THE EXTRARENAL RESPONSE TO ACUTE ACID-BASE DISTURBANCES OF RESPIRATORY ORIGIN [1]

By GERHARD GIEBISCH, LAWRENCE BERGER,[2] and ROBERT F. PITTS with the technical assistance of MARY ELLEN PARKS and MARTHA B. MacLEOD

(From the Department of Physiology, Cornell University Medical College, New York, N. Y.)

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The plasma electrolyte changes in acute respiratory acidosis and alkalosis cannot be explained solely on the basis of renal compensatory mechanisms. In acute respiratory acidosis, renal conservation of base is accomplished by means of increased excretion of ammonia and titratable acid (1) as well as by enhanced renal reabsorption of bicarbonate bound base (2, 3). However, under acute conditions, the degree of base saving effected by these means cannot account for the increment of bicarbonate bound base observed. This initial increment of bicarbonate bound base in the extracellular fluid which these renal tubular mechanisms then maintain, must be supplied from extrarenal sources. Similarly, extrarenal mediation must pertain in acute respiratory alkalosis, since altered renal excretion of base cannot alone account for the extracellular electrolyte changes which occur (4). Furthermore, renal mechanisms cannot explain completely the changes in plasma concentration of chloride, phosphate, and organic acids observed in acute respiratory acid-base disturbances.

The nature of the extrarenal compensation for acid-base imbalances has been studied by various approaches. In metabolic acidosis, direct tissue studies reveal alteration of the content of tissue phosphates (5), of sodium and potassium of bone and soft tissues (5), and of carbon dioxide stores of bone and muscle (6). The observed changes are interpreted to be evidence of direct tissue compensation. In addition, the buffering of infused acid or alkali cannot be accounted for by blood buffers alone (7–9), nor by the buffer capacity of the extracellular fluid (10). Further, altered organic acid metabolism has been invoked as capable of modifying significantly the response of the organism to an alkali invasion (11).

Fewer studies exist as to the nature of the tissue response to an acid-base disturbance of respiratory origin. In this regard three lines of evidence can be cited. First, studies in respiratory acidosis indicate that of the total carbon dioxide gained, 80 to 90 per cent is accounted for by extravascular sites, largely bone and muscle (12, 13); conversely, a loss of comparable degree from tissues occurs in respiratory alkalosis. Second, direct muscle analyses indicate changes in cell base in respiratory acid-base imbalance (14, 15), which may be taken to reflect tissue compensation. Finally, that tissue electrolytes may be made available for extracellular buffering, has been postulated. Thus, various investigations have revealed changes in carbon dioxide capacity (16, 17) of blood, and alterations in plasma concentration of various electrolytes of such magnitude and direction to be compatible with transfer of these constituents across an extracellular boundary (18, 19).

The present study is an attempt to delineate more precisely and completely the nature and extent of the tissue contribution to the extracellular compartment in the compensation for an acute respiratory acidosis or alkalosis.

Nephrectomized dogs were used to eliminate any factor of renal compensation. By the measurement of extracellular fluid volume and of plasma and red blood cell electrolyte concentration, an estimate could be obtained of transfer of ions into or out of the readily available extracellular fluid volume.

Tissue contribution to compensation in the extracellular fluid for respiratory acidosis or alkalosis was found to be mediated mainly by three means: Transfer of chloride across the erythrocyte membrane; shifts of base across an extracellular boundary other than the red blood cell

[1] Aided by grants from the National Heart Institute of the National Institutes of Health and the Life Insurance Medical Research Fund.

[2] Fellow of the National Heart Institute. Present address: Mt. Sinai Hospital, New York, New York.

membrane; and significant alterations in organic acid content of the extracellular fluid.

EXPERIMENTAL PROCEDURE

A total of 29 experiments was performed on 24 male dogs, ranging in weight from 14.6 to 35.9 Kg. Anesthesia was induced and maintained by sodium pentobarbital, given intravenously. Bilateral nephrectomy was performed by flank approaches. Blood was obtained from a needle indwelling in a femoral artery. A polyethylene catheter was inserted into the femoral vein, and used for administration of the various materials infused. An endotracheal airway with inflatable rubber cuff was introduced.

Following these procedures, appropriate amounts of sucrose, radiosulfate, and/or radiochloride (Cl^{36}) were infused in a manner previously reported from this laboratory (20). The total time for infusion including washout with 50 to 100 ml. of 3 per cent glucose in water never exceeded 15 minutes. In a total of three experiments the sucrose blank of plasma was followed over several hours of respiratory acidosis or alkalosis, and increase in blank with time was found to be comparable with that reported by Swan, Madisso, and Pitts (20). Appropriate blank correction was applied.

In some experiments radiosodium (Na^{22}) was infused. In such cases this material was given intravenously, 12 to 18 hours prior to the experiment to assure adequate equilibration. The dose used was usually 50 μc, equivalent to about 8×10^6 cpm. Sufficient carrier was added to make a final concentration of 0.107 M NaCl. Correction was applied for radiosodium lost into the urine prior to nephrectomy.

An equilibration time of two and one-half hours from the midpoint of infusion was used for sucrose, radiosulfate, and for radiochloride. In 16 experiments, sucrose and radiosulfate were used simultaneously to estimate readily available extracellular fluid volume.

Subsequent to equilibration time, blood was collected from the indwelling arterial needle at hourly intervals for two to three hours. The amounts drawn each time varied between 25 and 40 ml.

In acidosis experiments, following the control periods the dog breathed gas mixtures, usually containing 20 per cent CO_2 and 80 per cent O_2 for two to six hours. In a few experiments the gas mixture contained 12 per cent CO_2 and 88 per cent O_2. The gas was administered by attaching a Douglas bag containing the mixture to a non-rebreathing Digby-Leigh valve which was connected to the endotracheal tube.

In alkalosis experiments, hyperventilation was maintained for two to four hours by means of a Palmer-type respiration pump attached to the endotracheal tube. In these experiments room air was used. In a few studies the initially induced period of acidosis or alkalosis was followed by another set of control periods, during which the dog was allowed to breath room air spontaneously; these then were succeeded by two further periods during which the condition opposite to that of the initial experimental periods was induced: hyperventilation after initial CO_2 breathing or CO_2 breathing after hyperventilation.

ANALYTICAL METHODS

Plasma was analyzed for Na, K, Cl, CO_2, and PO_4 by methods described previously (21). Values for pH were determined on samples of whole blood in the manner described by these authors. Lactate of plasma was estimated by the method of Barker and Summerson (22). Sucrose, radiosulfate, and Cl^{36} of plasma were determined by methods described in a previous report from this laboratory (20). The gamma-emission of Na^{22} of plasma was measured in a lead-shielded scintillation counter, containing a Thallium activated sodium iodide crystal, and a steel filter for elimination of weak beta-emission. Determinations were performed on a 2 ml. wet sample in metal planchettes. A suitable Na^{22} standard in saline (0.107 M) was used. Comparison with standards in plasma revealed no significant difference. Adequate sample and background counts were performed to assure a consistency of counting to within ± 2 per cent. By the analytical methods used, complete separation of Na^{22} and Cl^{36} could be achieved. No such complete separation could be accomplished in the case of radiosulfate and Na^{22}, since the precipitates of radiosulfate gave excessively high counts, due to contamination with Na^{22}. Therefore, radiosulfate was not used in the few experiments in which Na^{22} was infused. Plasma volume was estimated by the T-1824 dye-dilution technique (23). A measure of total proteins of plasma was obtained by the copper sulfate method of Van Slyke and his associates (24). Hematocrit was measured in Wintrobe tubes, spun at 4,000 RPM in a centrifuge of 17 inch diameter for 30 minutes. Whole blood analyses for Na and K were performed using an internal standard flame photometer. Whole blood CO_2 content was determined according to the method of Van Slyke and Neill (25). Whole blood chlorides were determined on a Van Slyke-Hawkins protein-free filtrate (26), an aliquot of which was analyzed by the method of Van Slyke and Hiller (27).

CALCULATIONS

Symbols and formulas used are given in detail in the Appendix. Total amounts of ions in the readily available extracellular fluid (ECF) were calculated from data of plasma concentration, plasma volume, and radiosulfate space ($VS^{35}O_4$), with appropriate factors applied for Gibbs-Donnan distribution and water content of plasma. Total amounts of ions in the total red blood cell mass were calculated from data of plasma concentration, whole blood concentrations, plasma volume, and hematocrit. The increments or decrements of total extracellular or red cell ions were calculated in each experiment by averaging the total of each ion species in control periods and in periods of respiratory acidosis or

alkalosis. Increments are recorded as $+\Delta mM$, decrements as $-\Delta mM$. In experiments in which both acidosis and alkalosis were induced in the same animal, each pair of periods (control, acidosis, or alkalosis), was compared to the immediately preceding pair.

RESULTS

Readily available ECF in respiratory acidosis and alkalosis in nephrectomized dogs

Figure 1 shows the plasma disappearance curves of sucrose and radiosulfate of several representative experiments. Similar curves were obtained in all animals. It should be noted that the plot is a semi-logarithmic one. The vertical line indicates the time at which acidosis or alkalosis was induced.

It is apparent that in all cases a straight line could be fitted through all points, since the logarithm of plasma concentration decreases linearly with time.[8] This linearity is compatible with constant clearance from unchanging volume of distribution (29). The conclusion, therefore, seems warranted that neither the sucrose space (VS) nor radiosulfate space ($VS^{35}O_4$) changed after induction of respiratory acidosis or alkalosis. In 16 experiments the volumes of distribution of sucrose and radiosulfate were measured simultaneously. The average ratio $VS/VS^{35}O_4$ was found to be 0.90. This value is in good agreement with the value (0.91) recently given by Swan, Madisso, and Pitts (20). On the basis of the findings of the same authors, the radiosulfate space was selected as a measure of extracellular fluid. In 8 experiments where the radiosulfate space was not measured, its equivalent was obtained by multiplying the sucrose space by 1/0.90. This procedure appears permissible, since this ratio has been shown to remain unchanged in respiratory acidosis or alkalosis. From the data presented the conclusion is drawn that extracellular fluid volume did not change in respiratory acidosis or alkalosis.

[8] In the nephrectomized animal the slopes of the curves of disappearance of radiosulfate and sucrose from plasma are dependent on extrarenal removal of these substances from the extracellular fluid. This extrarenal loss, such as slow metabolic breakdown, secretion into the gastrointestinal tract, *etc.*, prevents these disappearance curves from reaching a plateau. Recent experiments by Mulrow, Oestreich, and Swan (28) show that the slopes of disappearance from plasma of radiosulfate and sucrose remain unchanged in the nephrectomized animal for as long as 30 hours. The fact that no plateau is reached and that no "flattening out" occurs, strongly indicates that decrease in plasma concentration of these substances is not indicative of progressive increase in extracellular fluid volume but of constant extrarenal removal of these substances from an unchanging volume of distribution.

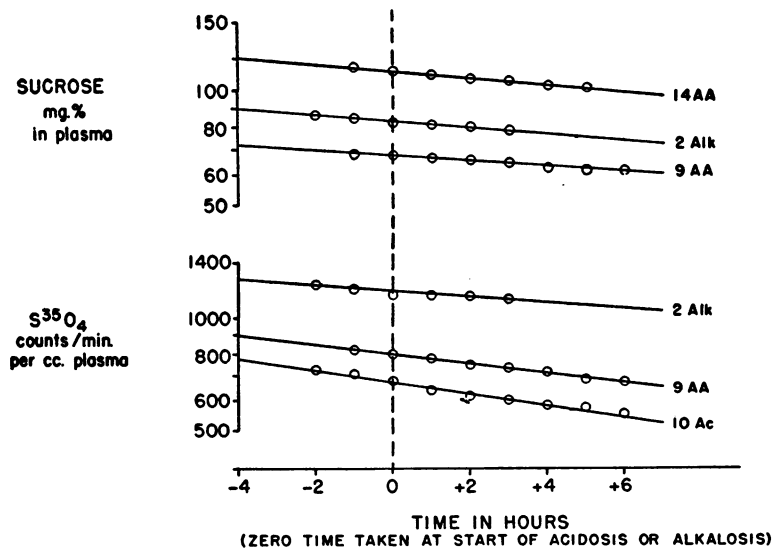

FIG. 1. DISAPPEARANCE OF RADIOSULFATE AND SUCROSE FROM PLASMA IN NEPHRECTOMIZED DOGS, SUBJECTED TO RESPIRATORY ACIDOSIS OR ALKALOSIS

TABLE I

Experiments illustrating the effect of acute respiratory acidosis and alkalosis on distribution of electrolytes

Elapsed time	pH	Art. pCO_2	$VS^{35}O_4$	Plasma volume	Conc. in plasma water				Quantity in readily available extracellular space				Quantity in total red blood cell volume			
					Na	K	Cl	HCO_3	Na	K	Cl	HCO_3	Na	K	Cl	HCO_3
min.		*mm. Hg*	*ml. H_2O*		*mM/L.*				*mM*				*mM*			
150	7.30	46.5	4,610	753	155	3.2	108	23.4	682	14	517	113	83	5	38	9
210	7.30	43.2	4,610	753	153	3.8	107	21.2	676	17	512	107	80	5	38	8
270	7.29	43.2	4,610	753	150	4.0	107	20.7	668	18	516	100	79	5	37	7
275		20% CO_2—80% O_2														
330	6.84	173	4,610	678	158	4.3	108	30.6	701	19	517	147	91	7	50	15
390	6.86	179	4,610	678	159	7.6	105	32.7	701	33	498	156	103	8	56	19
450	6.89	173	4,610	678	161	8.0	103	33.4	713	35	492	159	100	8	55	19
510	6.81	199	4,610	655	162	7.8	106	32.5	717	34	502	155	96	8	54	18
570	6.84	176	4,610	655	163	6.9	104	31.0	723	30	497	148	89	6	51	17
630	6.82	183	4,610	655	167	5.7	105	30.4	739	25	500	145	94	7	52	15
		Dog 10 Ac—20.5 Kg.						ΔmMols.	+41	+13	−14	+45	+15	+2	+15	+9
150	7.39	43.3	5,270	948	162	3.8	114	26.6	820	19	617	146	125	8	86	16
210	7.35	46.4	5,270	948	159	3.9	113	26.3	803	20	619	144	128	8	81	16
270	7.40	39.9	5,270	948	161	4.0	112	26.3	814	20	613	141	121	7	76	15
275		Hyperventilation														
330	7.74	6.6	5,270	854	159	3.3	113	8.9	800	17	619	49	116	8	74	7
390	7.75	6.6	5,270	854	156	3.3	117	8.6	787	17	644	47	100	8	61	3
450	7.73	6.6	5,270	854	159	2.8	120	8.1	803	14	659	44	94	8	57	3
		Dog 2 Alk—23.6 Kg.						ΔmMols.	−16	−4	+25	−97	−22	0	−17	−12

Changes in electrolyte distribution in respiratory acidosis and alkalosis

In Table I are presented data on electrolyte changes in the readily available extracellular fluid obtained in two typical experiments. Experiment 10 Ac is one of 18 experiments in which respiratory acidosis was induced. It is apparent that there was a marked drop in arterial pH and an elevation of pCO_2 to a level compatible with inspiration of a 20 per cent CO_2 mixture at atmospheric pressure. $VS^{35}O_4$ remained unchanged, as described above. Plasma volume was estimated once at the midpoint of each group of three periods. The value obtained was used in the calculations for the preceding and succeeding hourly period. A decrease in plasma volume was usually observed, possibly related to the repeated withdrawals of blood for analyses and to water transfer into red cells. Plasma electrolyte concentrations showed a progressive rise of sodium and potassium, a slight fall of chloride, and a significant increase of bicarbonate. The next four columns show total electrolytes in the extracellular fluid, and in the lowermost row are presented the increments and decrements of these ions in the ECF. It is evident that there was a transfer of sodium and potassium into the ECF, a significant increment of bicarbonate, and a movement of chloride out of the readily available extracellular fluid volume. The last four columns illustrate similar data of total electrolytes and net transfers for total red cell mass. Of special interest is the finding that the chloride increment is of the same order of magnitude as the simultaneous chloride decrement in the ECF.

Experiment 2 Alk shows similar data in a representative alkalosis study. The pH and pCO_2 changes here are typical of those in severe respiratory alkalosis. Again, $VS^{35}O_4$ remained unchanged. Plasma concentration data show changes opposite to those in respiratory acidosis; the balance of total ions in the ECF demonstrates a movement of sodium and potassium out of the ECF, a marked decrease in bicarbonate, and a significant increment of extracellular chloride. In this case, red cell data again reveal a chloride

TABLE II

Experiments illustrating the effect of acute respiratory alkalosis and acidosis, in the same animal, on distribution of electrolytes, including phosphate and lactate

Elapsed time	pH	Art. pCO_2	$V_{S^{35}O_4}$	Plasma volume	Conc. in plasma water						Quantity in readily available extracellular space						Quantity in total red blood cell volume			
					Na	K	Cl	HCO_3	PO_4	Lact.	Na	K	Cl	HCO_3	PO_4	Lact.	Na	K	Cl	HCO_3
min.		*mm. Hg*	*ml. H_2O*		*mM/L.*						*mM*						*mM*			
150	7.42	29.9	6,545	1,487	155	3.9	116	20.8	1.4	1.0	972	25	793	142	9	6				
210	7.45	26.7	6,545	1,487	154	3.9	115	19.8	1.5	1.2	969	26	781	136	10	8				
215		Hyperventilation																		
270	7.57	6.7	6,545	1,284	151	3.9	124	7.5	1.0	5.9	960	25	850	47	6	39				
330	7.54	6.1	6,545	1,284	150	3.5	125	6.9	0.7	6.7	952	22	854	43	5	44				
335		Spontaneous breathing of room air							ΔmMols.		−15	−2	+65	−94	− 4	+35				
390	7.30	33.2	6,545	1,281	154	3.8	115	17.2	1.6	3.1	974	24	785	118	10	20				
450	7.34	33.2	6,545	1,281	154	4.2	116	19.2	1.9	1.2	980	27	793	132	13	8				
455		20% CO_2—80% O_2							ΔmMols.		+21	+2	−63	+80	+ 6	−28				
510	6.87	146	6,545	1,233	160	4.7	113	28.0	3.4	1.4	1,006	29	777	190	22	9				
570	6.92	143	6,545	1,233	163	6.5	113	30.0	4.8	0.2	1,028	41	783	205	31	1				
		Dog 9 Alk-Ac—35.9 Kg.							ΔmMols.		+40	+9	− 9	+72	+15	− 9				
150	7.41	36.6	3,670*	718	158	4.7	110	23.8	2.1	1.6	555	16	423	91	8	6	102	8	59	14
210	7.37	36.6	3,670	718	158	4.5	112	22.0	2.0	1.9	555	16	428	84	8	7	118	9	62	13
215		Hyperventilation																		
270	7.61	10.0	3,670	706	154	4.2	114	11.5	0.7	8.5	546	15	438	44	3	31	108	8	54	6
330	7.63	10.0	3,670	706	150	4.7	113	11.6	0.8	6.4	535	13	434	44	3	24	100	9	49	5
335		Spontaneous breathing of room air							ΔmMols.		−14	−2	+10	−44	− 5	+21	−6	0	− 9	− 8
390	7.40	29.9	3,670	655	154	4.5	114	19.0	2.0	2.7	543	16	437	74	7	10	95	8	48	8
395		20% CO_2—80% O_2							ΔmMols.		+ 2	+2	+ 1	+30	+ 4	−18	−9	−1	− 4	+ 2
450	6.85	160	3,670	592	161	5.5	111	28.5	5.3	2.1	567	21	427	109	19	8	102	9	62	18
510	6.81	176	3,670	592	161	7.8	111	29.0	6.5	1.9	567	28	427	111	24	7	98	8	59	17
		Dog 14 Alk-Ac—21.0 Kg.							ΔmMols.		+24	+9	−10	+36	+15	− 2	+5	0	+13	+10

* $S^{35}O_4$ space estimated on the basis of sucrose space.

TABLE III

Net transfers of ions in acute respiratory acidosis

Experiment no.	Weight *Kilo.*	$V_{S^{35}O_4}$	$V_{sucrose}$	Balance in readily available extracellular space—*mM*					
		ml. H_2O		Na^+	K^+	Cl^-	HCO_3^-	PO_4	Lact.
1 Ac	25.0	4,680*	4,220	+ 8	+ 2	−25	+34		
2 Ac	14.9	3,150*	2,840	+22	+ 5	−10	+25		
3 Ac	17.0	4,025*	3,625	+22	+ 1	0	+32		
4 Ac	17.2	3,805*	3,425	+32	+ 4	−10	+24		
5 Ac	19.5	3,645	3,640	+16	+10	− 2	+30		
6 Ac	22.0	4,025	3,345	+15	+ 5	0	+36		
7 Ac	19.9	3,855	3,455	+22	+10	−16	+42		
8 Ac	21.1	4,415	3,805	+47	+ 7	− 9	+66		
8 A	17.0	2,780*	2,505	+27	+ 5	− 8†	+25		
9 Ac	23.5	3,255	3,340	+20	+ 5	+ 5†	+13		
10 Ac	20.5	4,610	—	+41	+13	−14†	+45		
11 Ac	28.1	5,370	4,700	+53	+ 7	− 8†	+55		
12 Ac	19.6	4,305	4,320	+24	+15	+ 8†	+39		
13 Ac	25.4	5,670	5,440	+29	+26	−19†	+43		
9 Alk-Ac	35.9	6,545	6,210	+40	+ 9	− 9	+72	+15	−9
11 Ac-Alk	23.6	5,040	—	+15	+13	−22	+38	+ 5	−1
13 Alk-Ac	14.6	3,625	—	+ 9	+ 2	−14†	+29	+ 6	+2
14 Alk-Ac	21.0	3,670*	3,300	+24	+ 9	−10†	+36	+15	−2

* $S^{35}O_4$ space estimated from sucrose space.
† Red blood cell data obtained.

change of comparable magnitude but opposite direction to that in the ECF.

It is of importance that only about half of the decrement in extracellular bicarbonate can be accounted for by the observed concomitant changes in sodium, potassium, and chloride. A similar but smaller discrepancy was evident in respiratory acidosis. This discrepancy was considered too large to be explained by alterations in known cations; therefore, the anions phosphate and lactate were studied in subsequent experiments.

The experiments listed in Table II illustrate two additional significant features. In these experiments both alkalosis and acidosis were induced in the same animal, with appropriate control periods interposed. The data show a marked rise of lactate associated with a slight fall of phosphate in respiratory alkalosis, and a significant increase in phosphate with concomitant fall in lactate in periods of respiratory acidosis. These data reduce the anion deficits formerly observed. Furthermore, the total amounts of ions transferred into or out of the readily available extracellular fluid are of comparable magnitude to those observed in separate experiments of acidosis or alkalosis. The net transfers of ions are reversible and reestablishment of control conditions occurs to a reasonable extent after the pCO_2 has been restored to normal. This therefore illustrates the ready mobility of a fraction of body electrolytes in

TABLE IV

Net transfers of ions in acute respiratory alkalosis

Experiment no.	Weight *Kilo.*	$V_{S^{35}O_4}$	$V_{sucrose}$	Balance in readily available extracellular space—*mM*					
		ml. H_2O		Na^+	K^+	Cl^-	HCO_3^-	PO_4	Lact.
1 Alk	22.3	4,040	3,555	−15	− 4	+17†	− 63		
2 Alk	23.6	5,270	4,390	−16	− 4	+25†	− 97		
3 Alk	20.9	4,755	4,150	−19	− 2	+12†	− 87		
5 Alk	24.1	6,400	5,965	−22	− 5	+20	−110		
6 Alk	22.5	4,575	3,940	−11	− 4	+13†	− 51		
7 Alk	19.9	4,470	3,695	−16	− 2	− 5†	− 95		
9 Alk-Ac	35.9	6,545	6,210	−15	− 2	+65	− 94	− 4	+35
11 Ac-Alk	23.6	5,040	—	− 3	−22	+43	− 79	−11	+32
12 Alk	16.4	2,685*	2,415	− 7	+ 9	+ 9†	− 60	+ 3	+27
13 Alk-Ac	14.6	3,625	—	−16	+ 1	+19†	− 58	− 6	+15
14 Alk-Ac	21.0	3,670*	3,300	−14	− 2	+10†	− 44	− 5	+21

* $S^{35}O_4$ space estimated from sucrose space.
† Red blood cell data obtained.

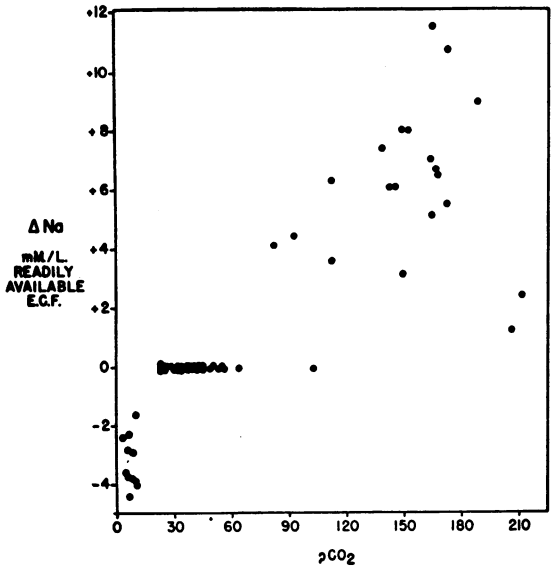

FIG. 2. TRANSFER OF SODIUM PER LITER OF READILY AVAILABLE EXTRACELLULAR FLUID, AS A FUNCTION OF ARTERIAL pCO_2

Each point represents a separate experiment. The values at zero on the ordinate scale represent control periods.

contributing to buffering within the extracellular fluid.

Experiment 14 Alk-Ac further demonstrates a similar lability of red cell electrolytes. Again, the extracellular chloride changes are of similar magnitude but opposite direction to those in the red cells.

In Tables III and IV are presented summaries of electrolyte transfers to or from the readily available extracellular fluid in all experiments performed. Shown are the results of 18 observations in respiratory acidosis and 11 observations in respiratory alkalosis. The consistency of the above described changes is evident. Variations in the absolute amounts of ions transferred in individual experiments are largely related to differences in the size of the extracellular fluid volumes and to differences in severity of the acidosis or alkalosis, *i.e.*, the extent of alteration of arterial pCO_2 (*vide infra*).

In a total of 8 acidosis experiments and 8 alkalosis experiments, red cell data were obtained. In the majority of observations the ΔCl of total red cell mass was of similar magnitude, but opposite sign to that of the ECF.[4]

[4] However, it should be pointed out that *in vivo* studies of transfers of ions across the red cell membrane have certain limitations. The possible sources of errors are: 1) Changes in total body hematocrit may not be closely reflected by the observed peripheral hematocrit (30); 2) errors derived from estimating red cell concentra-

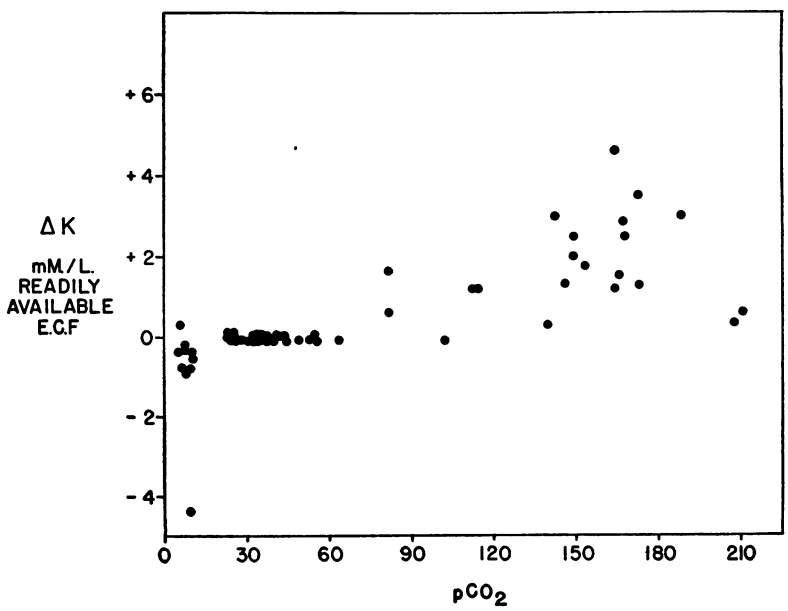

FIG. 3. TRANSFER OF POTASSIUM PER LITER OF READILY AVAILABLE EXTRACELLULAR FLUID, AS A FUNCTION OF ARTERIAL pCO_2

Each point represents a separate experiment. The values at zero on the ordinate scale represent control periods.

In Figure 2 the ΔNa per liter extracellular fluid is plotted as a function of arterial pCO_2. Each point represents a separate experiment. The points massed at zero on the ordinate scale represent pCO_2 values of control periods. It is evident that there is a direct and fairly linear relationship between the amounts of sodium transferred into or out of one liter of extracellular fluid, and deviations of pCO_2 from control values.

A relationship similar in direction but smaller in magnitude is presented for ΔK per liter extracellular fluid in Figure 3. Here again increasing amounts of base are transferred into the ECF with increasing arterial pCO_2.

Figure 4 depicts the interrelationship between ΔCl per liter ECF and arterial pCO_2. In this case the ΔCl per liter ECF is inversely related to deviations of pCO_2 from control values, increasingly more chloride disappearing from the extracellular fluid as the pCO_2 is raised.

tion of ions from whole blood, plasma, and hematocrit determinations may result in apparent transfers; 3) new cells which may be released into the circulation may not have the same average composition as those already in the blood stream (31).

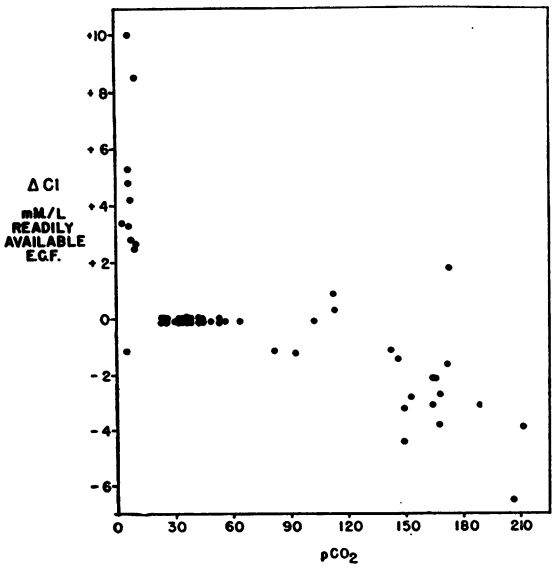

FIG. 4. TRANSFER OF CHLORIDE PER LITER OF READILY AVAILABLE EXTRACELLULAR FLUID, AS A FUNCTION OF ARTERIAL pCO_2

Each point represents a separate experiment. The values at zero on the ordinate scale represent control periods.

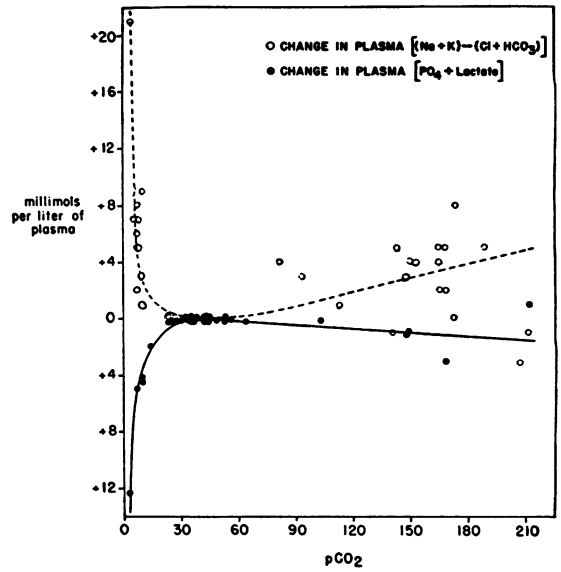

FIG. 5. VARIATIONS IN ANION DEFICIT, AS A FUNCTION OF ARTERIAL pCO_2

The points at zero on the ordinate scale represent control periods. The anion deficit $(Na + K) - (Cl + HCO_3)$, and the sum of plasma phosphate and lactate of control periods are arbitrarily taken as zero. Each point represents one experiment. The two curves are drawn free-hand.

Figure 5 illustrates the manner in which phosphate and lactate contribute to the diminution of the anion deficit previously discussed. This deficit, expressed as the function $[(Na + K) - (Cl + HCO_3)]$ of plasma, is found to increase both in acidosis and alkalosis, more so in the latter. In the experiments performed, the sum of plasma phosphate and lactate ($\Sigma[PO_4 + \text{lactate}]$) was found to rise with deviations from control pCO_2. The graph depicts the relationship between the increase of $[PO_4 + \text{lactate}]$ of plasma above control values and the increase of anion deficit. It should be noted that in respiratory acidosis the phosphate ion accounts for the greater part of this deficit, and in respiratory alkalosis the lactate ion is predominant. The degree to which phosphate and lactate do not completely account for the anion deficit indicates that other anions are involved.

In an attempt to elucidate further the mechanism of the observed ion shifts, experiments were performed in which exchangeable pools of sodium and chloride were determined. In some experiments the volume of distribution of radiochloride (VCl^{36}) was estimated. Table V illustrates ob-

TABLE V

Experiments illustrating the effect of acute respiratory acidosis or alkalosis on the exchangeable pool of sodium and chloride, and the volumes of distribution of radiochloride

Elapsed time *min.*	Exchangeable Na^+		Exchangeable Cl^-		$V_{Cl^{36}}$	
	mM	*mM/Kg.*	*mM*	*mM/Kg.*	*ml. H_2O*	*% Body wt.*
Experiment no. 8 A			Control			
150	684	40.4	508	30.3	4,170	24.7
210	713	42.2	500	29.6	4,100	24.3
			20% CO_2—80% O_2			
270	684	40.1	498	29.5	4,180	24.7
330	692	40.8	522	30.9	4,380	25.9
Experiment no. 14 Alk-Ac			Control			
150	994	47.3	586	27.9	5,090	24.2
210	1,002	47.8	603	28.7	5,170	24.6
			Hyperventilation			
270	997	47.4	603	28.7	5,075	24.2
330	969	46.1	606	28.9	5,155	25.5

servations in typical experiments. Table VI shows the deviations from control values in all acidosis and alkalosis experiments. The small and inconsistent changes observed are not regarded as significant.

DISCUSSION

Qualitatively, the observations presented above on ion shifts between extracellular and cellular or supportive structures in respiratory acidosis and alkalosis are independent of the nature of the substance employed in measuring extracellular fluid volume. Quantitatively, the magnitude of the shifts observed are of course dependent on the volume measured and thus on the nature of the substance employed. We have chosen the volume of distribution of radiosulfate as a measure of readily available extracellular fluid on the evidence of Swan, Madisso, and Pitts (20). We define readily available extracellular fluid as plasma and that fraction of the interstitial fluid with which it is in ready diffusion equilibrium.

TABLE VI

Summary of deviation from control values of exchangeable sodium, exchangeable chloride, and chloride^{36} space

Experiment no.	Average % deviation from control		
	Exchangeable Na	Exchangeable Cl	$V_{Cl^{36}}$
Respiratory acidosis			
1 Ac	+2.6	−2.0	+1.7
2 Ac	+3.1	+2.6	+5.0
3 Ac	+2.3	+0.5	+0.9
4 Ac	—	+1.7	+4.2
14 Alk-Ac	−2.5	+5.6	+7.3
8 A	−1.9	+0.7	+3.3
Respiratory alkalosis			
12 Alk	0.0	+1.7	−1.5
14 Alk-Ac	−1.9	+1.8	+2.0

Following nephrectomy, neither the measured volume of extracellular fluid (20), nor its electrolyte content, changes significantly over several hours (32). As shown above, the volume of distribution of radiosulfate remained unchanged in consequence of the induction of either severe respiratory acidosis or alkalosis. That extracellular fluid volume remained unchanged is further supported by the observation that the volumes of distribution of sucrose and radiochloride likewise remained unaltered. Possible errors in estimating extracellular fluid electrolyte content include changes of Gibbs-Donnan distribution factor with changes in pH, and changes in the readily avail-

able extracellular fluid volume by water transfers (33, 34) not detected by the methods used. In our experiments, the calculated transfer of water into red cells in respiratory acidosis, based on five observations, reveals a shift of the magnitude of about 2.0 per cent of the total ECF, a value well within the limits of error of estimating the volume of distribution of radiosulfate. In three alkalosis experiments the water shift out of red cells was similarly only 1.5 per cent of the measured extracellular fluid volume. These changes are believed to be too small to affect the conclusions drawn.

The data presented indicate that significant redistribution of electrolytes occurs in respiratory acidosis and alkalosis in the absence of renal compensatory mechanisms. In acute respiratory acidosis, the decrease of fixed acid and the increase of base in the extracellular fluid permit the expansion of buffer anions to a degree which diminishes considerably the severity of the developing acidosis. Similarly, in acute respiratory alkalosis of the severity induced in these experiments, compensatory reduction of buffer anions in the extracellular fluid occurred, thereby preventing the development of a lethal alkalosis.[5]

To depict the participation of these mechanisms in the changes of buffer content observed, Figures 6 and 7 are presented. These graphs are based on observations limited to those experiments in which all the respective ions have been determined. Since a fair degree of variation occurs, they indicate general trends of responses to acute respiratory acidosis and alkalosis. In Figure 6 the total increment of buffer anions of the extracellular fluid during acute respiratory acidosis is represented by the total height of the column. The largest fraction of this increment in buffer anions is due to the increase of bicarbonate and only to a slight extent to the increment in basic phosphate ($HPO_4^=$). The contributions of the various ion species to the total increase are expressed as percentages.

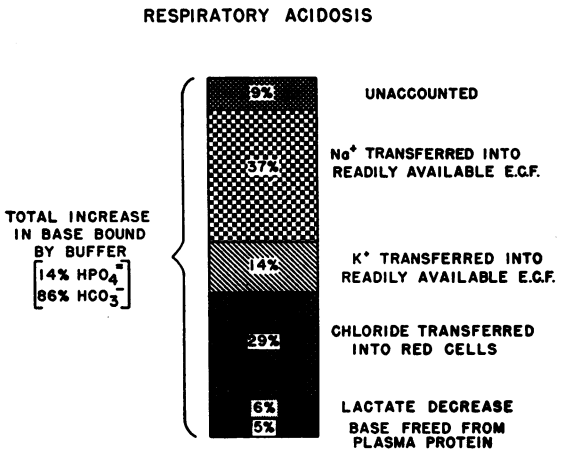

FIG. 6. THE MODE OF EXTRACELLULAR BUFFERING IN ACUTE RESPIRATORY ACIDOSIS

Data from four experiments are averaged (9 Alk–Ac, 11 Ac–Alk, 13 Alk–Ac, 14 Alk–Ac). Gas mixtures used were in all experiments 20 per cent CO_2 to 80 per cent O_2. In Figures 6 and 7 $HPO_4^=$ was calculated using the Henderson-Hasselbalch equation assuming a pK′ for phosphate of 6.8.

Only a small portion of the total increase is mediated by a decrease in base binding of plasma proteins (35) as consequence of the pH fall (5 per cent) and by decrease of the fixed acid anion lactate (6 per cent). As can be seen, the major portion of buffer anion increase is made possible by fixed acid chloride leaving the extracellular fluid (29 per cent) and base being transferred into it. The total cation increment is made up of sodium (37 per cent) and potassium (14 per cent). The fraction unaccounted for (9 per cent) probably represents changes in undetermined constituents, such as organic acids, calcium, and magnesium.

Figure 7 is a similar representation of changes observed in acute respiratory alkalosis. In this instance the total height of the column indicates the degree of buffer anion decrement in the extracellular fluid. Again, only a very small part is

[5] In the case of respiratory acidosis, induced by breathing a 20 per cent CO_2 mixture, the H_2CO_3 concentration of plasma would rise theoretically to about 5 mM per L. If $[BHCO_3]$ remained unchanged, then the normal ratio $[BHCO_3]/[H_2CO_3]$ of 20:1 would change to 4:1, resulting in a pH of 6.70. In most of our experiments, pH ranged about 6.88, indicating an alteration of this ratio to 6:1. Thus extrarenal compensation effected an increase in $[BHCO_3]$ of approximately 10 mM per L. of plasma. A similar analysis of the situation in acute respiratory alkalosis of the degree achieved in our experiments, where $[H_2CO_3]$ falls to approximately 0.2 mM per L., reveals that were no compensatory displacement of $[BHCO_3]$ to occur, a pH of 8.1 would result. That the pH rises only to approximately 7.7 indicates that extrarenal compensation has permitted a fall of $[BHCO_3]$ of about 12 mM per L. plasma.

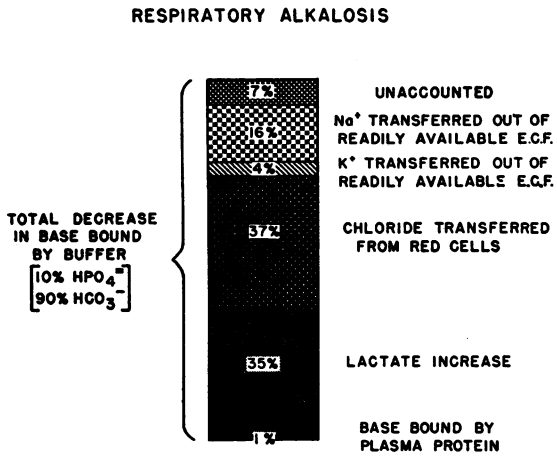

FIG. 7. THE MODE OF EXTRACELLULAR BUFFERING IN ACUTE RESPIRATORY ALKALOSIS

Data from five experiments are averaged (9 Alk–Ac, 11Ac–Alk, 12 Alk, 13 Alk–Ac, 14 Alk–Ac). The pCO_2 in these experiments uniformly fell to levels of 6 to 8 mm. Hg.

due to altered base binding of plasma proteinates (1 per cent). However, the increase in lactate markedly contributes to the displacement of buffer anion (35 per cent). Further displacement of buffer anion is effected by transfer of chloride into the extracellular fluid (37 per cent) and disappearance of sodium (16 per cent) and potassium (4 per cent) from it. The remaining undetermined 7 per cent may include changes in various other anions or cations, such as keto-acids and serum calcium (36).

In the following an attempt is made to analyze in somewhat more detail the pertinent changes in fixed acid and base which occur in the nephrectomized animal during respiratory acid-base disturbances.

Changes in blood *lactate* levels have been observed repeatedly in alkalosis of respiratory and metabolic origin (11, 37, 38). So far, the precise etiology of these alterations is not known, but several factors may be involved in its origin. Peters and Van Slyke (38) have suggested that in an alkaline medium, hemoglobin may deliver oxygen to the tissues less efficiently because of the shift to the left of its oxygen dissociation curve (39), thus favoring anaerobic glycolysis. Similarly, circulatory stasis, related to lowering of blood pressure (36) and peripheral vasoconstriction (40), both of which are known to occur in respiratory alkalosis, may lead to lack of available oxygen.

A somewhat different view is presented by Katzman, Villee, and Beecher (41). *In vitro* studies of tissue slices in an adequately oxygenated environment indicate that with bicarbonate concentration of the system kept constant, lactate production varies inversely with pCO_2. This may be interpreted to reflect a direct effect of carbon dioxide partial pressure on lactate metabolism. A further factor, suggested by several authors (42) has been increased epinephrine secretion. The markedly increased lactate levels observed in respiratory alkalosis are probably the result of a combination of the above factors, all of which favor anaerobic glycolysis. Our experiments, in which both alkalosis and acidosis were induced, indicate that these alterations in lactate metabolism are readily reversible.

Regarding the *chloride* changes observed, it has been found in animals with intact kidneys that total plasma anion concentration ($Cl + HCO_3$) is maintained in the presence of marked alterations of anion pattern (43). Thus, an inverse relationship between plasma chloride and bicarbonate has been observed in respiratory acid-base disturbances (44). Our experiments show that this condition holds in the arenal animal as well. Therefore, these changes must be due to transfers of chloride and bicarbonate across an extracellular boundary. Our experimental results indicate that the chloride shift across the red cell membrane, as classically described for whole blood by various authors (45, 46), appears of sufficient magnitude to account for all the chloride which leaves the extracellular fluid in respiratory acidosis, or conversely, enters it in respiratory alkalosis. In this manner the significant buffer capacity of the red cell, largely in the form of hemoglobin, is made available to the extracellular fluid. In some experiments, however, the chloride shift to or from the extracellular fluid seemed larger than could be accounted for by a red cell transfer alone. The possibility remains, therefore, that chloride shifts may occur across some other extracellular boundary. Our data certainly indicate that a significant fraction of exchangeable chloride does exist outside of the readily available extracellular space.

Alteration of extracellular *base* content was a regular finding in the studies reported. In respiratory acidosis a discharge of sodium and potassium into the readily available extracellular space occurred consistently. These moieties presumably were derived from cells or supportive structures. Our data indicate that the red cells are not responsible for the cation transfers observed. Conversely, extracellular cations must have entered cells or/and supportive structures in respiratory alkalosis. It seems likely that sodium and potassium were exchanged mole for mole for hydrogen ions in this process (10). The transfers of sodium occurred without alteration in the size of the exchangeable sodium pool as measured by radiosodium. The store of exchangeable bone sodium is potentially large (47). Evidence indicates that this reserve of base may be called upon in the maintenance of extracellular ion composition (48, 49) in acid-base disturbances. Under the acute conditions of our experiments, exchanges apparently represent redistribution of sodium without mobilization of previously non-exchangeable sodium. Balance studies conducted in human subjects by Singer, Elkinton, Barker, and Clark (50) (based on the chloride space), have revealed shifts of cell base in acute respiratory acidosis and alkalosis comparable to those reported here.

Significant changes in plasma inorganic *phosphate* levels were in accord with older observations of Haldane, Wigglesworth, and Woodrow (51) who found a significant rise in respiratory acidosis, and a fall in alkalosis. Similarly, Fitz, Alsberg, and Henderson (52) and Goto (5) showed earlier that the large intracellular stores of organic phosphate may be drawn upon in furnishing extracellular buffer anion. On the other hand, an increase in intraerythrocytic organic acid soluble phosphorus has been demonstrated in metabolic alkalosis (53). The etiology of these changes has been studied by Mackler and Guest (54). These authors observed that acidosis inhibits phosphorylation of glucose, both *in vitro* and *in vivo*. In this connection, the older finding of Lawaczeck (55), which showed that an increase of hydrogen ion concentration, induced by an increase in pCO_2, accelerates hydrolysis of red cell organic phosphates, may be pertinent.

Bicarbonate is the most important of the extracellular buffer anions; alterations of its content mirror changes in the reserve of physiologically available buffer alkali in the body (38). All our experiments reveal no significant further alteration of extracellular buffer content (largely bicarbonate) after the first hour. It therefore appears that in acute respiratory acidosis and alkalosis of the nature induced in these experiments, the major portion of extracellular buffering, and consequently the major portion of ion transfers, is achieved within this period of time.

SUMMARY AND CONCLUSIONS

1. In 29 experiments performed on nephrectomized dogs, acute respiratory acidosis and alkalosis were induced.

2. Volumes of distribution of radiosulfate, of sucrose, and of radiochloride did not change. From these findings, it is concluded that extracellular fluid volume remained unaltered.

3. Significant redistribution of electrolytes across the boundary of the readily available extracellular fluid volume was observed.

4. Tissue contribution to buffering within the extracellular fluid was found to be mediated by the following mechanisms:

a) Transfer of chloride across the erythrocyte membrane;
b) shift of sodium and potassium across an extracellular boundary other than the red cell membrane;
c) transfer of inorganic phosphate across an unknown extracellular boundary;
d) significant alterations in lactate metabolism.

ACKNOWLEDGMENTS

We would like to express our appreciation to Drs. H. D. Lauson and R. C. Swan for helpful advice.

APPENDIX

The following symbols and calculations were used to derive data on electrolyte content of extracellular fluid and red cells:

1) Water correction, when used, was calculated as:

$$W = 1 - \frac{P_s}{100}$$

where

W = plasma water correction factor

P_s = plasma proteins in grams per 100 ml. plasma

2) $C_W = \frac{C_P}{W}$

C_W = concentration in plasma water (mM per liter)

C_P = concentration in plasma (mM per liter)

3) $C_R = \frac{C_B - (1 - H)\, C_P}{H}$

where

C_R = concentration in red blood cells (mM per liter of cells)

C_B = concentration in whole blood (mM per liter whole blood)

H = Hematocrit, expressed as a decimal fraction. No correction was applied for trapped plasma.

4) $V_S = \frac{I_S}{C_0} \times W$

V_S = volume of distribution of sucrose (ml. H_2O)

I_S = amount of sucrose injected (mg.)

C_0 = plasma concentration of sucrose at zero time (mg. per ml.)

C_0 was obtained by extrapolating to zero time a straight line which could be fitted by eye to the values of the logarithm of successive plasma concentrations plotted against time.

5) The volume of distribution of radiosulfate was estimated by a slightly modified formula, suggested by Gamble and Robertson (56).

$$V_{S^{35}O_4} = W \cdot PV + \frac{W}{D}\left[\frac{I_{S^{35}O_4}}{C_0} - PV\right]$$

$V_{S^{35}O_4}$ = volume of distribution of $S^{35}O_4$ (ml. H_2O)

PV = plasma volume (ml. plasma)

D = Donnan factor for $S^{35}O_4$, taken as 1.1 (20)

$I_{S^{35}O_4}$ = amount of $S^{35}O_4$ injected (counts per min.)

C_0 = concentration of $S^{35}O_4$ at zero time, in counts per minute per ml. plasma. C_0 was obtained in a manner similar to that described for C_0 of sucrose.

6) $V_{Cl^{36}} = W \cdot PV + \frac{W}{D}\left[\frac{I_{Cl^{36}}}{C_t} - PV\right]$

Volume of distribution of Cl^{36} was determined by Formula *5*, save that D = 1.05, and C_t is taken as the concentration of Cl^{36} in counts per min. per ml. plasma at the hourly intervals.

7) Plasma volume was determined once in control periods and once every two to three hours thereafter.

$PV_C = PV \cdot W$

PV_C = volume of distribution of T-1824 (taken as estimate of plasma volume) in ml. H_2O

PV = plasma volume in ml. plasma

8) Total amounts of electrolytes in the readily available extracellular fluid (ECF) were obtained by use of data of plasma concentration, plasma volume, interstitial fluid volume, and appropriate Donnan and water correction factors. The following formula was used:

$$T_E = \frac{PV \cdot C_P}{1000} + [V_{S^{35}O_4} - PV_C]\frac{C_W \cdot D}{1000}$$

T_E = total mM in $V_{S^{35}O_4}$

D = Donnan factor, 0.95 for Na and K.
1.05 for Cl and HCO_3.

No Donnan factor was used for PO_4 and lactate.

9) Total amounts of electrolytes in the total red blood cell volume were calculated by use of data on red blood cell concentration, plasma volume, and hematocrit. No correction for excess plasma or excess red cells was applied because of the findings of Reeve, Gregersen, Allen, and Sear (30) that the ratio of total body hematocrit to peripheral hematocrit varied from 0.9 to 1.1 in dogs under different experimental conditions. The following formula was used to calculate total electrolytes in red cell volume:

$$T_C = \frac{C_R}{1000}\left[\frac{PV}{1 - H} - PV\right]$$

T_C = total red cell electrolytes in mM.

10) Base binding of plasma proteinate (BP_S in mEq. per L.) was estimated according to Singer and Hastings (35).

11) Exchangeable sodium and chloride were estimated in the usual way (57).

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