RENAL CONCENTRATING MECHANISMS IN NEWBORN INFANTS. EFFECT OF DIETARY PROTEIN AND WATER CONTENT, ROLE OF UREA, AND RESPONSIVENESS TO ANTI-DIURETIC HORMONE *

By C. M. EDELMANN, Jr.,† H. L. BARNETT and V. TROUPKOU

(From the Department of Pediatrics, Bronx Municipal Hospital Center-Albert Einstein College of Medicine, Yeshiva University, New York, N. Y.)

(Submitted for publication January 20, 1960; accepted March 17, 1960)

Measurements of the concentration of osmotically active solutes in the urine of dehydrated infants have demonstrated a gradual increase from birth through the first weeks of life, with adult levels being attained only after several months (1–13).

The minimal response noted in infants to administration of vasopressin has led many observers (2, 5, 8, 11, 12) to the conclusion that due to immaturity the renal tubules are relatively insensitive to antidiuretic hormone (ADH). However, failure to observe a decrease in rate of urine flow or an increase in urine osmolality in response to vasopressin cannot be interpreted simply as resistance to the hormone per se, since other aspects of the concentrating mechanism, quite distinct from the role of ADH (14–16), must also be taken into consideration. These include the concentration of sodium in renal tissue along an osmotic gradient from cortex to medulla by active sodium transport in Henle's loop, and maintenance of this gradient, despite the medullary blood flow, through the orientation of the postglomerular capillaries which perform as countercurrent exchangers.

The length of the loop of Henle in its function as a countercurrent multiplier is of considerable importance. The length of this segment in various species correlates well with maximal concentrating performance. It is short in early infancy and increases in length with advancing age (17).

The special role of urea in the concentrating process has been emphasized by Berliner, Levinsky, Davidson and Eden (14) and by Schmidt-Nielsen and O'Dell (18, 19). Protein or urea added to the diet of adults has been shown by Epstein, Kleeman, Pursel and Hendrikx (20) to produce a significant increase in maximal urine osmolality at low rates of urine flow during hydropenia and in the maximal rate of reabsorption of free water ($Tm^{c}_{H_2O}$) during mannitol diuresis. In rats fed urea, Crawford, Doyle and Probst (21) have demonstrated production of smaller daily urine volumes and higher concentrations of both urea and nonurea solutes than in rats treated similarly but not fed urea.

In observations in adults, Epstein and associates (22) and De Wardener and Herxheimer (23) have shown that the response to dehydration and vasopressin administration, as measured by maximal urine osmolality, decreases markedly following several days of forced drinking.

In the present investigation the effects of various levels of intake of protein, urea, sodium chloride, and water on the concentrating mechanism of young full-term and prematurely born infants were examined.

METHODS

Observations were made on full-term and prematurely born infants whose ages and weights are indicated in Table I.

Dietary loading. Paired observations, separated generally by 5 to 10 days, were made on each infant. For 3 to 7 days prior to each observation the infants were maintained on isocaloric diets, as specified in Table I.

In Group I, the diet was varied to provide a high protein intake in a low volume prior to the first observation, and a low protein intake in a high volume prior to the second. The diet of Group 2 was of constant volume but provided a high intake of protein prior to the first observation and a low intake prior to the second. Two infants (Group 2A) were observed while on uniform diets except for the addition of a daily supplement of urea or sodium chloride prior to the first observation.

* Presented in part at the Annual Meeting of the American Physiological Society, Atlantic City, N. J., April 16, 1959. Supported in part by research grants from the Kidney Disease Foundation of New York, and the Sylvan League, Inc.

† Postdoctoral Fellow, National Heart Institute (HTS 5267), Bethesda, Md.

TABLE I

Concentrating performance at low and high rates of urine flow

Subject	Birth wt.	Age	Weight	Daily dietary intake: Protein	Daily dietary intake: Volume	Daily dietary intake: Supplementary urea or NaCl	Observations during unmodified hydropenia: Serum P_{osm}*	Serum: Urea N	Urine: V	Urine: U_{osm} Total	Urine: U_{osm} Urea	Observations during i.v. infusion: C_{In}	C_{PAH}	$T_m{}^c{}_{H_2O}$
	g	*days*	*g*	*g/kg*	*ml/kg*		*mOsm/kg*	*mg/100 ml*	*ml/min/1.73 m²*	*mOsm/kg*		*ml/min/1.73 m²*		
Group 1														
Gi ♀	1,740	29	2,100	8.3	106		318	17.5	0.540	940	303	60.4	194	4.4
		38	2,310	2.5	241		315	13.0	1.13	425	15	51.4	193	0.0
Be ♂	1,640	13	1,740	8.6	114		308	23.0	0.297	857	326			
		20	1,980	2.4	209		313	6.0	0.396	508	17			
As ♀	2,800	25	2,880	9.2	121		310	45.0	0.533	993	214	47.5		4.2
		32	3,170	2.2	213		350	9.0	0.516	690	56	60.4	177	2.0
St ♀	2,800	21	2,900	8.9	117		360	30.0	0.688	933	197	49.9	223	5.2
		28	3,200	2.2	205		319	8.5	0.373	661	71	53.2	248	3.3
Group 2														
He ♀	3,100	7	2,900	8.1	161		340	17.8	0.444	834	298	58.4	161	4.2
		14	2,960	2.2	150		302	9.8	0.250	622	97	59.2	117	2.8
Wh ♂	1,980	11	2,000	9.0	178		312	35.0	0.411	707	336	41.0	93	1.9
		21	2,160	2.4	164		310	8.0	0.197	560	71	33.9	104	1.2
Ca ♂	1,980	11	1,840	9.0	180		308	30.0		712	336			
		22	2,090	2.5	175		295	7.0	0.193	580	54			
Or ♂	3,000	16	2,980	9.1	181		318	19.5	0.564	827	338	44.1	202	
		23	3,180	2.4	167		308	10.0	0.748	690	120	46.5	189	
		25	3,960	8.0	159		325	30.0	0.534	1,139	484	56.6	174	4.4
Mo ♂	3,620	32	4,260	2.6	165		300	12.0	0.380	834	158			
		39	4,440	2.5	165							57.9	203	3.6
Group 2A														
Ri ♀	2,060	9	1,840	2.6	180	Urea, 2.21 g/kg	325	34.5	0.327	825	425	31.3	82	
		14	1,960	2.6	180	None	323	10.0	0.166	730	40	27.5	108	
Pe ♀	2,260	9	2,220	2.3	160	NaCl, 250 mg/kg	312	11.0	0.473	616	69			
		12	2,180	2.3	160	None	330	9.4	0.293	931	124			
Group 3														
Ha ♂	2,140	12	1,880	4.0	117		300	19.3	0.113	615	86			
		19	2,000	3.9	200		310	17.0	0.184	573	183			
Wr ♀	2,560	6	2,440	4.2	132		321	19.2	0.314	825	338	52.2	130	4.6
		13	2,540	3.8	226		290	12.0	0.357	745	223	53.8	197	2.7
Ma ♂	3,240	20	3,510	4.4	127		300	19.0	0.492	864	330			
		27	3,650	4.2	237		312	20.0	0.564	808	376			
Sc ♂	1,960	13	1,970	4.3	124		320	17.0	0.290	657	178	20.9	71	1.6
		20	1,970	3.8	215		297	13.8	0.476	527	191	28.3	88	1.0
Bo ♀	1,900	24	2,240	7.5	123		319	32.0	0.293	670	263	53.7	197	3.4
		17	1,980	7.5	214		340	34.0	0.355	595	296	54.0	186	2.3

* Several values for P_{osm} are unexpectedly high. Although these values generally were associated with elevated serum electrolyte concentrations, later observations following the same degree of water deprivation failed to yield similarly elevated values, suggesting a methodological error. However, assuming such error, only slight and insignificant differences would result in derived data and therefore the figures for serum osmolality were used as shown.

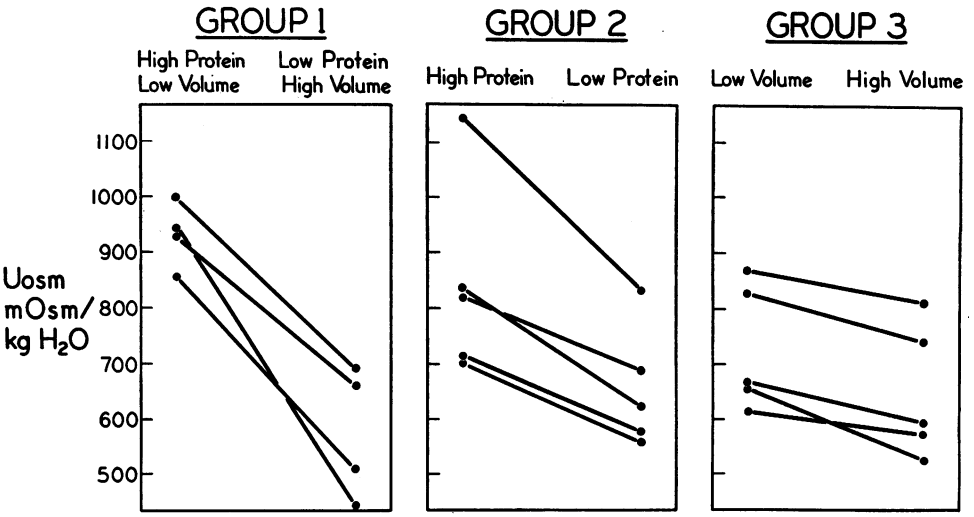

FIG. 1. EFFECT OF DIETARY PROTEIN AND WATER CONTENT ON MAXIMAL URINARY OSMOLALITY MEASURED DURING HYDROPENIA.

The diet of Group 3 was constant except for water content, providing a low volume prior to the first and high volume prior to the second observation.

The observations were arranged so that any increase in concentrating performance that might result from greater maturity with increasing age would be opposite to the effect expected from the particular diet. The infants were thirsted and fasted uniformly for 12 to 14 hours prior to each observation.

For the determination of maximal concentrating performance at low rates of urine flow, several timed urine specimens were obtained at the beginning of each observation, the infants being disturbed as little as possible. In order to obviate the introduction of a painful stimulus, which has been shown in infants to influence both glomerular filtration rate (GFR) and ADH release, vasopressin was not administered during the initial observation periods. However, it has been demonstrated repeatedly that vasopressin does not augment the maximal ADH effect resulting from dehydration. Following the initial collection periods, a majority of subjects was given a saline infusion and priming injection containing inulin, para-aminohippurate (PAH), and vasopressin. The priming dose of vasopressin was 50 mU per m^2, the sustaining dose 0.75 mU per minute per m^2. All fluids were infused at a constant rate of 10 to 12 ml per minute per 1.73 m^2. After equilibration and 2 to 4 additional collection periods, mannitol was added to the infusion in a concentration of 10 per cent and collections were continued until the rate of urine flow reached a plateau.

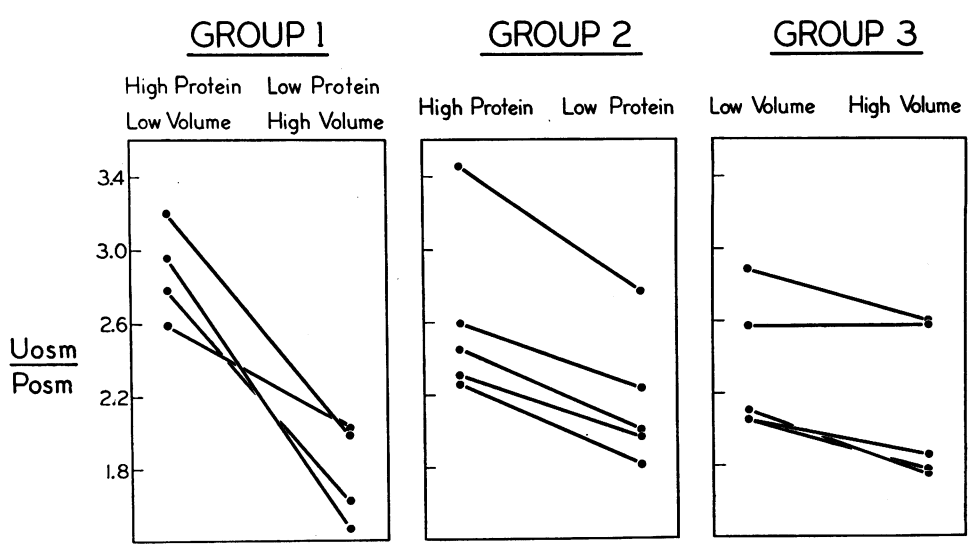

FIG. 2. EFFECT OF DIETARY PROTEIN AND WATER CONTENT ON MAXIMAL U/P OSMOLAL RATIO MEASURED DURING HYDROPENIA.

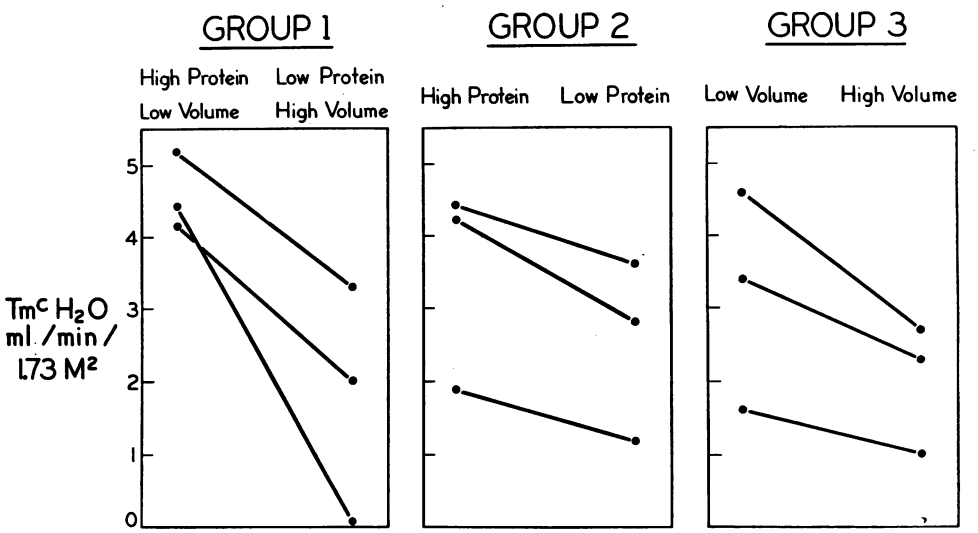

FIG. 3. EFFECT OF DIETARY PROTEIN AND WATER CONTENT ON $Tm^{c}_{H_2O}$ MEASURED DURING MANNITOL DIURESIS AND VASOPRESSIN ADMINISTRATION.

Acute loading. Several unpaired observations were carried out on infants maintained on a diet providing 2.3 to 2.6 g of protein per kg per day and 160 to 180 ml per kg per day. These observations likewise were preceded by a period of 12 to 14 hours of complete thirsting and fasting. After several hours of baseline observation, urea was administered through a stomach tube and additional urine samples were collected for 2 to 3 hours.

The osmolality of urine and serum was determined by measurement of the freezing point depression, using a Fiske osmometer with a semi-micro adapter. Urea nitrogen was determined by a modification of the methods of Friedman (24), Marsh, Fingerhut and Kirsch (25), and Natelson (26); inulin was determined by a modification of the method of Schreiner (27); and PAH by a modification of the method of Smith and co-workers (28).

RESULTS

Group 1: High protein, low volume diets and low protein, high volume diets (Figures 1, 2 and 3, Tables I-1 and II-1). Following water deprivation, significantly greater values for maximal urine osmolality, maximal urine-plasma (U/P) osmolal ratio, and $Tm^{c}_{H_2O}$ were observed in each infant following ingestion of the high protein, low volume

TABLE II

Statistical analysis of derived data

Group	Diet	Serum urea nitrogen	V	U_{osm}		$U_{osm}V$		C_{osm}	$T^{c}_{H_2O}$	$Tm^{c}_{H_2O}$
		mg/100 ml	*ml/min 1.73 m²*	Total	Urea	Total	Urea			
				mOsm/kg		*μOsm/min/1.73 m²*		*ml/min/1.73 m²*		
1	High protein, low volume	28.9	0.514	931	260	485	128	1.48	0.96	4.60
	Low protein, high volume	9.1	0.604	571	40	321	19	0.99	0.38	1.77
	p*	0.05	0.7	<0.01	0.02	0.1–0.2	<0.01	0.1	0.01	0.05–0.1
2	High protein	26.5	0.488	844	358	434	180	1.34	0.85	3.50
	Low protein	9.4	0.394	657	100	275	47	0.90	0.51	2.52
	p	0.01	0.4	<0.01	<0.001	0.10	0.01	0.1–0.2	0.05–0.1	0.05
3	Low volume	21.3	0.300	726	239	228	81	0.73	0.43	3.2
	High volume	21.4	0.387	650	254	258	104	0.84	0.45	2.0
	p	1.0	0.02–0.05	<0.01	0.7	0.02–0.05	0.10–0.20	0.05–0.1	0.5	0.05–0.1

* Student's *t* test for paired data (29).

diets. The concentration of blood urea nitrogen and the rates of excretion of osmotically active solutes were greater during observations following ingestion of the high protein diets, although the differences observed in rates of total solute excretion are of questionable significance.

Group 2: High protein and low protein diets (Figures 1, 2 and 3, Tables I-2 and II-2). Results in these infants were similar to those in the first group. Values for maximal urine osmolality, maximal U/P osmolal ratio, and $Tm^{c}_{H_2O}$ were consistently greater during hydropenia in infants given high protein feeding. In one 25 day old infant, the concentration of osmotically active urinary solutes was as high as 1,139 mOsm per kg. The decrease in maximal urine osmolality observed after low protein feeding paralleled the decrease in urine urea content.

Group 2A: Effect of adding urea or sodium chloride to the diet (Table I-2A). In one premature infant, the addition of urea to the diet, in an amount calculated to provide a rate of urea excretion comparable to that observed during high protein feeding, resulted in changes in concentrating performance similar to those observed following diets of high protein. As in the first and second group of infants, the rate of excretion of urea, the blood urea nitrogen concentration, and the urine urea nitrogen concentration were greater following ingestion of the diet with urea supplement.

The addition of sodium chloride to a basal diet in place of urea as a solute load was studied in one infant. Rates of excretion of total and nonurea solute were greater following sodium chloride supplementation, although the increases were slight and not as great as those after urea supplementation. However, maximal urine osmolality and U/P osmolal ratio were lower following ingestion of the diet with added salt.

Effect of acute urea loading (Figure 4). Administration of urea, in amounts ranging from 50 to 300 mg per kg body weight, resulted in three types of response; representative observations are diagrammed in Figure 4. Following small urea loads, no change was noted in urine volume or osmolality. In the majority of infants, striking and proportional increases in both rates of urine flow and rates of solute excretion were observed, but with no changes in the concentration of total urinary solute. Nevertheless, these infants did demonstrate an increase in the ratio of urea to nonurea solute. In several infants, increases in both rates of urine flow and of solute excretion were noted, accompanied by an increase in concentration of total urinary solutes. Increases in rates of solute excretion with urea loading were found to be the result primarily of increases in rates of urea excretion, since the increase in urine osmolality was almost exclusively in the urea fraction.

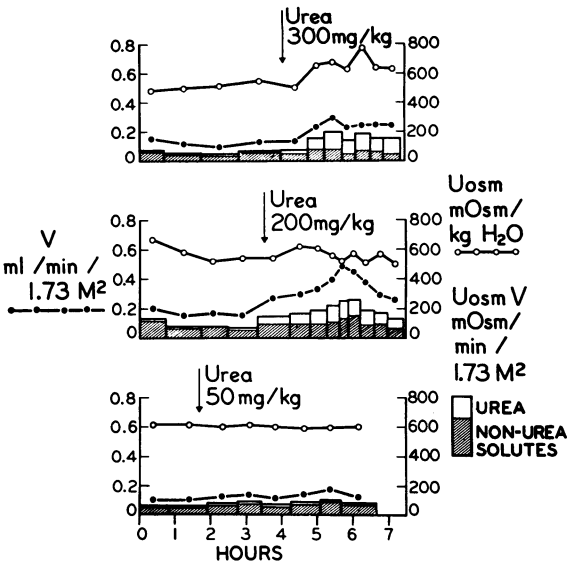

FIG. 4. EFFECT OF ACUTE UREA LOADING ON RATE OF URINE FLOW, RATES OF EXCRETION OF TOTAL, UREA, AND NONUREA SOLUTE, AND CONCENTRATION OF URINARY OSMOTICALLY ACTIVE SOLUTE.

Group 3: Low volume and high volume diets (Figures 1, 2 and 3, Tables I-3 and II-3). Values observed for maximal urine osmolality, maximal U/P osmolal ratio, and $Tm^{c}_{H_2O}$ were greater following feeding of low volume diets. In contrast with the other groups, these findings were accompanied by significantly lower rates of urine flow. The greater concentrations of osmotically active solutes in the urine were accompanied by decreased rates of total solute excretion and osmolal clearance, with no change in the rates of excretion of urea.

DISCUSSION

Current evidence suggests that the action of the antidiuretic hormone is to increase the permeability of certain segments of the renal tubule to

water and that urine hypertonic to plasma is produced by the abstraction of water from an isotonic precursor (14). During hydropenia, with minimal rates of urine flow, only very small volumes of osmotically free water must be reabsorbed at the concentrating site to produce urine several times hypertonic to plasma. Theoretically, reabsorption in the collecting tubule of only 2 ml of water per minute per 1.73 m^2, concomitant with a rate of urine flow of 0.5 ml per minute per 1.73 m^2, would permit formation of urine with a solute concentration of 1,500 mOsm per kg. Measurements in infants of the rate of reabsorption of osmotically free water during mannitol diuresis (Table I) yield values as high as 3 to 4 ml per minute per 1.73 m^2, indicating that in infants, as in adults, the factor limiting maximal urinary osmolality at low rates of urine flow is not the maximal rate at which tubular water can be reabsorbed. Thus it would appear that the failure of the young infant to achieve levels of urinary osmolality observed in the adult during prolonged dehydration is not due to inadequate permeability of the tubule to water in response to ADH. This suggests the possibility that differences may exist between infants and adults in the mechanisms responsible for the production of an area of hypertonicity around the collecting tubule.

The usual dietary intake of protein in infants is relatively high when compared with adults on the basis of body weight but is approximately the same when compared on the basis of surface area. It is not possible to establish a physiological standard of reference for such comparisons (30). However, independent of standards of reference, the infant excretes a considerably smaller percentage of the nitrogen of his diet than does the adult, due to a strongly anabolic state. In addition, in the very young infant the percentage of total nitrogen excreted as urea is lower than in the adult, due mainly to excretion of a larger proportion of nitrogen in the form of amino acids and ammonia (31, 32).

In the present observations, infants fed high protein diets demonstrated a consistently increased concentrating performance following water deprivation when compared with infants fed low protein diets. These findings in infants are in agreement with those reported in adults by Epstein and co-workers (20). In Group 2, values for P_{osm} were consistently greater following high protein intake, suggesting that the higher values for urinary osmolality observed may have resulted in part from a greater degree of dehydration or ADH release. As discussed earlier, however, it is believed that the experimental conditions resulted in all instances in maximal ADH release; analysis of body weight loss following dehydration indicated no differences with the various dietary regimens. Measurement of the osmotically active constituents of the urine, separated into urea and nonurea solute, showed that the decrease in urine osmolality from the first to the second observation was due primarily to a decrease in concentration of urea, resulting from a decrease in the rate of urea excretion, with no major change in concentration of nonurea solutes. The decrease in rate of urea excretion was due principally to the decreased concentration of blood urea nitrogen, rather than to changes in GFR or in rates of urea clearance, although decreases in the latter associated with lower rates of urine flow were observed in the second group of infants with low protein feeding.

Osmolal clearances were greater during the observations preceded by high protein feeding. Despite this presumably greater volume of isotonic fluid delivered to the concentrating site and the increased solute load, urine osmolality and urine U/P osmolal ratio were increased, suggesting (33) an enhanced rate of reabsorption of water in the collecting tubule ($T^c_{H_2O}$). Addition of urea to the diet, in place of protein, resulted in similar findings, indicating further that the increased concentrating performance observed following high protein feeding most likely resulted simply from the provision of additional urea for excretion.

When sodium chloride was added to the diet for several days as an osmotic load in place of increased protein or urea, the osmolal clearance was greater than that on the same diet without added salt. However, the maximal urinary osmolality and the U/P osmolal ratio in response to dehydration were decreased.

Thus the unique action of urea as a urinary solute, originally suggested by Gamble and associates (34, 35) and recently confirmed by Crawford and co-workers (21) and others (19, 20, 36–38) can be demonstrated in infants.

Epstein and co-workers (20) found in adults that acute urea loading had no effect on maximal urine osmolality or $Tm^{c}_{H_2O}$ and concluded that the effect of chronic administration of protein or urea was achieved by promoting an adaptive response by the renal tubules. In the present study similar results were obtained in infants given low or intermediate urea loads. However, these data are interpreted as showing that the effect of urea can be demonstrated not only after chronic administration but also with acute loading, since it would be expected *a priori* that the osmotic diuresis resulting from acute urea loading would be accompanied by a decrease in urinary osmolality (33), which did not occur. The demonstration in several infants of unchanged urine osmolality following urea loading, despite increased rates of solute excretion and urine flow, indicates an enhanced rate of reabsorption of water in the concentrating site. Furthermore, in three subjects an increase in urine osmolality following urea loading was observed, as recently reported in adults by Levinsky and Berliner (39), adding to the evidence supporting the unique role of urea.

The feeding schedule of the young infant, providing nourishment every 3 to 4 hours during the newborn period and allowing neither fasting nor thirsting for a period of more than 6 to 8 hours during the remainder of early infancy, contrasts markedly with the feeding schedule of the adult, in which complete thirsting and fasting commonly occur for 12 or more hours each day. The daily intake of fluid and the rate of turnover of body water is large in the infant when compared with the adult. In the present investigation the importance of fluid intake on the urinary concentrating performance in infants was demonstrated. Lower rates of urine flow and higher values for urinary osmolality were observed during dehydration following diets of low volume, similar to the findings previously reported in adults (22, 23).

Following ingestion of low volume diets, rates of osmolal clearance and of excretion of total osmotically active solutes, measured during hydropenia, were less than those after ingestion of high volume diets. Values for $T^{c}_{H_2O}$ were not different in infants previously fed low or high volume diets, suggesting that the smaller urine volumes noted after low volume diets might represent the net effect of reabsorption of a constant volume of osmotically free water from a smaller volume of isotonic precursor fluid in the collecting tubule.

SUMMARY AND CONCLUSIONS

1. Observations were made of the effects of various levels of intake of protein, urea, sodium chloride, and water on the concentrating mechanism of young full-term and prematurely born infants.

2. In terms of current concepts of renal concentrating mechanisms, the failure of the young infant consistently to produce urine as hypertonic to plasma as that seen in the adult appears to be explained best, not by differences in tubular permeability to water in response to antidiuretic hormone, but rather through differences in diet and metabolism of protein, and most likely through differences in other mechanisms involved in the production of an area of interstitial hypertonicity around the collecting tubule.

3. The young infant normally receives a diet high in protein content. However, by virtue of his strongly anabolic state, as well as the form utilized for urinary nitrogen, little urea is available for excretion. High protein intake, or supplementation of the diet with urea, provides a significantly increased amount of urea for excretion, and results during hydropenia in a greatly increased urinary osmolality and urine urea concentration.

4. The usual infant diet provides a large intake of water. Ingestion of a diet with low water content results in increased urinary osmolality in response to dehydration.

5. Values for net reabsorption of osmotically free water in infants approach those seen in adults, indicating no difference in the effect of antidiuretic hormone on the permeability of the collecting tubule to water.

REFERENCES

1. McCance, R. A., and Young, W. F. The secretion of urine by newborn infants. J. Physiol. (Lond.) 1941, **99**, 265.
2. Heller, H. The renal function of newborn infants. J. Physiol. (Lond.) 1944, **102**, 429.
3. Thomson, J. Observations on the urine of the newborn infant. Arch. Dis. Childh. 1944, **19**, 169.
4. Thomson, J. Urinalysis in dehydration fever. Arch. Dis. Childh. 1947, **22**, 226.

5. Barnett, H. L., Hare, K., McNamara, H., and Hare, R. Measurement of glomerular filtration rate in premature infants. J. clin. Invest. 1948, **27**, 691.
6. Pratt, E. L., Bienvenu, B., and Whyte, M. M. Concentration of urine solutes by young infants. Pediatrics 1948, **1**, 181.
7. McCance, R. A. Renal physiology in infancy. Amer. J. Med. 1950, **9**, 229.
8. Heller, H. The water metabolism of newborn infants and animals. Arch. Dis. Childh. 1951, **26**, 195.
9. Barnett, H. L., and Vesterdal, J. The physiologic and clinical significance of immaturity of kidney function in young infants. J. Pediat. 1953, **42**, 98.
10. Hansen, J. D. L., and Smith, C. A. Effects of withholding fluid in the immediate postnatal period. Pediatrics 1953, **12**, 99.
11. Ames, R. G. Urinary water excretion and neurohypophysial function in full term and premature infants shortly after birth. Pediatrics 1953, **12**, 272.
12. Calcagno, P. L., Rubin, M. I., and Weintraub, D. H. Studies on the renal concentrating and diluting mechanisms in the premature infant. J. clin. Invest. 1954, **33**, 91.
13. McCance, R. A., and Widdowson, E. M. Normal renal function in the first two days of life. Arch. Dis. Childh. 1954, **29**, 488.
14. Berliner, R. W., Levinsky, N. G., Davidson, D. G., and Eden, M. Dilution and concentration of the urine and the action of antidiuretic hormone. Amer. J. Med. 1958, **24**, 730.
15. Thorn, N. A. Mammalian antidiuretic hormone. Physiol. Rev. 1958, **38**, 169.
16. Lamdin, E. Mechanisms of urinary concentration and dilution. A. M. A. Arch. intern. Med. 1959, **103**, 644.
17. Peter, K. Studies of the Growth and Development of the Kidney. Jena, Gustav Fischer, 1927.
18. Schmidt-Nielsen, B. Urea excretion in mammals. Physiol. Rev. 1958, **38**, 139.
19. Schmidt-Nielsen, B., and O'Dell, R. Effect of diet on distribution of urea and electrolytes in kidneys of sheep. Amer. J. Physiol. 1959, **197**, 856.
20. Epstein, F. H., Kleeman, C. R., Pursel, S., and Hendrikx, A. The effect of feeding protein and urea on the renal concentrating process. J. clin. Invest. 1957, **36**, 635.
21. Crawford, J. D., Doyle, A. P., and Probst, J. H. Service of urea in renal water conservation. Amer. J. Physiol. 1959, **196**, 545.
22. Epstein, F. H., Kleeman, C. R., and Hendrikx, A. The influence of bodily hydration on the renal concentrating process. J. clin. Invest. 1957, **36**, 629.
23. De Wardener, H. E., and Herxheimer, A. The effect of a high water intake on the kidney's ability to concentrate the urine in man. J. Physiol. (Lond.) 1957, **139**, 42.
24. Friedman, H. S. Modification of the determination of urea by the diacetyl monoxime method. Analyt. Chem. 1953, **25**, 662.
25. Marsh, W. H., Fingerhut, B., and Kirsch, E. Determination of urea nitrogen with the diacetyl method and an automatic dialyzing apparatus. Amer. J. clin. Path. 1957, **28**, 681.
26. Natelson, S. Microtechniques of Clinical Chemistry for the Routine Laboratory. Springfield, Ill. Charles C Thomas, 1957, p. 381.
27. Schreiner, G. E. Determination of inulin by means of resorcinol. Proc. Soc. exp. Biol. (N. Y.) 1950, **74**, 117.
28. Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B., and Graber, M. The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. J. clin. Invest. 1945, **24**, 388.
29. Mainland, D. Elementary Medical Statistics; The Principles of Quantitative Medicine. Philadelphia, W. B. Saunders Company, 1952, p. 149.
30. Edelmann, C. M., Jr., and Barnett, H. L. Role of the kidney in water metabolism in young infants. Physiologic and clinical considerations. J. Pediat. 1960, **56**, 154.
31. Barlow, A., and McCance, R. A. The nitrogen partition in newborn infants' urine. Arch. Dis. Childh. 1949, **24**, 225.
32. Widdowson, E. M., Dickerson, J. W. T., and McCance, R. A. The excretion of urea, ammonia and purine end-products by the newborn animal. Biochem. J. 1958, **69**, 421.
33. Raisz, L. G., and Scheer, R. L. Studies on the renal concentrating mechanism. II. Effect of small acute changes in solute excretion. J. clin. Invest. 1959, **38**, 1.
34. Gamble, J. L., Putnam, M. C., and McKhann, C. F. The optimal water requirement in renal function. 1. Measurements of water drinking by rats according to increments of urea and of several salts in the food. Amer. J. Physiol. 1929, **88**, 571.
35. Gamble, J. L., McKhann, C. F., Butler, A. M., and Tuthill, E. An economy of water in renal function referable to urea. Amer. J. Physiol. 1934, **109**, 139.
36. Kellogg, R. H., and Koike, T. I. Difference between mannitol and urea diuresis in the rat (abstract). Amer. J. Physiol. 1955, **183**, 633.
37. Rubini, M. E., Blythe, W. B., and Meroney, W. H. Effect of the type of urinary solute on the process of urine concentration (abstract). J. clin. Invest. 1957, **36**, 926.
38. Hendrikx, A., and Epstein, F. H. Effect of feeding protein and urea on renal concentrating ability in the rat. Amer. J. Physiol. 1958, **195**, 539.
39. Levinsky, N. G., and Berliner, R. W. The role of urea in the urine concentrating mechanism. J. clin. Invest. 1959, **38**, 741.