ELECTROLYTE EQUILIBRIA IN ERYTHROCYTES DURING DIABETIC ACIDOSIS^{1, 2}

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It has been suggested that changes in the electrolyte composition of the erythrocyte may parallel such changes in other body cells (1). The main objections to this theory have been that the red cell has not only a slower metabolic rate (2) but also an anion pattern that differs markedly from that of other cells (3). In the studies here reported, erythrocyte base and water were measured during diabetic acidosis and recovery, and an attempt made to interpret the observed data in terms of a logical and orderly sequence of events, both within the erythrocyte and within other body compartments. The shifts found in base and water were qualitatively similar to those demonstrated previously by other workers in total intracellular base and water, using balance techniques (4-6). Furthermore, in most instances they were quantitatively reconcilable with changes that have been demonstrated in the anion composition of the erythrocyte (7). This suggests that this readily available cell may serve as an indicator of intracellular base and water shifts in disease.

METHODS

Blood samples were drawn from patients admitted in severe diabetic acidosis before the institution of any therapy, at 6 and 12 hours after the beginning of therapy, and at 24-hour intervals thereafter until the patient's discharge from the hospital. Blood was collected in 20-ml. syringes containing 0.02 ml. of heparin ("Liquaemin," Hoffmann-LaRoche, sodium content 0.0035 mEq./20 ml. of blood). Measurements of water, sodium, potassium, and hematocrit were made on whole blood. Plasma was separated immediately for the determination of pH, water, sodium, potassium, and chloride.

Intake and urinary output of water, sodium, potassium and chloride were measured on all patients for the first 24 hours. The urinary excretion of these elements was measured throughout the patient's hospital stay.

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The water content of whole blood and plasma was determined by delivering 1 ml. into a tared bottle, weighing and drying to constant weight.

Chlorides were done by the method of Schales and Schales (8).

Sodium and potassium were measured photometrically by an internal standard flame photometer (9) built by the authors. Standards were compounded to approximate the levels of interfering ions encountered in the material being analyzed. Recovery experiments for both plasma and whole blood showed the instrument to have an accuracy of ± 1 per cent for sodium and ± 1.5 per cent for potassium. Plasma was analyzed without precipitation of protein. Whole blood was prepared for analysis by a modified Neumann wet-ash procedure (10).

Plasma pH was measured with a Beckman model G pH meter.

Hematocrit determinations were done in duplicate on carefully mixed blood. The tubes were spun for 45 minutes at 3000 RPM in a centrifuge with a head radius of 16 cm. They were respun for successive 15-minute periods until a constant value was obtained. Readings were made to the bottom of the buffy coat and a correction factor for trapped plasma of 0.98 applied (11, 12). Duplicate hematocrit determinations checked within 0.5 per cent.

Intracellular sodium, potassium and water were calculated by the formula (10):

$$C_c = \frac{C_b - C_p(1 - V)}{V}$$

in which

C_c = concentration in cells in mEq./L.

C_b = concentration in whole blood in mEq./L.

C_p = concentration in plasma in mEq./L.

V = corrected hematocrit expressed as a decimal.

Values for sodium and potassium per kilogram of water were calculated from the formula:

$$C/\text{Kg H}_2\text{O} = \frac{C_L \times 1000}{\text{Gm. H}_2\text{O}}$$

where

$C/\text{Kg H}_2\text{O}$ = concentration per kilogram of water

C_L = concentration per liter of substance

Gm. H_2O = grams of water per liter of substance.

RESULTS

Table I presents the mean results on 21 normal individuals whose blood was analyzed to test the

TABLE I
Plasma and red cell Na and K in 21 normal human subjects

	Sodium		Red cells		H ₂ O in Gm./L		HCT. Corr.	Potassium		Red cells	
	Plasma	Red cells	Plasma	Red cells	Plasma	Red cells		Plasma	Red cells	Plasma	Red cells
	mEq./L	mEq./Kg H ₂ O	mEq./L	mEq./Kg H ₂ O				mEq./L	mEq./Kg H ₂ O	mEq./L	mEq./Kg H ₂ O
Average	139.8	150.0	16.3	21.5	927	721	<i>r</i> = .98	4.0	4.31	104.5	146.0
S.D.	2.5	3.1	3.6	5.0	15.7	17.3	3.3			6.4	7.9

reproducibility of the methods employed; and to obtain a set of values for comparison with those obtained in diabetic acidosis and recovery.

Table II shows the results obtained in 9 patients during severe diabetic acidosis and for several days following recovery. Fifteen patients were studied but 6 are excluded from this report; one because of death four hours after admission, one because of inadequate blood samples, and four because of evidence of long-standing renal damage. In the 9 remaining patients, analyses of the urine and of the blood non-protein nitrogen on recovery from acidosis gave normal results. All these patients, as can be seen from Table II, presented data which, while differing quantitatively, showed uniform qualitative changes. The variations from patient to patient are undoubtedly the outcome of differences in the duration and cause of the acidosis in each case.

Table III shows the mean results of the determinations on the 9 patients on admission to the hospital in severe acidosis, following 6 to 12 hours of therapy, following 36 hours of therapy, and on discharge from the hospital. Values for 6 to 12 hours are averaged, since some patients showed maximum changes at 6 and others at 12 hours, as can be seen from Table II.

The changes in plasma electrolytes and water in these patients on admission to the hospital in severe diabetic acidosis were similar to those found by other investigators (4-6, 13-15) and were in keeping with a loss of water due to glucose diuresis and a parallel loss of fixed base. Plasma dehydration was demonstrated by a fall in plasma water from 930 to 910 Gm./L, while a normal concentration of sodium per kilogram of plasma water reflected the parallel loss of base. Since this base was excreted largely in combination with organic acids a relative excess of chloride would be expected in the plasma and this was found. The average chloride: sodium ratio in the 9 patients on

admission was 0.86 (Table II) in contrast with the normal ratio of 0.74. The mean concentration of potassium in the plasma water was slightly elevated, and was highest in those patients in whom there had been a decreasing urine output in the hours preceding admission. This suggests that high levels of serum potassium in untreated diabetic acidosis are due to failure of the kidney (embarrassed by low blood pressure and dehydration) to clear the large amounts of potassium being released from the cells.

During the first 12 hours of treatment all patients received large amounts of insulin and 0.85 per cent sodium chloride solution administered parenterally. The values for plasma water and sodium at 6 to 12 hours demonstrate the efficiency of the intravenous administration of salt solution in combating dehydration. Plasma water was normal at 12 hours, and despite the large amounts of fluid administered (up to 8 liters) no patient showed chemical or clinical evidence of overhydration. The mean plasma sodium value was slightly elevated at 12 hours. Two patients exhibited hypernatremia, which slowly subsided. One (E. G.) had an initial plasma sodium of 165 mEq./Kg H₂O; the other had a normal plasma sodium concentration initially which rose to 168 mEq./Kg H₂O during treatment. One undesirable effect of 0.85 per cent sodium chloride solution in the treatment of this condition was seen in the accentuation, in all cases, of the already existing hyperchloremia. It is not possible to say how much this perpetuated the acidosis. However, plasma pH and CO₂ had returned to normal in all cases by 36 hours (between 18 and 24 hours in most cases) after admission so that the acidotic effect, if any, was not of long duration in these patients with adequate renal function.

The concentration of plasma potassium decreased an average of 20 per cent in all patients in the first 12 hours of treatment. This has been de-

scribed by many investigators (6, 14-16) and does not correlate with changes in blood sugar, plasma bicarbonate or pH. Many explanations have been offered for this decrease, including dilution of extracellular fluid, intracellular shifts and urinary losses. Since no measurements of extracellular volume were made on these patients, no exact figures for total extracellular potassium are

TABLE II
Analytical data on nine patients during recovery from diabetic coma

Patient	Time from start of treatment	pH	CO ₂ mM/L	Blood sugar mgm. %	HCT. (corr.)	Plasma			H ₂ O Gm./Kg	RBC		
						Na mEq./Kg H ₂ O	K mEq./Kg H ₂ O	Cl mEq./Kg H ₂ O		Na mEq./Kg H ₂ O	K mEq./Kg H ₂ O	H ₂ O Gm./Kg
A. B. 65	0 hours	—	8	805	47.4	140	6.0	110	915	50.0	129	724
	6	—	20	400	38.6	145	3.8	125	937	13.0	108	692
	12	—	24	325	36.8	154	4.3	127	937	0	136	649
	36	—	—	82	39.1	140	4.6	114	929	13.0	119	738
	3 days	—	21	211	42.8	145	4.6	—	930	6.8	102	740
B. F. 19	2 hours*	7.25	6	292	52.9	156	3.8	128	861	20.5	123	735
	6	7.50	11	290	43.1	146	3.4	127	918	26.0	128	705
	12	7.49	17	275	42.7	152	2.8	135	922	9.0	126	719
	36	7.49	29	87	41.2	151	2.8	121	930	11.3	134	709
	4 days	7.40	23	143	40.1	148	3.4	107	927	7.5	145	691
E. G. 35	0 hours	7.02	8	515	57.9	165	4.4	141	910	—	—	—
	6	7.05	—	400	37.8	173	3.2	160	930	11.0	146	702
	12	7.20	17	—	33.5	178	3.1	166	931	2.6	143	690
	36	7.48	27	157	30.7	172	3.3	157	941	18.4	158	658
	60	7.45	—	—	31.6	157	3.7	137	937	11.5	131	690
	3 days	—	—	—	33.8	150	4.7	128	933	27.2	134	728
H. H. 21	5	7.40	21	257	33.3	147	4.5	131	932	27.9	140	709
	0 hours	7.15	7	417	51.9	149	6.1	129	902	4.3	128	729
	6	7.40	7	363	41.9	149	4.7	140	926	0	137	707
	12	7.42	11	183	41.0	143	3.6	137	936	13.6	122	720
	48	7.53	21	106	41.6	144	3.9	128	922	7.0	134	678
E. P. 18	3 days	7.42	—	—	40.0	144	4.2	129	927	21.3	122	737
	0 hours	—	4	1160	45.7	140	7.6	120	916	12.5	110	750
	6	—	5	750	39.3	158	3.2	144	926	20.1	118	736
	12	—	14	255	36.1	168	3.3	147	928	1.2	139	645
	36	—	23	280	33.5	158	3.3	128	932	9.4	136	699
R. P. 49	5 days	—	—	383	38.1	155	3.5	116	931	0	135	710
	9	—	22	190	38.3	150	4.3	120	925	14.7	132	742
	0 hours	7.00	8	383	48.2	142	5.2	127	911	26.2	116	730
	6	7.20	17	186	38.7	150	3.1	141	927	0	155	685
	20	7.36	20	230	40.2	141	3.9	127	935	4.0	142	649
M. P. 59	48	7.30	—	373	43.6	145	4.2	126	929	22.7	134	697
	6 days	7.41	21	—	40.6	150	4.4	127	925	22.5	144	703
	0 hours	—	10	475	48.5	141	6.0	—	916	25.0	145	707
	6	7.35	16	162	44.5	139	3.9	—	935	37.5	150	714
	12	—	22	30	43.1	149	3.9	—	927	27.0	147	720
B. R. 16	3 days	7.38	20	109	48.3	141	3.2	—	925	14.0	141	714
	0 hours	7.14	5	271	46.2	146	4.4	127	916	21.5	130	695
	6	7.25	13	122	33.6	148	2.4	138	944	13.9	123	643
	12	7.40	22	160	34.2	147	2.6	138	940	14.2	117	658
	5 days	—	29	264	33.9	149	2.8	116	930	21.0	148	666
J. R. 37	7	—	29	256	34.1	153	3.7	119	930	28.1	142	652
	11	7.36	27	90	37.2	152	4.4	122	932	30.0	139	697
	0 hours	6.80	6	350	46.4	153	5.0	141	905	11.5	123	748
	6	—	6	154	38.1	157	3.8	162	925	0	133	728
	12	7.30	10	154	38.5	154	2.8	145	930	14.7	127	727
J. R. 37	36	—	20	182	31.4	144	3.0	134	933	33.0	124	716
	3 days	—	23	104	32.9	149	2.6	129	929	—	130	736
	7	7.41	—	—	31.3	148	3.8	116	925	28.0	138	754

* This patient had received 60 units of insulin 2 hours before the first blood sample was drawn.

TABLE III
Plasma and red cell Na and K in 9 diabetic subjects

	Sodium				H ₂ O in Gm./L		Potassium			
	Plasma		Red cells		Plasma	Red cells	Plasma		Red cells	
	mEq./L	mEq./Kg H ₂ O	mEq./L	mEq./Kg H ₂ O			mEq./L	mEq./Kg H ₂ O	mEq./L	mEq./Kg H ₂ O
Admission	135	148	15.3	21	910	728	5.0	5.5	89	123
6 and 12 hours	144	154	6.5	9.3	930	689	3.2	3.4	91	130
36 hours	140	150	9.6	14.0	931	688	3.2	3.5	92	132
Discharge	138	148	14.4	19.8	928	712	3.8	4.1	96	134

available. However, assuming an extracellular volume of 30 per cent of body weight at 12 hours and estimating the initial extracellular volume by correcting the 12-hours volume for the plasma water change between admission and 12 hours, and multiplying each value by the appropriate potassium concentration, an approximation of the total extracellular potassium change may be obtained. Using this calculation and the average values from all 9 patients, the mean loss of extracellular potassium was 33 mM—approximately one-third of the initial total extracellular content of 93 mM—whereas the average negative balance of potassium was 35 mM during this 12-hour period. Thus the urine contained all the potassium lost from the extracellular space during this period of treatment.

This loss of potassium from the body at a time when there is a large demand for the ion due to the deposition of glycogen and protein in cells may be due to failure of the renal mechanism to change rapidly from a state in which large amounts of potassium must be excreted to one in which the ion must be conserved. On the other hand, in the absence of any specific metabolic dysfunction which disturbs this relationship, the plasma potassium concentration may be related to the total body content of this ion. Since these patients had a low total body potassium (4, 15), and since insulin had restored their metabolic processes towards normal, the kidney may have been excreting potassium during these hours to restore the normal potassium ratio between plasma and cells. In defense of this hypothesis was the slow and parallel rise in serum and erythrocyte potassium concentrations during the later phases of recovery.

No potassium was given to any of these patients parenterally, but fluids or foods high in potassium were given orally as soon as tolerated. This was possible in all cases between 12 and 18 hours after the start of therapy. No patient ex-

hibited signs or symptoms characteristic of hypokalemia at any time nor were electrocardiograms during recovery characteristic of this syndrome. Plasma potassium concentrations rose slowly until at discharge they approached the normal value of 4.3 mEq./Kg H₂O. Values for plasma water and sodium remained normal (with the exception of 2 cases of hypernatremia) from 12 hours after hospital admission until discharge.

On admission, prior to any therapy, the red cells had a mean deficit of potassium of 23 mEq./Kg of cell water, 16 per cent less than the normal. This deficit is comparable to that which occurred in the first patient reported by Atchley and his associates (4), a patient in whom 13 per cent of the estimated total body potassium was lost during the induction of experimental diabetic acidosis. However, the water content of the red cells was normal, despite loss of potassium, dehydration of the surrounding plasma, and the obviously dehydrated state of the patient. The mean cell sodium concentration appears normal in Table III but if one excludes a single value of 50 mEq./Kg H₂O, the mean was 16.1 mEq./Kg of cell water, or 20 per cent below the normal value, confirming the findings of Danowski and his co-workers (13).

Within the first 12 hours of treatment the red-cell potassium values increased from 123 to 130 mEq./Kg H₂O. However, this change could be entirely accounted for by the loss of water from the cells and did not indicate an intracellular shift of this ion since, despite the dehydration of the extracellular fluid, the mean red-cell water content decreased during this period from a normal of 728 to 689 Gm./L. Sodium values fell to an average of 55 per cent of the admission value, and in 6 of the 9 patients reached 0 at either 6 or 12 hours.

At 36 hours the potassium concentration had begun to rise slightly. However, the increase of

2 mEq./Kg H_2O which had occurred was far smaller than the potential exchange of 1.5–2 mEq. of potassium per liter of red cells per hour which has been demonstrated *in vitro* (2). Whether this slow accumulation of potassium in the erythrocyte is due to competition for the ion with other cells having a more active glycogen metabolism or whether it is related to the reaccumulation of water and phosphate in the cell (17) cannot be ascertained from the data on these patients. Red-cell water at 36 hours remained decreased. Red-cell sodium had increased and was returning to normal more rapidly than potassium.

At the time of the patient's discharge from the hospital, total red-cell potassium had increased considerably, although the increase in cell water, which had risen to a mean value of 712 Gm./L, masked much of this rise. Only two patients left the hospital with a normal concentration of red-cell potassium. There was a direct correlation between the level of potassium and the duration of the patient's hospital stay. Red-cell sodium,

on discharge, had almost reached the normal concentration of 21 mEq./Kg of cell water, having a mean value of 19.8 mEq./Kg H_2O .

DISCUSSION

The data in Table I show that there is a higher total base concentration in the red cells than in the plasma in keeping with the normal cation distribution between all cells and their surrounding media (3, 18). However, Table III shows that in diabetic acidosis and in the post-acidotic state this distribution is reversed and the total base concentration in the red cells is lower than in the surrounding plasma. This difference requires examination in light of the need for osmotic equality between cells and plasma, as well as the need for electroneutrality in each phase. Knowledge of the concentrations of anions as well as cations in the red cell is required. Since no measurements of erythrocyte anions were made on these patients, it was necessary to turn to the literature for these data in order to explain the changes observed.

FIG. 1

Figure 1 has been constructed according to the manner of Gamble (3) to demonstrate the electrolyte composition of the red cell water in the normal, the diabetic in acidosis, and the diabetic 36 hours after treatment for acidosis. In the left hand columns of each section the ions are plotted as millimols and the total osmolarity of the cell water is shown by superimposing total anions on total cations. In the right hand columns the ions are plotted in terms of milliequivalents to demonstrate electroneutrality. Concomitant values for plasma pH and osmolarity and cell pH are shown above each diagram. The values for cell sodium and potassium and plasma pH are from Tables I and III. The hemoglobin has been assumed to be 300 Gm./L of red cells with a molecular weight of 67,000. All other values are from the literature (3, 7, 13, 14, 19–28). While the application of such derived data, obtained in part from studies of normal cells, in conjunction with actual analytical data is open to criticism on obvious grounds, its use seems justified since it explains the changes observed in a manner consistent with the fundamental concepts of osmotic equality and electroneutrality.

It can be seen from these diagrams that electroneutrality is maintained at all times within the cell, despite the large changes in cell base. This is made possible by changes in valency and concentration among the anions, notably hemoglobin and phosphate, in response to changes in cell pH (7, 19, 23, 24). Osmotic equality obtains in the normal and the diabetic in acidosis. In the latter, the replacement of polyvalent anions by univalent chloride—a change caused by the reduction in cell pH—accounted for the normal total osmolarity of the cells and provided an explanation for the normal water content of the cells at this time. Thirty-six hours after the start of therapy both total cations and anions were reduced since neither potassium nor phosphate had yet reaccumulated in the cells, but, with the return of pH towards normal, chloride had left the cell. The total osmolarity of the cell water was then only 274 as compared with a plasma osmolarity of 302.

There is a definite mathematical relationship between this reduced osmolarity and the observed fall in erythrocyte water. Total osmolarity fell from 292 to 274 (6 per cent decrease) and water fell from 728 to 688 Gm./L (5.5 per cent de-

crease). However, to accept this value for cellular osmolarity we must assume either an unstable state or osmotic inequality between two contiguous phases of body water. The rapid diffusibility of water across cell membranes makes the existence of an unstable state unlikely, while the magnitude of the pressure implied by a difference of 26 milliosmols (500 mm. of mercury) makes a state of osmotic inequality equally unreasonable. It is more likely that knowledge of the osmotically active components of abnormal red cells is incomplete and that organic compounds exist within the cell at this time which account for the discrepancy observed. These compounds must be electrically neutral or equally divided between anions and cations if electroneutrality is to be preserved.

The low potassium values observed in the erythrocytes of these patients on admission were consistent with the available indirect evidence of low intracellular potassium (4–6). Balance data usually reveal a continuing small loss of potassium during the early hours of treatment (5, 6), but the data reported here indicate that, at least within the erythrocyte, there was a prompt cessation of potassium loss on the initiation of therapy. The subsequent slow rise in red cell potassium paralleled calculated rises in total intracellular potassium (4–6, 15).

Fewer data are available on the movements of sodium in and out of cells, and no clear concept of the nature or causes of these movements has yet been advanced. However, 7 of the 8 patients of Danowski and his associates (5), studied by balance technique, showed a marked loss of intracellular sodium during the 22 to 37 hour period following the beginning of therapy. The difference in time between this loss and our findings may be due to a delay in renal excretion, a difference in the time of discharge of sodium between the erythrocyte and other body cells, or the administration of glucose during the preceding period. Nine of the ten patients presented by Seldin and Tarail (6) showed negative intracellular balance of sodium during one or more periods of study and 8 presented this finding in the first 7 hours of treatment.

Correlation of shifts in total intracellular water with shifts observed in red cell water was also significant. Calculation of the balances of intracel-

lular water from the data of the two groups of investigators referred to above (5, 6)³ revealed that, in most instances, there was a transient period of loss of water from the total intracellular mass during the early hours of treatment. Six of Danowski's patients showed this at some time during the first 37 hours of treatment, and in the 10 patients of Seldin and Tarail all but one lost intracellular water at some time during the first 5 hours of treatment and the one remaining patient showed this by 17 hours.

This correlation between changes in total intracellular base and water and changes in these elements within the erythrocytes appears to be more than fortuitous, particularly in view of the different therapeutic measures employed in the three groups of patients. Our patients received only insulin and saline. Danowski's group received glucose and large amounts of potassium in addition to insulin and saline, and Seldin and Tarail's patients received insulin, saline, and small to moderate amounts of glucose.

SUMMARY

1. Values for plasma and erythrocyte sodium, potassium, and water in 9 patients during diabetic acidosis and recovery are reported. For comparison, values in 21 normal subjects are presented.

2. On admission in diabetic acidosis, plasma base concentration was normal and plasma water was decreased. Erythrocyte sodium and potassium concentrations were decreased while the water content was normal.

3. Following therapy with insulin and 0.85 per cent sodium chloride solution, the patients showed rapid clinical improvement with return of plasma pH, bicarbonate and water to normal. Undesirable side effects of therapy were occasional hypernatremia, the constant occurrence of transient hyperchloremia and hypokalemia persisting for several days.

4. With treatment, the erythrocytes lost no further potassium, but did lose both water and sodium. Sodium reaccumulated in the cells more rapidly than potassium or water. In most patients both cell water and potassium were lower than normal at the time of discharge from the hospital.

5. The mechanism of these changes is discussed.

6. There is a parallelism between water and base shifts in the erythrocytes and in the total cell mass during recovery from diabetic acidosis.

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³ (5) Calculated from the data of Tables I and III.

(6) Calculated from the data of Tables I, II and III.

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