STUDIES ON THE RENAL CONCENTRATING MECHANISM.
II. EFFECT OF SMALL ACUTE CHANGES IN SOLUTE EXCRETION *

By LAWRENCE G. RAISZ † AND ROBERT L. SCHEER

(From the Medical and Radioisotope Services, Veterans Administration Hospital, and Department of Medicine, State University of New York Upstate Medical Center, Syracuse, N. Y.)

The renal concentrating mechanism is considered to be limited by a maximal urine osmolal concentration or a maximal osmotic urine/plasma (U/P) ratio at low rates of solute excretion (1). When solute excretion is increased by the administration of large osmotic loads to hydropenic subjects urine osmolality decreases (2–4). This decrease has recently been ascribed to the limitation imposed by the existence of a maximal rate of net water reabsorption (termed $T_m^c H_2 O$) at high rates of solute excretion and urine flow (5–7). When no osmotic load is given, solute excretion is low in hydropenic subjects so that the net rate of water reabsorption is much below its maximum. Under these circumstances the existence of a constant maximal "osmotic ceiling" independent of small changes in solute excretion and urine flow has been postulated (1, 7), but such a limit has not yet been clearly demonstrated experimentally. The present study was therefore undertaken to assess the effects of small, acute changes in solute excretion on the osmotic U/P ratio observed in hydropenic normal men. Osmotic U/P ratio was not constant but tended to vary inversely with the concurrent rate of solute excretion.

METHODS

The subjects were four paid volunteer male medical students who were selected for their ability to void small volumes of urine so that catheterization could be avoided.[1]

On the day prior to the experiment subjects took no fluids after lunch and had a standard dry supper consisting of toast, meat, cheese, jelly, butter and cake. Experiments were begun at 6:00 to 7:00 a.m. the following morning. The subjects remained recumbent except to void or exercise. Urine samples were obtained at intervals of 30 minutes and blood samples approximately every two hours. The procedures described below were begun at 8:00 or 8:30 a.m. after three to four preliminary urine samples had been obtained. All experiments were performed from November to March except the urea series which was conducted in May. The following experiments were performed:

1. Resting state. Each subject remained recumbent for five to six hours.
2. Light exercise. Two subjects rode a stationary bicycle for one-half hour at 14 miles per hour against minimal resistance.
3. Venesection. In three subjects, 500 to 600 ml. of whole blood was removed in four to 20 minutes.
4. Mannitol infusion. Each subject received an infusion of 5 or 8 per cent mannitol in isotonic NaCl solution at 1 ml. per minute for two hours and at 2 ml. per minute for the next two hours.
5. Urea ingestion. Each subject ingested four doses of urea (25 Gm. per 100 ml. dissolved in orange juice) at intervals of one hour. The first two doses were 5 Gm. and the next two were 10 Gm. of urea.

Chemical methods. Creatinine was determined by the Jaffe reaction (8) using adsorption with Lloyd's reagent for the serum (9). Urea was determined by the method of Van Slyke and Cullen (10). Mannitol was determined by a chromotropic acid method (11). Sodium and potassium were determined with an internal standard flame photometer. Osmolality was measured with a Fiske freezing-point osmometer. Duplicate determinations of osmolality agreed within one per cent.

Calculations. Data on urine concentrating ability are presented in terms of the osmotic U/P ratio, and data on the mean in six periods. Subsequently, one subject showed considerable variation in creatinine clearances and his data were discarded. In 72 preliminary periods of 21 experiments on the four remaining subjects, the average deviation of the creatinine clearance from the day's mean was ± 6 per cent.

TABLE I

Summary of results of individual experiments

Experiment, subject, date	No. of periods	Range of values*						Values for regression equation†			
		V	U_{osm}	P_{osm}	Osmotic U/P ratio	C_{osm}	C_{cr}	a	b	r	p
		ml./min.	mOsm./Kg.	mOsm./Kg.		ml./min.	ml./min.				
Resting state											
G. C. 2/21/57	10	0.24–0.47	1,098–1,175	296–302	3.70–3.90	0.90–1.91	100–156	4.47	0.308	+0.554	>0.10
L. G. 11/24/56	10	0.51–1.07	1,038–1,223	299–304	3.40–3.99	2.09–3.60	127–145	4.59	0.473	−0.752	<0.02
R. L. 2/8/57	11	0.30–0.53	1,041–1,201	294–297	3.55–4.05	1.21–1.93	126–165			−0.732	<0.02
J. P. 11/26/56	11	0.32–0.48	1,287–1,345	301‡	4.20–4.37	1.60–2.04	131–158			+0.164	>0.10
Light exercise											
G. C. 11/17/56	8	0.34–0.82	903–1,096	288–292	3.23–3.79	1.25–2.64	102–161	4.17	0.373	−0.795	<0.02
R. L. 11/16/56	9	0.24–0.62	1,000–1,221	293–294	3.40–4.13	0.99–2.12	89–139	4.90	0.728	−0.889	<0.01
Average								4.53	0.551		
Venesection											
L. G. 12/18/56	9	0.25–0.51	1,248–1,383	297–304	4.13–4.65	1.13–2.16	120–163	5.06	0.393	−0.726	<0.05
R. L. 2/14/57	12	0.32–0.56	1,045–1,135	287–292	3.61–3.89	1.08–2.13	111–151	4.15	0.251	−0.810	<0.01
J. P. 2/12/57	10	0.28–0.59	1,214–1,313	296–303	4.05–4.40	1.67–2.30	117–153	4.92	0.361	−0.623	<0.10
Average								4.71	0.335		
Mannitol											
G. C. 12/14/56	12	0.35–1.33	880–1,097	296–302	2.94–3.63	1.26–3.95	120–156	3.94	0.236	−0.924	<0.001
L. G. 2/19/57	10	0.50–1.46	962–1,175	295–298	3.36–3.94	1.97–4.79	159–203	4.34	0.207	−0.986	<0.001
R. L. 12/15/56	11	0.29–1.31	864–1,113	290–296	2.96–3.81	1.08–3.85	109–156	4.07	0.287	−0.973	<0.001
J. P. 3/19/57	11	0.30–1.40	931–1,265	292–297	3.16–4.28	1.30–4.47	134–167	4.56	0.314	−0.956	<0.001
Average								4.23	0.261		
Urea											
G. C. 5/31/57	10	0.25–1.24	988–1,247	291–298	3.35–4.28	1.07–4.13	144–174	4.44	0.277	−0.918	<0.001
L. G. 5/30/57	9	0.35–1.27	1,075–1,359	292–303	3.56–4.65	1.63–4.88	179–225	5.09	0.304	−0.943	<0.001
R. L. 5/21/57	11	0.42–1.60	956–1,155	291–300	3.21–3.97	1.65–5.10	138–189	4.31	0.206	−0.970	<0.001
J. P. 5/29/57	11	0.39–1.71	898–1,221	296–306	2.95–4.13	1.62–5.10	132–178	4.52	0.307	−0.974	<0.001
Average								4.59	0.271		

* Abbreviations are as follows: V, urine flow; U_{osm}, urine osmolality; P_{osm}, plasma osmolality; U/P, urine/plasma; C_{osm}, osmolal clearance; and C_{cr}, creatinine clearance.

† The values given are for the constants (a and b), correlation coefficient (r) and probability (p) for the regression equation, $U/P = a - b\ C_{osm}$.

‡ No change.

solute excretion as the osmolal clearance (C_{osm}). The former expression is preferred to the use of urine osmolality alone since the data are thus corrected for variations in plasma osmolality. Osmolal clearance is calculated as: $C_{osm} = U_{osm} V/P_{osm}$, where U_{osm} and P_{osm} are urine and plasma osmolality and V is urine flow. This term is preferred to $U_{osm}V$ alone, not only because it corrects the data for variations in P_{osm} but also because it would measure the volume of the isotonic fluid which, according to recent evidence (12), is delivered from the distal tubules to a concentrating site in the collecting tubules.

The statistical methods used are described by Snedecor (13). The use of correlation coefficients may be questioned since the variables used contain common terms. While certain correlations might be due in whole or in part to such a common term, the negative correlation coefficients obtained in the present study do not depend on this term.

RESULTS

The results are summarized in Table I and illustrated for one subject in Figure 1. After 18 hours of water deprivation osmotic U/P ratios ranged from 3.63 to 4.65, urine flow was less than 0.5 ml. per minute and C_{osm} was 1 to 2 ml. per minute (except in Subject L.G. in whom initial values of C_{osm} were 1.6 to 3.3 ml. per minute). The U/P ratio and C_{osm} were fairly constant during the first one and one-half to two hours after waking, but subsequently a sustained increase in solute excretion was observed in the resting state in three of four experiments.
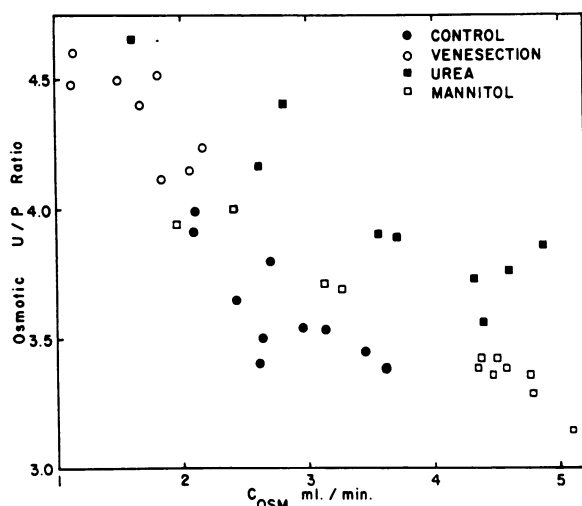

FIG. 1. OSMOTIC U/P RATIO VS. OSMOLAL CLEARANCE (C_{osm}) IN SUBJECT L. G.

Each point represents a half-hour urine collection period. The closed circles ("control") are for the resting state experiment.

After venesection, C_{osm} showed a distinct and persistent decrease in the experiment on Subject L.G., a small transient decrease in J.P., and an increase followed by a decrease in R.L., so that it was possible to observe the effects of a decreasing C_{osm}. Light exercise was followed by a sustained increase in C_{osm} in G.C. and by a transient increase in R.L. Mannitol and urea loading were used to increase C_{osm} in a predictable fashion (Figure 2). The range of C_{osm} for all data was 0.9 to 5.1 ml. per minute and the range of urine flow 0.24 to 1.71 ml. per minute. In most experiments, observations were made while C_{osm} was increasing and in this circumstance it was found that osmotic U/P ratio decreased. After venesection (and in one light exercise experiment) observations were obtained when C_{osm} was decreasing and in this circumstance osmotic U/P ratio increased. Such an inverse relationship between osmotic U/P ratio and C_{osm} was observed in each experiment except the studies on Subjects G.C. and J.P. in the resting state.[2] The linear regression equation, $U/P = a - b\ C_{osm}$, was calculated as a simple expression of this relationship. Table I gives the values of a and b, correlation coefficients (r), and probability (p) values for the linear regression equations of each experiment. The negative correlation coefficient between osmotic U/P ratio and C_{osm} was usually significant, although the constants a and b showed considerable variation. The value of the intercept a appeared chiefly to reflect variations in osmotic U/P ratio achieved at the beginning of the experiment. Variations in the slope b on the other hand are a measure of the effect of changes in solute excretion on osmotic U/P ratio. The values were between 0.206 and 0.473, except for the light exercise experiment in Subject R.L. in whom there was a more marked decrease in osmotic U/P ratio in association with a small increase in solute excretion ($b = 0.728$). This experiment may indicate that there is some impairment of the con-

---

[2] The lack of inverse correlation in Subject J.P. was not unexpected since changes were so small. The control data on G.C. are unexplained. The U/P ratio showed a small increase during the experiment which may have represented the effect of further dehydration, although in a previous study (14) 18 hours of water deprivation usually led to maximal osmotic U/P ratios.
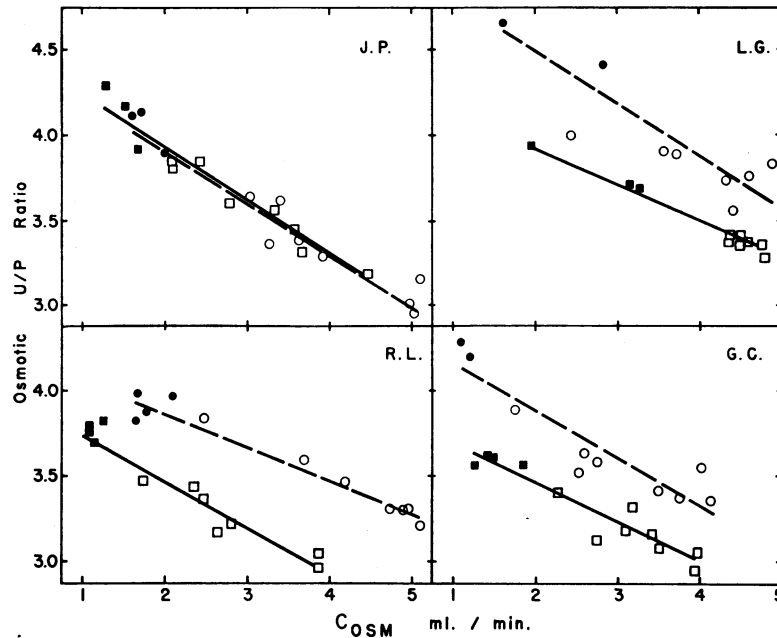
FIG. 2. OSMOTIC U/P vs. C_{osm} BEFORE AND AFTER THE ADMINISTRATION OF
UREA (CIRCLES) AND MANNITOL (SQUARES)

Closed symbols represent the preliminary periods before loading. The regression lines for mannitol (solid) and urea (dashed) are drawn between the lowest and highest value for C_{osm} obtained in that experiment.

centrating mechanism with light exercise such as is seen more strikingly after heavy exercise (15). The values for the slope b were higher in some of the resting state, light exercise and venesection experiments than after mannitol and urea loading. The former experiments usually included a lower range of C_{osm} than that achieved with mannitol and urea loads and also differed in that the changes in C_{osm} were chiefly due to changes in Na and K excretion. Since the values

TABLE II

Total and partial correlations (r) and their significance (p) between osmotic urine/plasma ratio (U/P), osmolal clearance (C_{osm}), and creatinine clearance (C_{cr}) for each subject and for the massed data

Subject, age; Surface area; No. of periods	Type of correlation	U/P vs. C_{osm}		U/P vs. C_{cr}		C_{osm} vs. C_{cr}	
		r	p	r	p	r	p
G. C., 28 1.92 40	Total	−0.736	<0.001	+0.142	n.s.	+0.330	<0.05
	Partial	−0.839	<0.001	+0.604	<0.001	+0.650	<0.001
L. G., 25 1.96 38	Total	−0.785	<0.001	−0.281	n.s.	+0.696	<0.001
	Partial	−0.855	<0.001	+0.595	<0.001	+0.799	<0.001
R. L., 24 1.88 54	Total	−0.743	<0.001	−0.315	<0.05	+0.667	<0.001
	Partial	−0.753	<0.001	+0.360	<0.01	+0.680	<0.001
J. P., 26 1.90 43	Total	−0.937	<0.001	−0.614	<0.001	+0.590	<0.001
	Partial	−0.903	<0.001	−0.216	n.s.	+0.054	n.s.
All data No. periods = 175	Total	−0.635	<0.001	−0.100	n.s.	+0.625	<0.001
	Partial	−0.737	<0.001	+0.493	<0.001	+0.731	<0.001

for C_{osm} were similar in the mannitol and urea experiments, direct comparison is possible (Figure 2). The slopes of the regression line are nearly identical in the two series although osmotic U/P ratios were higher in the urea experiments in three of four subjects. In these instances, higher osmotic U/P ratios were observed in the preliminary periods before loading was begun.

There was no striking effect of variations in creatinine clearance (C_{cr}) in the present studies. There was a positive correlation between C_{cr} and C_{osm} in each subject but there was no consistent pattern of total correlation between C_{cr} and osmotic U/P ratio (Table II). When the effect of C_{osm} on osmotic U/P ratio was eliminated by determining the coefficient of partial correlation,[3] a significant positive partial correlation between C_{cr} and U/P ratio was found in three subjects. In the fourth (J.P.) the total correlation was significantly negative and the partial correlation was not significant.

Changes in plasma osmolality were small and variable, except for the consistent increase after urea loading. There was no significant correlation between the changes in plasma osmolality and the changes in osmotic U/P ratio in any group of experiments.

DISCUSSION

The acute changes in osmotic U/P ratio observed in the present study are considered to be a characteristic of the intrinsic response of the renal concentrating mechanism to small changes in solute excretion and not due to influence of extrinsic factors which might alter concentrating ability. Such extrinsic factors include a change in the production of antidiuretic hormone (ADH), variations in the prior state of hydration and variations in the prior protein intake. There is little reason to suppose that ADH production should decrease in the present experiments since dehydration was not relieved. In previous studies (14) the administration of vasopressin did

[3] The coefficient of partial correlation is a convenient measure of the significance of association between two independent variables when one or more other known variables are held constant. Methods of correlation analysis rather than of multiple regression analysis were used since it could not be predetermined which of the variables was dependent.

not increase osmotic U/P ratio in subjects deprived of water for 18 hours; in fact, there was usually a decrease in osmotic U/P ratio after vasopressin, associated with an increase in solute excretion. Prior water loading or water deprivation (16–18) has been shown to influence the concentrating capacity despite the administration of "maximal" amounts of exogenous vasopressin. This observation has been taken to indicate that the concentrating mechanism can be influenced by the duration or degree of dehydration through the operation of some factor other than the amount of ADH immediately available. Such a factor might be directly related to the effective osmolality or inversely related to the volume of the body fluids. The present study did not test these possibilities, since most changes in osmotic U/P ratio occurred when both effective osmolality and extracellular fluid volume were nearly constant.

Although variations in protein or urea intake have been shown to alter concentrating ability (19) these changes were not observed in acute experiments. Small urea loads did not alter urine osmolality in the subjects of Epstein, Kleeman, Pursel and Hendrikx (19) who were on a low protein diet. It is possible that the decrease in U/P ratio which accompanies an increase in solute excretion was obscured by an effect of urea itself in subjects on a restricted protein intake. In the present study, U/P ratios were higher after urea loading than after mannitol loading in three of four experiments. However, this difference was associated with higher osmotic U/P ratios in the preliminary periods before the load was actually administered. Such variations in the osmotic U/P ratio after 18 hours of water deprivation have been previously observed (14) and cannot be related either to variations in solute excretion or plasma osmolality. The series of urea experiments was done in May after the other experiments of the present study had been completed so that adaptation or seasonal variation might have affected the response to 18 hours of water deprivation. With acute changes in solute excretion in an individual experiment an inverse relationship between osmotic U/P ratio and osmolal clearance was observed whether the change in solute excretion was chiefly of electrolytes, urea or mannitol. Thus the present data

confirm those previous studies (3, 4) which indicate that the concentrating mechanism operates independently of the contemporaneous pattern of solute reabsorption and excretion, presumably because the urine is concentrated at a site impermeable to solutes.

The decrease in osmotic U/P ratio as C_{osm} increases over the range of 1 to 5 ml. per minute has been presented as a linear regression because this expression was appropriate for most experiments. However, in some experiments (G.C., mannitol; L.G, control) the slope was somewhat steeper over a lower range of C_{osm}, suggesting a curvilinear relationship. Moreover when C_{osm} is increased progressively above 5 ml. per minute the relationship between osmotic U/P ratio and C_{osm} is clearly curvilinear (4) and U/P ratio appears to approach one as an asymptote. Attempts to extend the observations on the relationship between C_{osm} and osmotic U/P ratio to very low rates of solute excretion have thus far been unsuccessful. Such low rates can be achieved in chronic experiments by restricting dietary protein or in acute experiments after heavy exercise. Both procedures lead to lowering of the osmotic U/P ratio in hydropenic men (15, 19). This change is taken to indicate impairment of the concentrating mechanism rather than an effect of low solute excretion itself, since under these circumstances concentrating ability (as measured by net water reabsorption) is also impaired during osmotic diuresis.

The concept that the concentrating mechanism is limited by a maximum osmotic U/P ratio at low rates of solute excretion and a maximum net water reabsorption ($T_m^cH_2O$) at high rates of solute excretion and urine flow has been presented by Smith and co-workers (6, 7). This concept implies that there should be an appreciable range of solute excretion over which U/P ratio is constant. Boyarsky and Smith (7) reported that in their hospitalized patients (age, 24 to 74 years) who had no history of renal disease, the maximal osmotic U/P ratios after 12 to 14 hours of water deprivation were 2.2 to 3.4 and were constant up to an average urine flow of about 1 ml. per minute. There are several possible explanations for the differences between these data and the present observations. The shorter period of prior de-

hydration, the older age group, and possibly a poor prior protein intake may have led to the lower initial osmotic U/P ratios in Boyarsky and Smith's subjects. Moreover the conclusion that osmotic U/P ratio was constant at urine flows below 1 ml. per minute appears to be based on preliminary collection periods which show little variation in urine flow in most subjects.

The striking inverse relationship between osmotic U/P ratio and osmolal clearance obtained in the present study makes it difficult to assess the effect of other variables. While a positive partial correlation between osmotic U/P ratio and creatinine clearance was obtained in three of our subjects, the techniques used are too inaccurate to determine the effect of changes in glomerular filtration rate on concentrating ability. On the other hand, a marked decrease in glomerular filtration rate with heavy exercise (15) or constriction of the renal artery (20) is associated with definite impairment of concentrating ability.

SUMMARY

The effect of small changes in osmolal clearance on the osmotic urine/plasma ratio achieved after 18 hours of water deprivation has been studied in healthy men. Changes in osmolal clearance over the range of 0.9 to 5.1 ml. per minute and in urine flow over the range of 0.24 to 1.71 ml. per minute were observed in the resting state, after venesection, after light exercise and during mannitol and urea loading. The osmotic urine/plasma ratio was not constant. There was a significant inverse relationship between osmotic urine/plasma ratio and osmolal clearance at low rates of solute excretion and urine flow.

ACKNOWLEDGMENT

REFERENCES

1. Smith, H. W. Principles of Renal Physiology. New York, Oxford University Press, 1956, pp. 122 ff.
2. McCance, R. A. The excretion of urea, salts, and water during periods of hydropaenia in man. J. Physiol. (Lond.) 1945, 104, 196.

3. Hervey, G. R., McCance, R. A., and Taylor, R. G. O. Forced diuresis during hydropenia. Nature (Lond.) 1946, 157, 338.
4. Rapoport, S., Brodsky, W. A., West, C. D., and Mackler, B. Urinary flow and excretion of solutes during osmotic diuresis in hydropenic man. Amer. J. Physiol. 1949, 156, 433.
5. Wesson, L. G., Jr., and Anslow, W. P., Jr. Effect of osmotic and mercurial diuresis on simultaneous water diuresis. Amer. J. Physiol. 1952, 170, 255.
6. Zak, G. A., Brun, C., and Smith, H. W. The mechanism of formation of osmotically concentrated urine during the antidiuretic state. J. clin. Invest. 1954, 33, 1064.
7. Boyarsky, S., and Smith, H. W. Renal concentrating operation at low urine flows. J. Urol. (Baltimore) 1957, 78, 511.
8. Bonsnes, R. W., and Taussky, H. H. On colorimetric determination of creatinine by Jaffe reaction. J. biol. Chem. 1945, 158, 581.
9. Hare, R. S. Endogenous creatinine in serum and urine. Proc. Soc. exp. Biol. (N. Y.) 1950, 74, 148.
10. Van Slyke, D. D., and Cullen, G. E. The determination of urea by the urease method. J. biol. Chem. 1916, 24, 117.
11. MacFadyen, D. A. Estimation of formaldehyde in biological mixtures. J. biol. Chem. 1945, 158, 107.
12. Wirz, H. The location of antidiuretic action in the mammalian kidney in The Neurohypophysis, Proceedings of the Eighth Symposium of the Colston Research Society, H. Heller, Ed. New York, Academic Press, 1957, pp. 157 ff.
13. Snedecor, G. W. Statistical Methods, 5th ed. Ames, Iowa, Iowa State College Press, 1956, p. 534.
14. Raisz, L. G., McNeely, W. F., and Saxon, L. G. Studies on the renal concentrating mechanism. I. Effect of vasopressin. J. Lab. clin. Med. 1958, 58, 437.
15. Raisz, L. G., Au, W. Y. W., and Scheer, R. L. Studies on the renal concentrating mechanism. III. Effect of exercise. J. clin. Invest. 1959, 38, 000.
16. West, C. D., Traeger, J., and Kaplan, S. A. A comparison of the relative effectiveness of hydropenia and of Pitressin® in producing a concentrated urine. J. clin. Invest. 1955, 34, 887.
17. Jones, R. V. H., and de Wardener, H. E. Urine concentration after fluid deprivation or Pitressin Tannate in Oil. Brit. med. J. 1956, 1, 271.
18. 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.
19. 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.
20. 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.

SPECIAL NOTICE TO SUBSCRIBERS