Nature of the Renal Concentrating Defect in Sickle Cell Disease *

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Summary. Free water reabsorption ($T_{H_2O}^c$) measured during 10% mannitol diuresis and subsequently during 3% saline diuresis was compared in patients with sickle cell anemia and in normal subjects. During mannitol infusion, $T_{H_2O}^c$ progressively rose with increasing osmolar clearance (C_{osm}) and reached a maximal level in both groups studied. During hypertonic saline diuresis, $T_{H_2O}^c$ progressively rose in the normal subjects and exceeded the maximal levels attained during mannitol diuresis, with no evidence of a maximal $T_{H_2O}^c$ level appearing. In contrast, none of the saline curves significantly exceeded the mannitol curves in the sickle cell patients but tended to parallel the mannitol curves at comparable rates of solute clearance.

Since $T_{H_2O}^c$ is an index of both solute (sodium) transport from the loop of Henle and solute accumulation in the hypertonic medullary interstitium, tubular sodium handling was examined in both sickle cell patients and control subjects alike. No difference in the tubular transport of sodium could be demonstrated either under conditions of sodium loading or under conditions in which the tubular sodium load was low (water diuresis).

These data support the conclusion that the defect in urinary concentration in sickle cell patients is caused by a limitation in maintaining a high concentration of solute in the medullary interstitium, thus limiting the rate of $T_{H_2O}^c$ from the collecting duct.

Introduction

Understanding of the pathogenesis of sickle cell anemia has advanced rapidly in recent years with the development of hemoglobin electrophoresis and the identification of the genetic abnormality responsible for this disease. New knowledge concerning the sickling phenomenon and the

vagaries of the affected erythrocyte has paved the way for a better correlation of the protean clinical manifestations frequently observed.

The renal involvement in sickle cell anemia has interested investigators for a number of years because of the almost universal presence of a defect in urinary concentration, often without evidence of additional renal functional impairment. Furthermore, despite the inability to attain a maximally concentrated urine, $T_{H_2O}^c$ as measured during mannitol diuresis has been reported to be normal (1, 2).

Although pathological evidence of capillary blockage, vascular congestion, medullary scarring, and tubular damage has been observed (3-5), these morphologic changes are not uniformly pres-

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ent in kidneys of sickle cell patients. Moreover, the hyposthenuria in these patients is present almost from birth and persists throughout the life of the individual (6). A correlation between progression of the concentrating defect and advancing age has been noted (6), as well as the slow decline in renal function after the third decade of life, when all clearance values tend to become significantly diminished (7). Continuing vascular damage in the kidney probably plays an important role in the early decline of renal function, as well as in the more advanced changes seen in those patients manifesting evidence of overt renal disease [proteinuria (8), hematuria (9, 10), or papillary necrosis (4)]. However, this does not necessarily implicate vascular damage as the etiologic agent in the mechanism of the concentrating defect in children and young adults, which is consistently present despite otherwise normal renal function.

The present studies were designed to investigate the nature of the concentrating defect in sickle cell anemia. A comparison of $T^c_{H_2O}$ was made in patients with sickle cell anemia and in normal subjects during both mannitol and hypertonic saline diuresis. Whereas similar $T^c_{H_2O}$ curves were obtained during mannitol diuresis, hypertonic saline diuresis revealed marked differences in the capacity for free water reabsorption in the two groups studied as saline loading was progressively increased. Since $T^c_{H_2O}$ is a reflection of the rate of tubular sodium transport into the medullary interstitium, tubular sodium handling subsequently was examined under conditions of both high and low sodium loads.

Methods

Fifty-eight acute experiments were performed in 13 patients with sickle cell anemia (SS hemoglobin) and 14

FIG. 1. CHARACTERISTIC $T^c_{H_2O} : C_{osm}$ (FREE WATER REABSORPTION : OSMOLAR CLEARANCE) CURVES MEASURING FREE WATER REABSORPTION DURING 10% MANNITOL AND 3% SALINE DIURESIS. Note the divergence in the mannitol and the saline $T^c_{H_2O}$ curves in the normal subjects with increasing C_{osm} when compared to the "parallel" curves in the sickle cell patients.

TABLE I
Protocol of representative experiments during mannitol and saline diuresis*

10% Mannitol infusion					3% Saline infusion				
Time	V	UV	C _{osm}	T _{H₂O}	Time	V	UV	C _{osm}	T _{H₂O}
min	ml/min	mOsm/min	ml/min	ml/min	min	ml/min	mOsm/min	ml/min	ml/min
Hemoglobin AA									
0-16	1.1	889	3.0	1.9	0-14	0.4	481	1.6	1.2
16-26	4.7	2,340	8.1	3.4	14-24	0.7	678	2.3	1.6
26-36	7.3	3,416	11.7	4.4	24-35	0.8	763	2.6	1.8
36-46	9.0	3,968	13.5	4.5	35-45	0.9	853	2.9	2.0
46-56	13.1	5,425	18.4	5.3	45-55	1.1	1,023	3.5	2.4
56-66	16.1	6,239	21.0	4.9	55-65	1.5	1,364	4.5	3.0
66-76	20.9	7,643	25.5	4.6	65-75	1.5	1,207	4.0	2.5
					75-89	2.3	1,810	6.0	3.7
					89-99	3.6	2,537	8.3	4.7
					99-119	5.0	3,128	10.2	5.2
					119-129	7.3	4,172	13.5	6.2
					129-144	8.7	4,752	15.5	6.8
					144-160	11.2	5,835	18.8	7.6
					160-170	11.9	6,080	19.4	7.5
Hemoglobin SS									
0-15	1.3	581	2.0	0.7	0-10	2.6	1,158	3.9	1.3
15-25	6.6	2,794	9.6	3.0	10-21	3.7	1,664	5.5	1.8
25-35	7.9	3,337	11.2	3.3	21-30	5.7	2,490	8.2	2.5
35-45	9.3	3,843	12.9	3.6	30-40	8.5	3,618	11.9	3.4
45-55	9.8	4,033	13.5	3.7	40-50	11.1	4,577	14.9	3.8
55-65	11.3	4,591	15.4	4.1	50-65	13.5	5,396	17.5	4.0
65-81	13.2	5,261	17.4	4.2	65-75	16.3	6,394	20.6	4.3
81-91	16.2	6,257	20.7	4.5	75-86	17.2	6,738	21.5	4.3
91-102	18.3	6,971	22.8	4.5					
102-112	17.9	6,771	22.0	4.1					
112-122	18.8	7,170	23.1	4.3					

* Abbreviations: V = urine flow; UV = solute excretion; C_{osm} = osmolar clearance; T_{H₂O} = free water reabsorption. All values corrected to 1.73 m² BSA.

normal subjects in the Renal Metabolic Laboratory and the Sickie Cell Center at the University of Tennessee. There were 13 female and 14 male subjects ranging from 14 to 26 years of age. Glomerular filtration rates were assessed simultaneously in all subjects during each experiment by measuring plasma inulin clearance. After appropriate priming doses, a continuous infusion of inulin was administered at a constant rate to maintain adequate blood levels. No evidence of renal disease was present except the concentrating defect, which was found in all of the sickle cell group.

T_{H₂O} studies. All food and fluids were withheld for 12 to 15 hours before each test, and 0.5 U vasopressin was administered intramuscularly 30 minutes before the study. A solution containing 10% mannitol in 0.9% saline and aqueous vasopressin in a dosage of 30 mU per kg per hour was infused at a constant rate of 8 to 12 ml per minute with a Sigamotor infusion pump. Urine specimens were collected from the bladder at approximately 10-minute intervals through an indwelling catheter, and venous blood samples were withdrawn from the forearm through an indwelling needle at the midpoint of each collection period. The infusion was continued until a definite maximal T_{H₂O} level (T_{mH₂O}) was established.

In subsequent experiments a similar test was performed with 3% saline as the osmotic diuretic. The in-

fusion was administered at a rate of 8 to 14 ml per minute until the T_{H₂O} either reached a maximal level or surpassed that obtained during mannitol diuresis at comparable solute clearances. At least 2 days elapsed between consecutive studies on any individual subject.

Sodium transport studies. Subjects were studied under conditions of sodium loading after 15 hours of dehydration. After the priming dose of inulin had been administered, the sustaining solution containing inulin in 0.9% saline and 10% mannitol (10 studies) or in 0.9% saline alone (5 studies) was infused at a constant rate of 10 ml per minute. Urine specimens were collected every 10 to 20 minutes, and blood samples were withdrawn at the midpoint of each collection period.

Additional experiments were performed under conditions of low tubular sodium loads (water diuresis). Patients were hydrated by oral ingestion of water at the beginning of the study, and water was ingested throughout the experiment in amounts sufficient to maintain increasing urine flow rates. Urine and blood samples were collected as outlined above. Each experiment was continued until the urinary osmolality attained a minimal value and until solute-free water clearance (C_{H₂O}) of at least 13 ml per minute was established.

Chemical methods. Blood and urinary osmolalities were determined by the freezing point depression method

TABLE II
Summary of free water reabsorption studies in normal subjects and sickle cell patients during mannitol and saline diuresis*

Subject	Age	Sex	Weight	GFR†	U _{max}	Highest T _{H₂O}	Subject	Age	Sex	Weight	GFR†	U _{max}	Highest T _{H₂O}
	years		kg	ml/min	mOsm/kg	ml/min		years		kg	ml/min	mOsm/kg	ml/min
Hemoglobin AA							Hemoglobin SS						
10% Mannitol													
W.W.	15	M	58	111	647	4.9	W.R.	26	M	56	140	431	3.4
J.R.	17	M	73	115	772	6.2	N.P.	18	F	49	164	441	5.9
G.B.	16	M	59	129	840	5.1	L.B.	16	M	45	117	415	4.2
J.J.	14	F	58	113	961	5.9	P.S.	17	F	52	149	439	4.5
J.C.	15	M	57	137	1,060	8.1	L.R.	18	M	52	140	393	3.4
J.W.	21	F	61	106	774	4.5	B.W.	23	F	47	163	387	3.6
J.T.	19	F	58	76	816	5.4	G.D.	24	F	63	151	398	4.1
							O.H.	21	F	53	175	401	3.8
							E.H.	22	F	57	192	395	3.6
							W.P.	23	M	56	205	409	6.0
							I.W.	16	F	42	142	420	4.1
3% Saline													
W.W.	15	M	58	111	878	6.3	W.R.	26	M	56	140	428	3.1
J.R.	17	M	73	115	1,014	7.8	N.P.	18	F	49	164	472	5.4
G.B.	16	M	59	129	1,176	7.6	L.B.	16	M	45	117	450	4.2
J.J.	14	F	58	113	793	7.0	P.S.	17	F	52	149	450	4.3
J.C.	15	M	57	137	1,012	9.8	L.R.	18	M	52	140	388	2.3
R.J.	14	M	55		1,142	7.3	B.W.	23	F	47	163	386	3.7
L.P.	26	M	68	120	1,178	7.4	I.W.	16	F	42	142	429	3.9
E.M.	21	M	57		862	6.9	R.S.	25	M	50	126	424	4.5
J.H.	22	M	65		1,144	8.5							
P.T.	14	F	57	120	1,070	6.9							

* Abbreviations as in Table I. GFR = glomerular filtration rate; U_{max} = maximal urinary osmolality.

† Corrected to 1.73 m² BSA.

TABLE III
Effect of sodium loading on tubular sodium transport in normal subjects and sickle cell patients*

Time	V†	GFR†	FN _a †	UN _a V†	TN _a †	TN _a /FN _a
min	ml/min	ml/min	mEq/min	mEq/min	mEq/min	%
Hemoglobin AA						
0-20	2.4	175	23.57	0.25	23.32	98.9
20-30	5.0	136	18.24	0.38	17.86	97.9
30-40	5.9	125	16.25	0.45	15.80	97.2
40-50	7.0	136	17.69	0.54	17.15	96.9
50-60	9.2	142	18.28	0.71	17.57	96.1
60-70	10.5	137	17.49	0.84	16.65	95.2
70-80	12.2	128	16.08	0.99	15.09	93.8
80-90	14.8	134	17.02	1.23	15.79	92.8
90-100	18.3	140	17.81	1.59	16.22	91.1
100-110	20.8	133	16.65	1.90	14.75	88.6
Hemoglobin SS						
0-41	2.4			0.34		
41-61	5.3	217	28.90	0.52	28.38	98.2
61-71	8.8	166	22.02	0.70	21.32	96.8
71-81	11.3	169	21.97	0.84	21.13	96.1
81-91	12.8	168	22.08	0.94	21.14	95.7
91-101	15.2	184	24.01	1.10	22.91	95.5
101-111	16.8	168	21.77	1.28	20.49	94.1
111-121	19.0	174	22.23	1.43	20.80	93.6
121-131	19.8	166	21.25	1.46	19.79	93.1
131-141	20.6	165	21.34	1.50	19.84	93.0

* Abbreviations as in preceding Tables. FN_a = filtered sodium load; UN_aV = urinary sodium excretion; TN_a = tubular sodium reabsorption; TN_a/FN_a = fraction of the filtered load of sodium reabsorbed. All experiments contained 10% mannitol to permit observations of tubular sodium transport at varying rates of solute excretion. Since mannitol may limit tubular sodium reabsorption, additional experiments with infusion of 0.9% saline alone were performed, which revealed no difference in sodium transport in the two groups studied.

† Corrected to 1.73 m² BSA.

During 3% saline diuresis in the normal group, $T_{H_2O} : C_{osm}$ curves initially rose in a similar manner to the mannitol curves but exceeded the maximal levels attained during mannitol diuresis as C_{osm} increased from 13 to 20 ml per minute. At higher rates of C_{osm} , the saline $T_{H_2O} : C_{osm}$ curves continued to rise above the mannitol curves with no evidence of a maximal T_{H_2O} level appearing. The saline curves in the patients with SS hemoglobin, however, closely followed their mannitol curves, both in configuration and maximal T_{H_2O} levels. None of the saline curves exceeded their mannitol curves significantly, even at C_{osm} values as high as 32 ml per minute, but tended to parallel

T_{C_{H2O}} studies. The characteristic $T_{C_{H_2O}}:C_{osm}$ curves during 10% mannitol and 3% saline diuresis during studies in each group of patients are shown in Figure 1. The protocols of representative experiments are outlined in Table I, and the results of all such studies are summarized in Table II. During mannitol diuresis in both normal subjects and in sickle cell patients a progressive rise

TABLE IV
Summary of individual studies on tubular sodium transport under conditions
of sodium loading and water diuresis*

Subject	FN _a †	Slope UN _a V vs. V‡	TN _a †	TN _a /FN _a †	Subject	FN _a †	Slope UN _a V vs. V‡	TN _a †	TN _a /FN _a †
	<i>mEq/min</i>		<i>mEq/min</i>	%		<i>mEq/min</i>		<i>mEq/min</i>	%
Hemoglobin AA					Hemoglobin SS				
Sodium loading									
J.R.	14.00	65.13	13.12	93.8	W.P.	26.35	69.02	24.80	94.1
P.T.	12.00	66.90	10.73	89.4	L.R.	16.71	83.49	15.06	90.1
J.J.	12.96	58.30	12.08	93.2	B.W.	17.78	93.22	15.27	85.8
J.C.	16.89	88.37	15.46	91.6	D.H.	22.09	65.69	20.87	94.5
					G.D.	18.94	56.46	18.06	95.3
					N.P.	25.27	68.12	23.75	94.0
Mean	13.96	69.68	12.85	92.0		21.19	72.67	19.64	92.3
SD	2.11	13.00	2.00	1.9		4.02	13.31	4.19	3.7
p§						<0.05	>0.50	<0.05	>0.50
Water diuresis									
J.R.	16.07	7.81	15.78	98.2	W.P.	31.31	18.35	30.54	97.5
P.T.	16.55	5.64	16.15	97.6	L.R.	18.04	13.43	17.41	96.5
G.O.	17.09	11.24	16.72	97.8	B.W.	22.48	27.43	21.60	96.1
J.J.	18.79	9.39	18.28	97.3	O.H.	24.96	9.58	24.54	98.3
J.H.	19.54	10.97	19.40	99.3	G.D.	23.34	14.13	22.71	97.6
					N.P.	21.96	11.02	21.56	98.2
Mean	17.61	9.01	17.27	98.0		23.68	15.66	23.06	97.4
SD	1.49	2.33	1.53	0.8		4.39	6.36	4.35	0.9
p§						<0.05	>0.05	<0.05	>0.10

§ The p values pertain to the mean values in the control and in the sickle cell groups during 1) sodium loading and 2) water diuresis.

TABLE V

*Effect of water diuresis on tubular sodium transport in normal subjects and sickle cell patients**

Time	V†	GFR†	F _{Na} †	U _{Na} V†	T _{Na} †	T _{Na} /F _{Na}	C _{H₂O} †	C _{H₂O} /GFR	(GFR-V)/GFR	V/GFR	U _{osm}
min	ml/min	ml/min	mEq/min	mEq/min	mEq/min	%	ml/min	%	%	%	mOsm/kg
Hemoglobin AA											
0-20	2.7	116	16.04	0.24	15.80	98.5	0.1	0.1	97.7	2.3	271
20-40	8.3	127	18.02	0.33	17.69	98.2	4.7	3.7	93.5	6.5	122
40-60	13.5	127	17.15	0.28	16.87	98.4	10.6	8.3	89.4	10.6	60
60-80	16.3	120	16.07	0.34	15.73	97.9	13.1	10.9	86.4	13.6	56
80-90	16.7	136	18.11	0.36	17.75	98.0	13.4	9.8	87.7	12.3	55
90-110	17.6	125	16.75	0.41	16.34	97.6	14.0	11.2	85.9	14.1	57
110-130	20.0	131	17.38	0.51	16.87	97.1	15.7	11.9	84.7	15.3	60
Hemoglobin SS											
0-10	2.5			0.16			0.5				223
10-20	8.0	147	19.21	0.28	18.93	98.5	5.1	4.2	94.6	5.5	100
20-30	8.8	179	23.58	0.36	23.22	98.5	5.3	3.5	95.1	4.9	113
30-40	12.9	163	21.38	0.37	21.01	98.3	9.4	6.9	92.1	7.9	75
40-50	16.9	157	20.49	0.38	20.11	98.1	13.5	10.3	89.2	10.8	56
50-60	21.0	172	22.24	0.42	21.82	98.1	17.5	12.2	87.8	12.2	46
60-70	21.2	156	20.30	0.40	19.90	98.0	17.8	13.6	86.4	13.6	45
70-80	22.0	163	21.20	0.42	20.78	98.0	18.3	13.4	86.5	13.5	46
80-90	23.4	202	26.17	0.45	25.72	98.3	19.6	11.6	88.4	11.6	45

* C_{H₂O} = free water clearance; C_{H₂O}/GFR = fraction of the filtered sodium reabsorbed in the distal tubule; (GFR - V)/GFR = fraction of glomerular filtrate reabsorbed in the proximal tubule; V/GFR = fraction of glomerular filtrate excreted into the urine; U_{osm} = urinary osmolality. Other abbreviations as in preceding Tables.

† Corrected to 1.73 m² BSA.

the mannitol curves at comparable rates of solute clearance (Figure 1).

Sodium transport studies. The protocol of representative experiments during sodium loading is shown in Table III. The results of all experiments are summarized in Table IV. The filtered sodium load (F_{Na}) was significantly greater in the sickle cell patients because of the consistently higher glomerular filtration rate (GFR) ($p < 0.05$). As the anticipated rise in urine flow was observed, however, the increased sodium excretion (U_{Na}V) was approximately equal in the two groups. Because of the greater flow attained by sickle cell patients in some experiments, the slopes of the regression line of U_{Na}V vs. V were calculated for both groups (Table IV) and were found to be similar ($p > 0.50$). In addition, when the absolute tubular reabsorption of sodium (T_{Na}) was compared, it was consistently increased in the sickle cell patients because of the greater F_{Na} ($p < 0.05$), but when T_{Na} was factored by F_{Na}, the fraction of the filtered load of sodium reabsorbed (T_{Na}/F_{Na}) did not differ significantly from the controls ($p > 0.50$).

During the course of water diuresis (Tables IV and V) the GFR, and consequently F_{Na}, also were

appreciably greater in the sickle cell patients ($p < 0.05$); whereas, when U_{Na}V and the slopes of U_{Na}V vs. V were compared, the two groups were observed to be similar ($p > 0.05$). T_{Na} in the study group was invariably higher ($p < 0.05$),

FIG. 2. FRACTIONAL EXCRETION OF FREE WATER (C_{H₂O}/GFR) AT VARYING FRACTIONAL RATES OF URINE FLOW (V/GFR) IN NORMAL SUBJECTS AND SICKLE CELL PATIENTS DURING WATER DIURESIS. GFR = glomerular filtration rate.

but T_{Na}/F_{Na} revealed no difference in the fraction of filtered sodium reabsorbed ($p > 0.10$).

The ability to dilute the urine was compared during water diuresis as shown in Table V. Both the fraction of glomerular filtrate reabsorbed in the proximal portions of the nephron $[(GFR - V)/GFR]$ and the fraction of glomerular filtrate excreted into the urine (V/GFR) revealed similar values in sickle cell patients and control subjects. U_{osm} fell correspondingly in both groups, whereas C_{H_2O} progressively rose. The absolute values of C_{H_2O} became higher in the sickle cell group, but the fractional excretion of free water (C_{H_2O}/GFR) was approximately equal. When C_{H_2O}/GFR was plotted vs. V/GFR in order to compare the ability to generate free water at any given flow rate, no significant difference was found (Figure 2).

Discussion

Maximal free water reabsorption levels during loading with nonreabsorbable solute (mannitol) have been reported to be within normal limits in children with sickle cell anemia (1, 2). A normal $T^c_{m_{H_2O}}$ in the presence of a defect in urinary concentration is in contrast with the findings in most renal disease states, in which an inability to concentrate the urine is accompanied by an impairment in free water reabsorption.

During mannitol loading in normal hydropenic subjects increasing rates of C_{osm} are associated with a progressive rise in $T^c_{H_2O}$ until a maximal $T^c_{H_2O}$ is reached that levels off in plateau fashion or slowly declines (13). Since $T^c_{H_2O}$ reflects the passive movement of water from the collecting ducts into the hypertonic medullary interstitium, it is dependent on continuing solute accumulation in the medulla to maintain hypertonicity in the face of increasing water reabsorption. It has been shown that the osmotic effect of mannitol decreases tubular sodium concentration (14), which is thought to impose a limitation on tubular sodium transport from the ascending limb of the loop of Henle into the medulla (15). Accordingly, the collecting duct fluid will approach osmotic equilibrium with the medullary interstitium, resulting in a maximal $T^c_{H_2O}$ level.

During hypertonic saline diuresis, on the other hand, $T^c_{H_2O}$ normally continues to rise above the maximal level of mannitol diuresis (15). Recent

evidence indicates that during hypertonic saline infusion, the reabsorption of sodium along the loop of Henle increases proportionately to sodium load (16, 17). Since the sodium concentration in the early distal tubule remains hypotonic, no tubular transfer maximum for sodium in the loop is evident. With increasing rates of sodium reabsorption across the ascending limb, solute accumulation in the medulla is enhanced, thus establishing the conditions necessary for unlimited free water abstraction.

When $T^c_{H_2O}:C_{osm}$ curves during hypertonic saline diuresis in sickle cell patients are examined, the curves are seen to resemble closely those of mannitol diuresis and to reach maximal $T^c_{H_2O}$ levels in similar fashion at corresponding rates of solute clearance. Since $T^c_{H_2O}$ is an index of both solute (sodium) transport from the loop of Henle and its accumulation in the hypertonic medullary interstitium, the tubular sodium transport system was examined under conditions of high and low sodium loads.

During sodium loading, when the tubular sodium concentration was high, the filtered load of sodium in the sickle cell patients exceeded that in normal subjects (Tables III and IV), reflecting the higher GFR in the former group. Levitt, Hauser, Levy, and Polimeros, however, observed that lowering the GFR decreases sodium excretion to the same degree in both groups (18). Moreover, the present studies revealed no difference in the rate of sodium excretion, despite the difference in filtered load. Although the tubular reabsorption of sodium was increased in sickle cell patients during sodium loading, no difference in the fraction of the filtered load of sodium reabsorbed by the tubules occurred (Table IV). These observations provide strong evidence that tubular sodium excretion in sickle cell patients is normal during sodium loading. Although a defect in loop sodium transport cannot be excluded completely by these studies, it would be necessary to postulate increased sodium reabsorption in the distal tubule to account for comparable rates of sodium excretion. Such a conclusion does not seem necessary or warranted, since $T^c_{m_{H_2O}}$ is usually normal in young sickle cell patients during mannitol diuresis, implying no difference in sodium reabsorption in the loop of Henle.

When the sodium transport system was exam-

ined under conditions in which the tubular sodium load was low (water diuresis), similar results were obtained as with sodium loading (Tables IV and V). Despite an increase in filtered load in the sickle cell patients, the rate of sodium excretion revealed no significant difference. Similarly, the tubular reabsorption of sodium was greater in the study group, but the difference in the fraction of the filtered load of sodium reabsorbed was negligible. U_{osm} fell to correspondingly low levels in all patients studied as water diuresis progressed, indicating a normal ability to dilute the urine in sickle cell anemia, as reported previously (19, 20). Comparison in the two groups of both the percentage of glomerular filtrate reabsorbed in the proximal portions of the nephron and the percentage of glomerular filtrate excreted into the urine revealed no difference in the pattern of water excretion during water diuresis (Table V).¹

Additional evidence for normal tubular sodium transport was obtained when C_{H_2O} was examined. Since C_{H_2O} is dependent on active sodium transport from the ascending limb of Henle's loop and distal tubule without an equivalent movement of water (23), it is evident that any defect in sodium transport at these sites would impair C_{H_2O} . In this regard, C_{H_2O} reflects ascending limb and distal sodium transport. Similarly, C_{H_2O}/GFR directly expresses the fractional excretion of free water and indirectly reflects the fraction of the filtered sodium load reabsorbed in the distal tubules. When these parameters were compared, absolute values of C_{H_2O} were seen to be greater in the sickle cell patients because of the higher rate of V , but C_{H_2O}/GFR was quite similar to that of the control subjects (Table V).

To compare further free water generation we plotted C_{H_2O}/GFR vs. V/GFR , since V more accurately reflects the rate of delivery of glomerular filtrate to diluting sites than C_{osm} during water diuresis, when the distal tubule and collecting duct are relatively impermeable to water. The re-

sults demonstrated that C_{H_2O} was formed equally at corresponding flow rates in the two groups studied (Figure 2).

The renal concentrating defect has been attributed to renal damage due to intravascular sickling, stasis, and vascular occlusion (1, 6), which have been seen to occur in the kidney as well as many other organs of the body in sickle cell patients. In support of this concept Perillie and Epstein (24) have demonstrated increased sickling *in vitro* when erythrocytes containing S-hemoglobin are immersed in hyperosmotic saline solutions, suggesting that the milieu of the renal medulla enhances sickling, which in turn decreases medullary blood flow. No increase in sickling was noted below an osmolality of 600 mOsm per kg, however, with a maximal increase being reached at 600 to 1,000 mOsm per kg. Since patients with SS hemoglobin are seldom able to concentrate their urine (U_{max}) over 600 mOsm per kg even in infancy and rarely over 500 mOsm per kg beyond the age of 7 years (6, 25) (Table II), and since the difference in osmolality between the final urine and the medullary interstitium is small to negligible except in water diuresis (26), it appears that hypertonic concentrations necessary to increase the sickling phenomena are infrequently attained *in vivo*. Although vascular damage undoubtedly plays an increasingly important role in sickle cell nephropathy with advancing age, it does not seem to explain adequately the renal concentrating defect that is present almost from birth, often without accompanying demonstrable vascular changes.

The medullary interstitium maintains its hypertonicity to plasma through a combination of loop solute (primarily sodium) transport into the medulla and the action of the vasa recta acting as countercurrent diffusion exchangers to minimize solute loss from the medulla. In addition, the removal of water by the vasa recta is essential if hypertonicity is to be maintained in the face of increasing water reabsorption from the descending limb of the loop of Henle and the collecting ducts. Since no defect in tubular sodium transport has been found in sickle cell patients, a limitation in maintaining a high concentration of solute in the medullary interstitium appears to be of primary importance in the mechanism responsible for the $T_{H_2O}:C_{osm}$ curves during hypertonic saline diure-

¹ Even though some back diffusion of water from the collecting duct is known to occur during water diuresis despite lack of antidiuretic hormone (21, 22), the relatively water impermeable distal tubular segments minimize the effect of medullary solute accumulation on the pattern of water excretion. Any alteration from the normal under these conditions would more likely reflect abnormal sodium transport.

sis as well as the concentrating defect in sickle cell anemia.

The reason for this limitation on medullary solute concentration must remain speculative at this time. Indirect evidence suggests a possible increase, rather than a decrease, in medullary blood flow, which has been shown to produce a loss in renal concentrating ability, presumably through a decrease in medullary solute concentration (27). Large juxtamedullary glomeruli with dilatation of both the afferent and efferent arterioles have been described in the kidneys of children with sickle cell anemia (3, 28). Furthermore, Etteldorf, Smith, Tuttle, and Diggs have shown that both the ratios of effective renal plasma flow/maximal transport of *p*-aminohippurate (Tm_{PAH}) and of effective renal blood flow/ Tm_{PAH} as measurements of tubular perfusion by plasma are supranormal in children with sickle cell anemia, indicating increased vascularity or vasodilatation or both (7). Recent hemodynamic evidence from our laboratory, showing the extraction of PAH to be normal or decreased and the noncortical plasma flow to be normal or increased in young sickle cell patients, offers additional support to the concept of increased medullary blood flow (29).

A second factor that probably influences medullary solute accumulation pertains to the vascular changes that begin in early childhood. The medullary capillaries become dilated and tortuous (3), and their spatial configuration becomes anatomically distorted. The effect of these changes on renal concentration may result in a less efficient countercurrent exchange system, which could then fail to prevent solute loss from the medullary interstitium. These changes advance with age and become increasingly important in the early decline of renal function.

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