	Δ[Na] _r	[Cl] _r	[H ₂ O] _p	[Na] _r	[Cl] _r	[HCO ₃] _p	[HCO ₃] _r		Pco ₂	Δ(Buf) _b	Δ(HCO ₃) _r	"New" (HCO ₃) _r	"New" [HCO ₃] _r	"Corrected" [HCO ₃] _r
<i>min</i>																								
-4 (Control)	313	151.8	104.5	7.34	23.2	6.3	1.21	mg/100 ml																
0 (Start infusion)								106																
15	340	166.9	122.1	7.28	20.6	5.9																		
30 (End infusion)																								
44	364	182.3	138.4	7.21	20.0	4.6																		
75†	361	177.2	133.9	7.26	20.3	5.1	0.98	99																
105	359	176.9	133.1	7.25	20.2	5.3		90																
145	360	176.3	133.6	7.23	20.1	5.2	0.89																	
<i>min</i>																								
-4 (Control)																								
0 (Start infusion)																								
15	939	153.6	116.8	12.3	21.9	24.5	41.9																	
30 (End infusion)																								
44	952	181.9	152.7	8.7	18.6	20.5	46.5																	
75†	948	177.6	148.4	8.1	19.0	21.1	41.8																	
105	947	177.4	147.6	8.8	18.9	21.0	42.8																	
145	947	176.8	148.2	7.7	18.8	20.9	44.4																	

* LNa = 15.1 mEq per kg body weight; L = 27.9 mOsm per kg body weight; () represent concentration; () indicate a total amount per kilogram body weight; subscripts p, b, e, r, and csf indicate plasma, whole blood, extracellular fluid, erythrocytes, and cerebrospinal fluid, respectively; Pr indicates protein; W is the volume of total body water before infusion; U.A. indicates undetermined anion; But indicates the nonbicarbonate buffers of the blood.

† Values selected as steady state and used in subsequent calculations.

functions of L using mean values (Table IV) for W and A . Since V_e/V_e' is equivalent to the dilution ratio of an inert solute (X) confined to the extracellular fluid (i.e., $V_e/V_e' = [X]_e/[X]_e'$), by setting any given value for V_e at 1.00, the corresponding relative values for V_e' in the above ratio define the dilution ratios of such a solute. These ratios constitute the reference lines in Figure 3 against which the observed dilution ratios for bicarbonate are compared. Conversely, any given dilution ratio for bicarbonate fixes a specific value for V_e , providing there are no shifts of bicarbonate into or out of the extracellular fluid. In Table IV, V_e for each experiment was calculated from the observed dilution ratio for bicarbonate. For this purpose Equation 2 was modified as follows:

$$V_e = \frac{WL}{RWA + RL - WA}, \quad [3]$$

where $R = V_e'/V_e$ and is equal to the reciprocal of the bicarbonate dilution ratio on the assumption that there are no bicarbonate shifts.

Results

Eleven dogs were infused with hyperosmotic saline. Detailed results of a representative ex-

periment are shown in Table I and illustrate the calculations. A summary of all experiments is shown in Table II. In that table only the immediate preinfusion control values and the post-infusion steady-state values are shown. In general a new steady state was reached only about 45 minutes after completion of the infusion, a finding in general agreement with others (8). Samples taken at intervals less than 45 minutes after completion of the infusion usually showed higher values for osmolality and plasma sodium and chloride and lower values for plasma total CO_2 content and blood pH than did values obtained subsequently (see, e.g., Table I), signifying lack of complete equilibration of the infusate. In every experiment, measurements were continued for at least 90 to 120 minutes after completion of the infusion to assure that the values at 45 minutes were indeed representative of the steady state.

In all dogs receiving hyperosmotic saline, the concentrations of sodium and chloride in plasma

TABLE II
Observed data from 19 dogs receiving hyperosmotic infusions*

		[Osm] _p		[Na] _p		[Cl] _p		pH _b		[CO ₂] _p		[Pr] _p			
		<i>L</i> Na	<i>L</i>	<i>a</i>	<i>p</i>	<i>a</i>	<i>p</i>	<i>a</i>	<i>p</i>	<i>a</i>	<i>p</i>	<i>a</i>	<i>p</i>		
		<i>mEq/kg body wt</i>	<i>mOsm/kg body wt</i>	<i>mOsm/kg water</i>		<i>mEq/L</i>		<i>mEq/L</i>		<i>mmoles/L</i>		<i>g/100 ml</i>			
Sodium chloride exp'ts															
Dog	1	9.3	17.2	304	340	149.2	165.2	107.8	126.1	7.28	7.22	21.8	19.7	5.8	4.8
	2	12.1	22.4	299	338	143.2	163.9	102.8	127.1	7.36	7.30	20.2	18.2	6.4	5.4
	3	13.7	25.4	292	338	137.8	161.0	96.1	122.1	7.37	7.32	25.3	22.7	6.1	4.9
	4	14.1	26.2	303	348	144.7	164.3	101.5	126.5	7.37	7.31	25.6	21.4	6.2	4.9
	5	14.5	26.8	305	355	146.3	171.9	103.7	133.9	7.34	7.28	22.7	20.3	7.0	5.5
	6	15.8	29.2	300	358	144.0	170.4	104.3	135.3	7.39	7.30	20.9	17.3	6.9	5.5
	7	15.3	28.2	298	350	143.1	170.0	104.1	133.6	7.39	7.34	18.9	16.3	6.6	5.0
	8	18.1	33.4	300	361	140.6	171.1	100.5	132.6	7.32	7.25	21.5	18.9	6.7	5.3
	9	22.0	40.8	303	362	143.8	176.0	103.2	137.6	7.35	7.25	20.6	17.6	6.8	4.9
	10	20.2	37.4	311	377	150.1	181.8	105.8	140.4	7.35	7.24	20.6	17.3	6.2	5.5
	11	15.1	27.9	313	361	151.8	177.2	104.5	133.9	7.34	7.26	23.2	20.3	6.3	5.1
Mannitol exp'ts															
Dog	12		35.2	301	361	147.1	111.8	101.9	78.6	7.40	7.25	19.0	16.0	6.5	5.4
	13		36.0	300	360	142.3	110.6	109.1	83.1	7.34	7.22	17.8	15.1	6.5	5.2
	14		32.1	293	343	139.8	117.0	100.9	84.6	7.38	7.30	18.0	15.8	6.3	5.0
	15		35.0	294	350	139.4	110.3	106.2	82.9	7.38	7.25	18.8	16.0	6.4	5.2
	16		22.6	310	348	140.2	120.2	97.4	84.1	7.38	7.32	22.2	19.9	7.0	5.0
Urea exp'ts															
Dog	17		38.2	298	358	148.8	149.0	106.8	107.6	7.38	7.38	23.7	23.4	6.1	6.2
	18		30.5	301	352	144.3	145.1	105.4	106.0	7.35	7.35	20.3	20.2	6.4	6.3
	19		34.0	295	349	143.1	144.0	102.9	102.6	7.31	7.32	19.1	19.3	6.3	6.3

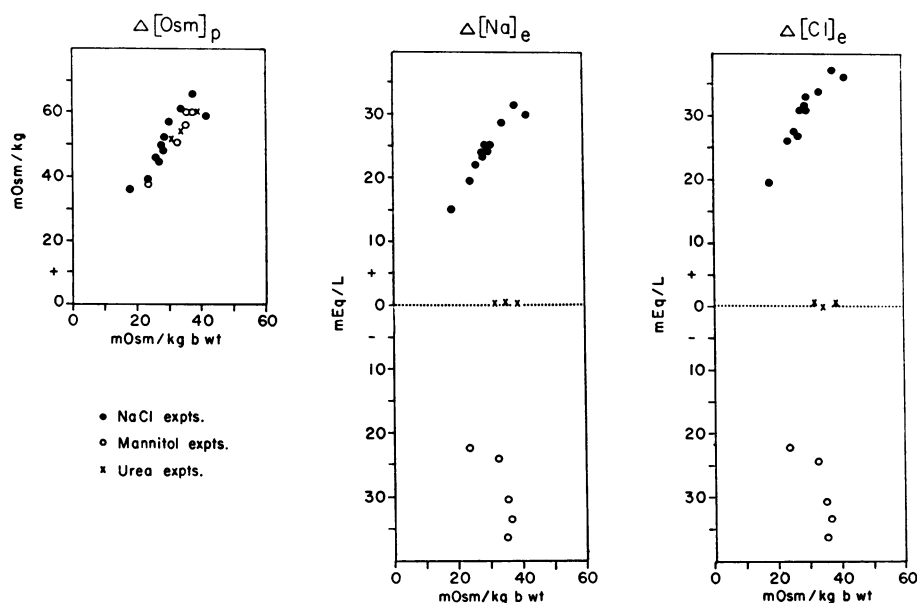


FIG. 1. STEADY-STATE CHANGES IN $[\text{Osm}]_p$, $[\text{Na}]_e$, AND $[\text{Cl}]_e$ AS A FUNCTION OF THE LOAD OF SOLUTE INFUSED.¹

and the plasma osmolality rose (Table II), the degree of rise bearing a close relationship to the load of solute infused (Figure 1). Acid-base data from these 11 dogs demonstrated that in every experiment total CO_2 content and blood pH fell, but because of the design of the experiments there were only small and inconsistent changes in plasma PCO_2 . The variation in the degree of fall of $[\text{HCO}_3]_e$ and of blood pH is in great part explained by the variation in the load of solute infused (Figure 2).

The effects of infusing hyperosmotic mannitol in five dogs are also shown in Table II. Plasma

osmolality rose in these experiments while the concentrations of sodium and chloride in plasma fell markedly. Figure 1 shows the relationship between changes in osmolality, $[\text{Na}]_e$ and $[\text{Cl}]_e$, and the load of solute administered. Each of the five dogs receiving mannitol developed an acid-base disturbance similar to that seen in the sodium chloride-loaded animals in that total CO_2 content and blood pH fell. Changes in blood pH and of $[\text{HCO}_3]_e$ as a function of load of solute administered are shown in Figure 2 and demonstrate the same general relationship as was presented with sodium chloride loading.

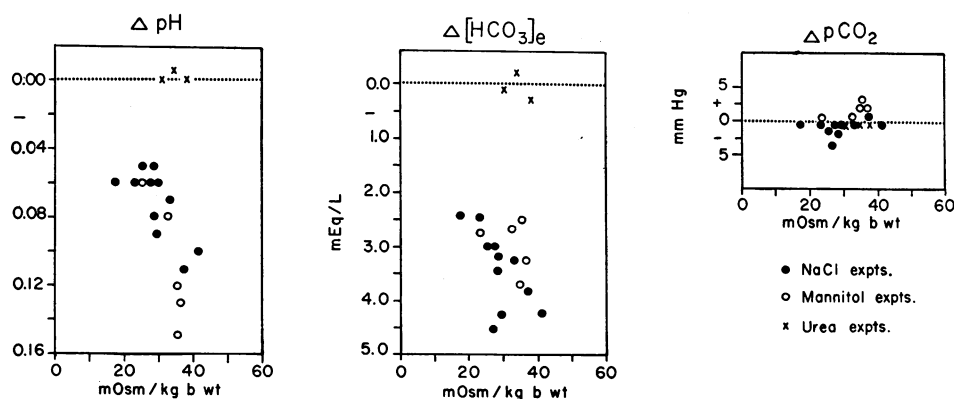


FIG. 2. STEADY-STATE CHANGES IN BLOOD pH, $[\text{HCO}_3]_e$, AND PLASMA PCO_2 AS A FUNCTION OF THE LOAD OF SOLUTE INFUSED.

TABLE III
Acid-base changes in plasma and cerebrospinal fluid in four dogs receiving hyperosmotic saline*

Dog	pH _b		pH _{cst}		[CO ₂] _p		[CO ₂] _{cst}		[Pco ₂] _p		[Pco ₂] _{cst}		[HCO ₃] _p		[HCO ₃] _{cst}		[Osm] _p Ratio†		[HCO ₃] _{cst} Ratio‡	
	<i>a</i>	<i>p</i>	<i>a</i>	<i>p</i>	<i>a</i>	<i>p</i>	<i>a</i>	<i>p</i>	<i>a</i>	<i>p</i>	<i>a</i>	<i>p</i>	<i>a</i>	<i>p</i>	<i>a</i>	<i>p</i>	<i>a</i>	<i>p</i>	<i>a</i>	<i>p</i>
2	7.36	7.30	7.30	7.33	20.2	18.2	23.7	25.4	34.9	34.5	48.5	46.8	21.4	19.0	22.2	23.9	0.885	0.929		
7	7.39	7.34	7.38	7.41	18.9	16.3	21.3	23.0	30.7	28.9	36.4	35.7	20.2	17.0	20.1	21.9	0.851	0.918		
8	7.32	7.25	7.28	7.30	21.5	18.9	21.5	22.3	40.6	39.9	45.7	43.8	22.8	19.6	20.0	20.9	0.831	0.957		
10	7.35	7.24	7.28	7.32	20.6	17.3	21.9	24.0	36.4	37.4	46.6	45.3	21.8	18.0	20.4	22.6	0.825	0.903		

* *a* represents control value; *p* represents the postinfusion steady-state values (see text). Other abbreviations as in Table I.

† Ratio = control [Osm]_p/postinfusion [Osm]_p (see Table II for data).

‡ Ratio = control [HCO₃]_{cst}/postinfusion [HCO₃]_{cst}.

Three dogs were given hyperosmotic urea infusions in amounts sufficient to raise the total osmolality of the plasma to levels comparable to those in the sodium chloride and mannitol experiments (Table II). None of these animals developed any significant changes in acid-base equilibrium or in the concentrations of sodium or chloride in plasma.

In both the sodium chloride and the mannitol experiments, plasma protein concentrations fell (Table II), whereas no significant changes occurred in the urea experiments. [U.A.]_e fell in the sodium chloride experiments by -3.1 ± 0.35 mEq per L and in the mannitol experiments by -2.9 ± 1.5 mEq per L. In six dogs receiving hyperosmotic sodium chloride, blood lactate changed by -0.11 ± 0.10 mEq per L and in four dogs plasma glucose changed by -5.1 ± 6.8 mg per 100 ml.

In four dogs receiving hyperosmotic saline, samples of cerebrospinal fluid were obtained before and 45 minutes after the infusion. Observed and derived acid-base data on plasma and the cerebrospinal fluid of these dogs are shown in Table III. In all four experiments the pH of the cerebrospinal fluid rose slightly while blood pH fell. [HCO₃]_{cst} also rose while [HCO₃]_e fell. The Pco₂ in the two phases showed only small and inconsistent changes as the result of the infusions.

Discussion

The results of these experiments confirm many previous observations (5-10) that the infusion of a hyperosmotic solution of sodium chloride can lead to the development of an acidosis in the extracellular fluid. This acidosis is not specifically dependent upon a rising sodium or chloride concentration, since in the mannitol experiments the concentration of these two ions fell. The lack of significant change in acid-base equilibrium after the infusion of urea, a solute which penetrates readily into most compartments of the body water, suggests that the acidosis occurs only with infusions of solutes that can osmotically condition the redistribution of body water between the intracellular and the extracellular compartments. Indeed, the simplest and most satisfactory explanation of the acidosis is that extracellular bi-

carbonate concentration falls as the result of a dilution of the extracellular fluid by a bicarbonate-poor fluid from the intracellular compartment. If, as in our experiments, P_{CO_2} remains unchanged, then extracellular pH must fall.

If this hypothesis is correct, the fall in "corrected" bicarbonate concentration should be accounted for by the increase in the volume of extracellular fluid incident to the osmotic transfer of water. If we assume perfect osmometric behavior, V_e' can be derived from Equation 2 (see Calculations) providing W and V_e are known. W can be calculated from the osmotic distribution of infused sodium, since previous work by others has demonstrated that this corresponds closely to the volume of total body water (7, 8). In nearly all experiments of the present study the osmotic distribution of sodium was within the range of 60 to 65% of body weight (Table IV), which is in accord with the expected volume of body water in the dog (22). In the mannitol experiments, to-

tal body water, approximated by the osmotic distribution of infused solute, gave values between 59 and 64% of body weight. It is more difficult to assign a proper value for the initial volume of the extracellular fluid. V_e is in fact the volume of fluid that is available for dilution by the osmotically induced transfer of water from the intracellular compartment. Although it is reasonable to assume that this volume is conceptually identical to the volume of the extracellular fluid, this assumption cannot be precisely established because of the uncertainties of definition and measurement of the volume of the extracellular fluid.

Because of these vagaries concerning the volume of the extracellular fluid, no exact quantitative formulation of the effects of dilution is possible. A more general approach can be made, however, by comparing the observed data with those expected under different assumptions as to the value for V_e . Figure 3 shows the expected fall in the concentration of an inert solute confined

TABLE IV
Calculation of V_e from bicarbonate dilution for eleven hyperosmotic saline experiments and for five hyperosmotic mannitol experiments*

	Control [HCO ₃] _e	"Corrected" [HCO ₃] _e	Dilution ratio	V_e'/V_e ratio	W	A	L	V_e
	mEq/L	mEq/L			L/kg body wt	mOsm/kg	mOsm/kg body wt	L/kg body wt
Sodium chloride exp'ts								
Dog 1	22.7	19.7	0.868	1.152	0.620	304	17.2	0.220
2	21.4	18.4	0.860	1.162	0.620	299	22.4	0.248
3	26.8	23.3	0.869	1.151	0.623	292	25.4	0.279
4	27.1	21.7	0.801	1.248	0.608	303	26.2	0.203
5	24.2	20.6	0.851	1.175	0.604	305	26.8	0.254
6	22.3	17.3	0.776	1.289	0.632	300	29.2	0.200
7	20.2	16.5	0.817	1.224	0.610	298	28.2	0.229
8	22.8	19.0	0.833	1.201	0.628	300	33.4	0.269
9	21.9	16.9	0.767	1.304	0.731	303	40.8	0.247
10	21.8	17.1	0.784	1.276	0.631	311	37.4	0.232
11	24.5	20.4	0.833	1.201	0.629	313	27.9	0.240
				Mean	0.631	303		0.238
				±SE	±0.011	± 2		±0.008
Mannitol exp'ts								
Dog 12	20.3	15.5	0.764	1.309	0.587	301	35.2	0.205
13	18.8	14.6	0.777	1.287	0.600	300	36.0	0.220
14	19.1	15.8	0.827	1.209	0.642	293	32.1	0.264
15	20.0	15.5	0.775	1.290	0.625	294	35.0	0.222
16	23.6	20.2	0.856	1.168	0.595	310	22.6	0.234
				Mean	0.610	300		0.229
				±SE	±0.009	± 3		±0.010

* Calculation carried out using Equation 2. V_e and V_e' are the volumes of extracellular fluid before and after infusion; A indicates the initial osmolality of the extracellular fluid; L is the load of osmotically active solute administered. Other abbreviations as in Table I.

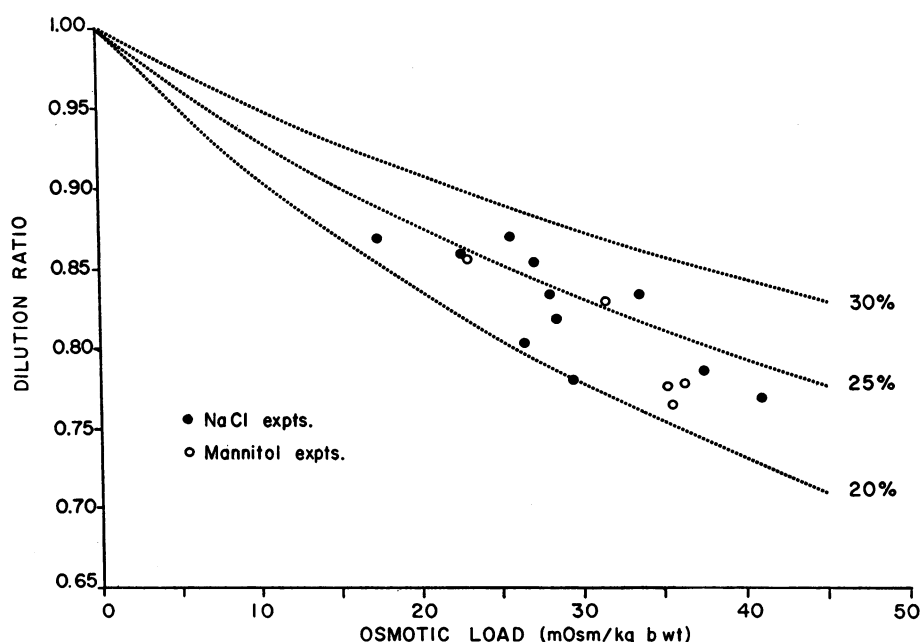


FIG. 3. DILUTION RATIO OF $[\text{HCO}_3]_e$ AS A FUNCTION OF THE LOAD OF SOLUTE INFUSED. The dashed lines represent dilution ratios expected for an inert solute confined to initial volumes of 20, 25, and 30% of body weight (see Calculations for details).

to initial volumes of the extracellular fluid of 20, 25, and 30% of body weight. These values were chosen, since they span most of the reported volumes of distribution of different so-called "extracellular" substances in the dog (22). The points plotted on this figure represent the "corrected" bicarbonate concentration for the 11 sodium chloride experiments and for the 5 mannitol experiments. In all cases these observed points fall within the range of 20 to 30% of the body weight. From these data the value for V_e in each experiment can be calculated (see Table IV) on the assumption that the fall in "corrected" bicarbonate concentration is due entirely to dilution and that there are no transfers of bicarbonate between the intracellular and the extracellular spaces. In the sodium chloride experiments the mean value obtained for V_e was $23.8 \pm 0.8\%$ of body weight, whereas in the mannitol experiments it was $22.9 \pm 1.0\%$. This difference is not statistically significant.²

² All of the above calculations ignore the small effects on bicarbonate concentration of the continuing production of acid which could not be excreted because of the nephrectomy. In 11 subsequent experiments, this effect has been quantitated by serial study of the acid-base equilibrium of blood under conditions duplicating those

These values for the initial volume of extracellular fluid calculated from the data on the bicarbonate dilution are not unreasonable. They are higher than the values usually reported (22) for inulin space in dogs (approximately 15 to 20% of the body weight) as might be expected, since inulin does not penetrate the water of connective tissue unless there is a prolonged period for equilibration (24, 25). On the other hand, they are generally lower than the average figure of about 30% of body weight for bromide or chloride space observed in dogs (22). This difference may indicate the penetration by chloride or bromide of compartments of the body water that are not readily available to extracellular bi-

employed in the present study except that no infusions were given. Over a period of 75 minutes, the magnitude of this effect expressed in terms of the change in standard bicarbonate concentration of the plasma (23) was -0.25 ± 0.21 mEq per L. In the sodium chloride and mannitol experiments, the magnitude of this effect upon bicarbonate concentration would be even less and dependent upon the degree of dilution induced. At relatively small osmotic loads (e.g., 20 mOsm per kg body weight) ignoring this effect overestimates the fall in bicarbonate concentration attributed to dilution alone by about 8%, whereas at higher osmotic loads (e.g., 36 mOsm per kg body weight) the error is about 4%.

carbonate and in addition may reflect the fact that the connective tissue water has a higher chloride concentration than does an ultrafiltrate of plasma (25, 26).

In all of the calculations presented above it has been assumed that there were no shifts in bicarbonate between the extracellular and intracellular compartments as the result of the infusions. Such an assumption may not be fully warranted, but in the absence of any precise independent method for measurement of initial extracellular bicarbonate space, the direction and extent of possible bicarbonate shifts cannot be ascertained. However, if one assumes possible limits for the initial volume of distribution of extracellular bicarbonate of 20% of body weight on the low side, as defined by the inulin space,

and 30% of body weight on the high side, as defined by the chloride space, then the magnitude of possible bicarbonate shifts can be estimated. For example, if the true initial volume of the extracellular fluid were 20% of the body weight, the dilution induced by a given load of sodium chloride would be greater than if the true volume were 23.8% as calculated above. A bicarbonate shift from the extracellular to the intracellular compartment would therefore have occurred in order to account for the observed result. Similarly, if the true initial volume were 30% of the body weight, a shift of bicarbonate in the opposite direction would have occurred in order to explain the observed result.

This calculation is illustrated in Figure 4 for a single large load of sodium chloride. Under the

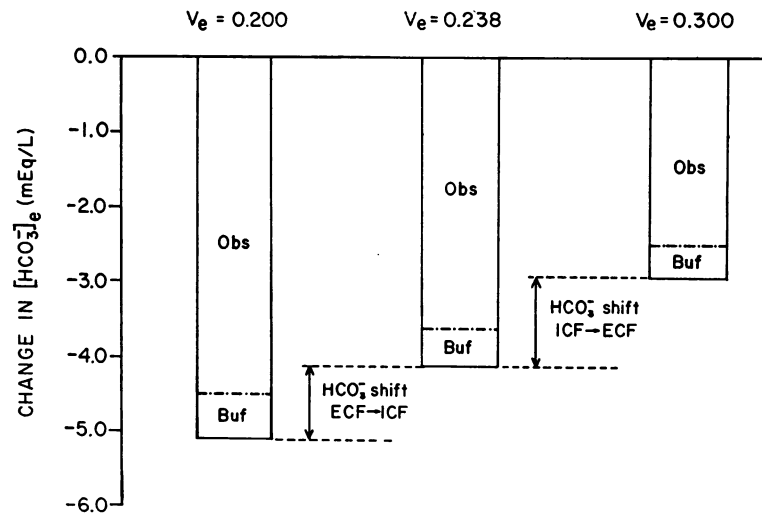


FIG. 4. MAGNITUDE AND DIRECTION OF POSSIBLE SHIFTS OF BICARBONATE RESULTING FROM A LARGE INFUSION OF HYPEROSMOTIC SALINE. The center column represents data that would be obtained if no bicarbonate shifts occurred and if $V_e = 0.238$ L per kg body weight, the mean value calculated from the 11 NaCl experiments (Table IV). The column on the left shows the result that would obtain if $V_e = 0.200$ L per kg body weight, while that on the right shows the result if $V_e = 0.300$ L per kg body weight. The differences in terms of its effect upon $[\text{HCO}_3^-]_e$ between the center column and each of the others therefore represent the magnitude of bicarbonate shift necessary to explain the observed results. The portion of each column labeled Obs represents the observed fall in $[\text{HCO}_3^-]_e$; the portion labeled Buf represents the effects of "new" $[\text{HCO}_3^-]_e$. Conditions of calculation: initial $[\text{HCO}_3^-]_e = 20$ mEq per L; $\Delta\text{pH} = -0.10$; $L = 36$ mOsm per kg body weight of NaCl. For each column Equation 2 was used to calculate V_e' with mean values for W and A observed in the NaCl experiments (Table IV). From V_e'/V_e the "corrected" $[\text{HCO}_3^-]_e$ for each column was calculated by using the value for initial $[\text{HCO}_3^-]_e$ as above. The amount of "new" $(\text{HCO}_3^-)_e$ was computed as outlined in the section on Calculations and was converted to a concentration using the value for $V_e'^{.1}$

rather extreme conditions selected, bicarbonate shifts into or out of the extracellular fluid could have occurred, but in no instance does the magnitude of possible bicarbonate shifts approach the degree of fall in bicarbonate concentration observed. It, therefore, seems fair to conclude that dilution is the most important factor conditioning the acid-base status following infusion of hyperosmotic saline. Recalculation of the previously published acid-base data of McDowell and co-workers (8) also gives results generally supporting this concept of the pathogenesis of the acidosis.

The data presented here neither prove nor disprove the hypothesis that the body fluids exhibit perfect osmometric behavior. McDowell and co-workers (8) reported that deviations from perfect behavior occurred under conditions similar to those used in the present experiments. These deviations were in the direction as to suggest that new osmols were generated intracellularly as a result of the infusion of hypertonic solutes. The effect of such "idiogenic" osmols would be to reduce the degree of dilution of the extracellular fluid compared to that expected for a perfect system. With equations given by McDowell and associates (8) and the same initial conditions as in the calculation in Figure 4, the initial volume of the extracellular fluid would be 23.2% of the body weight rather than the 23.8% calculated from perfect osmometric considerations. This discrepancy is small, and deviations of the magnitude reported by McDowell and co-workers for loads of solute used in the present study (8) would not, therefore, appreciably alter the conclusions reached.

The acidosis observed in our experiments differs in origin from that reported by Sotos and co-workers (10). The two sets of experiments are hardly comparable, however, since Sotos and co-workers used unanesthetized rabbits with intact kidneys (so that consistent expansion of the extracellular fluid was prevented) in which extreme degrees of hyperosmolality were achieved (400 to 500 mOsm per kg water) and in which convulsions, hypotension, and respiratory depression were observed.

One of the implications of the concept of "dilution acidosis" as developed above is that the bicarbonate concentration in the cells should rise

as water is lost from the intracellular compartment. If P_{CO_2} remains constant (i.e., with controlled ventilation as in our experiments) then intracellular pH should rise as extracellular pH falls. In other words, "dilution acidosis" in the extracellular fluid should be accompanied by a "concentration alkalosis" in the intracellular fluid.

It seems likely that intracellular pH rose in our experiments, since even in the unlikely case that the bicarbonate transfer from cells was as large as calculated in Figure 4, the virtual concentration of bicarbonate in the fluid leaving the cells would be about 6.7 mEq per L. This concentration in turn is lower than the estimates of intracellular bicarbonate concentration in muscle water (27) and is also less than the intracellular bicarbonate concentration of the whole body as calculated by Robin, Wilson, and Bromberg (28) from measurements of the distribution of 5,5-dimethyl-2,4-oxazolidinedione (DMO). Hence, the intracellular compartment would show an increased concentration of bicarbonate, and accordingly intracellular pH would rise.

Although no attempt was made in these experiments to measure intracellular pH, the observed acid-base changes in cerebrospinal fluid are in the direction expected of a compartment that rapidly equilibrates with the extracellular fluid with respect to P_{CO_2} and water but not with respect to bicarbonate. The degree of rise of $[HCO_3]_{csf}$, however, was less than would have been predicted solely on the basis of a diminution of cerebrospinal fluid volume. This discrepancy can be calculated on the assumption that cerebrospinal fluid was in osmotic equilibrium with the plasma both before and after completion of infusion, since under this circumstance, the ratio of initial to final cerebrospinal fluid bicarbonate concentration should be equal to the ratio of initial to final plasma osmolality. The data in Table III demonstrate that in every experiment the ratio for $[HCO_3]_{csf}$ exceeded that for $[Osm]_p$. Such a discrepancy could be explained if there were an admixture of newly formed cerebrospinal fluid of lower bicarbonate concentration with the original cerebrospinal fluid. Hence, the cerebrospinal fluid findings cannot be considered solely as a consequence of passive water transfer from one compartment to the other.

Summary

The changes in acid-base equilibrium of blood following the infusion of hyperosmotic solutions have been studied in nephrectomized dogs with Pco_2 held constant. The infusion of hyperosmotic sodium chloride or mannitol solutions consistently leads to a fall in plasma bicarbonate concentration and hence in blood pH. The magnitude of the fall in plasma bicarbonate concentration when corrected for erythrocyte bicarbonate readjustment and for the effects of nonbicarbonate buffers can be largely accounted for by the magnitude of the osmotically induced transfer of water from the intracellular to the extracellular space. Some transfers of bicarbonate between these two compartments cannot be excluded, but even under rather extreme assumptions, the effect of such transfers on bicarbonate concentration is relatively small compared to the effect of dilution. A corollary of these results is that intracellular pH should rise, since bicarbonate concentration within the cells should rise as water is withdrawn. Although no studies of intracellular pH were performed in these experiments, studies of the acid-base changes in cerebrospinal fluid, another compartment that apparently permits rapid equilibration of water and Pco_2 but not of bicarbonate, show that cerebrospinal fluid pH rises as blood pH falls and that this rise is due to an increased concentration of cerebrospinal fluid bicarbonate.

Acknowledgment

Acknowledgement is made to Dr. Ralph B. Dell, who as a medical student working with the senior author in the Department of Physiology of the University of Pennsylvania School of Medicine, initiated preliminary studies that led to the present experiments.

References

1. Winters, R. W., P. R. Scaglione, and G. G. Nahas. Dilution acidosis in the dog. *Proc. int. Union physiol. Sci.* 1962, **2**, no. 227.
2. Essen, H., F. Kounders, and O. Porges. Die Beziehungen der CO_2 Spannung der Alveolarluft zu den Chloriden des Blutserums. *Wien. Arch. inn. Med.* 1923, **5**, 499.
3. Stewart, J. D., and G. M. Rourke. The effects of large intravenous infusions on body fluid. *J. clin. Invest.* 1942, **21**, 197.
4. Shires, G. T., and J. Holman. Dilution acidosis. *Ann. intern. Med.* 1948, **28**, 557.
5. Odaiva, T. Influence of some neutral salt solutions intravenously administered on the alkali reserve of the blood. *Tohoku J. exp. Med.* 1923, **4**, 523.
6. Winkler, A. W., J. R. Elkinton, J. Hopper, Jr., and H. E. Hoff. Experimental hypertonicity: alterations in the distribution of body water, and the cause of death. *J. clin. Invest.* 1944, **23**, 103.
7. Wolf, A. V., and M. E. McDowell. Apparent and osmotic volumes of distribution of sodium, chloride, sulfate and urea. *Amer. J. Physiol.* 1954, **176**, 207.
8. McDowell, M. E., A. V. Wolf, and A. Steer. Osmotic volumes of distribution. Idiogenic changes in osmotic pressure associated with administration of hypertonic solutions. *Amer. J. Physiol.* 1955, **180**, 545.
9. Neely, W. A. Apparent sodium space of the dog. *Amer. J. Physiol.* 1959, **196**, 1333.
10. Sotos, J. F., P. R. Dodge, and N. B. Talbot. Studies in experimental hypertonicity. II. Hypertonicity of body fluids as a cause of acidosis. *Pediatrics* 1962, **30**, 180.
11. Peters, J. P., and D. D. Van Slyke. *Quantitative Clinical Chemistry. Interpretations*, 1st ed. Baltimore, Williams & Wilkins, 1931, vol. 1.
12. Barker, S. B., and W. H. Summerson. The colorimetric determination of lactic acid in biological material. *J. biol. Chem.* 1941, **138**, 535.
13. Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. *J. biol. Chem.* 1944, **153**, 375.
14. Gornall, A. G., C. J. Bardawill, and M. M. David. Determination of serum proteins by means of biuret reaction. *J. biol. Chem.* 1949, **177**, 751.
15. Severinghaus, J. W., M. Stupfel, and A. F. Bradley. Accuracy of blood pH and pCO_2 determinations. *J. appl. Physiol.* 1956, **9**, 189.
16. Severinghaus, J. W., M. Stupfel, and A. F. Bradley. Variations of serum carbonic acid pK' with pH and temperature. *J. appl. Physiol.* 1956, **9**, 197.
17. Alexander, S. C., R. Gelfand, and C. J. Lamberts. The pK' of carbonic acid in cerebrospinal fluid. *J. biol. Chem.* 1961, **236**, 592.
18. Harned, H. S., and R. Davis, Jr. The ionization constant of carbonic acid in water and the solubility of carbon dioxide in water and aqueous salt solutions from 0 to 50°. *J. Amer. chem. Soc.* 1943, **65**, 2030.
19. Harned, H. S., and B. B. Owen. *The Physical Chemistry of Electrolytic Solutions*, 2nd ed. New York, Reinhold, 1950.
20. Singer, R. B., J. K. Clark, E. S. Barker, A. P. Crosley, Jr., and J. R. Elkinton. The acute effects in man of rapid intravenous infusion of hypertonic sodium bicarbonate solution. I. Changes in acid-base balance and distribution of the excess buffer base. *Medicine (Baltimore)* 1955, **34**, 51.
21. Eisenman, A. J., L. B. Mackenzie, and J. P. Peters. Protein and water of serum and cells of human

- blood, with a note on the measurement of red blood cell volume. *J. biol. Chem.* 1936, **116**, 33.
22. *Blood and Other Body Fluids*. Washington, D. C., Federation of American Societies for Experimental Biology, 1961.
23. Jørgensen, K., and P. Astrup. Standard bicarbonate, its clinical significance, and a new method for its determination. *Scand. J. clin. Lab. Invest.* 1957, **9**, 122.
24. Cotlove, E. Mechanism and extent of distribution of inulin and sucrose in chloride space of tissues. *Amer. J. Physiol.* 1954, **176**, 396.
25. Nichols, G., Jr., N. Nichols, W. B. Weil, and W. M. Wallace. The direct measurement of the extracellular phase of tissues. *J. clin. Invest.* 1953, **32**, 1299.
26. Manery, J. F., I. S. Danielson, and A. B. Hastings. Connective tissue electrolytes. *J. biol. Chem.* 1938, **124**, 359.
27. Fenn, W. O. Carbon dioxide and intracellular homeostasis. *Ann. N. Y. Acad. Sci.* 1961, **92**, 547.
28. Robin, E. D., R. J. Wilson, and P. A. Bromberg. Intracellular acid-base relations and intracellular buffers. *Ann. N. Y. Acad. Sci.* 1961, **92**, 539.