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PERSISTENT 24-HOUR RENAL EXCRETORY RHYTHM ON A 12-HOUR CYCLE OF ACTIVITY

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Many investigations have defined the principal features of the diurnal rhythm of electrolyte and water excretion without contributing significantly to an understanding of its causes or mechanisms (Kleitman, 1939). Beyond its association with and apparent dependence upon the alternating rhythm of sleep and wakefulness, little is known of the renal or extrarenal influences which determine the rhythmic excretory change. Of the many concomitants of habitual sleep and wakefulness, change of posture can be eliminated as causing the renal changes, since assumption of recumbency during the daytime leads to increased water and electrolyte excretion (Wendt, 1876; White, Rosen, Fischer & Wood, 1926; Brun, Knudsen & Raaschou, 1945), whereas the recumbency of normal nocturnal sleep is associated with oliguria and diminished electrolyte excretion (Quincke, 1877; Laehr, 1890; Bazett, Thurlow, Crowell & Stewart, 1924; Simpson, 1924, 1926, 1929; Norn, 1929). It has also been shown that the excretory rhythm is not dependent upon periodicity of food intake, since it persists when identical meals are taken at regular intervals throughout the twenty-four hours (Borst & de Vries, 1950; and others).

Several attempts have been made to explain the diurnal excretory rhythm in terms of known renal mechanisms. Sirota, Baldwin & Villarreal (1950) showed that the glomerular filtration rate fell slightly during sleep, and Wesson, Anslow & Smith (1948) suggested that this might be responsible for the diurnal variations in sodium output. Neither of these groups of workers published any data on sodium excretion or on plasma sodium concentration. Borst & de Vries (1950), who measured neither filtration rate nor plasma electrolyte concentrations, concluded that the diurnal excretory rhythm reflected changes in renal tubular function secondary to a rhythm of adrenocortical activity. Stanbury & Thomson (1951) gave reasons for rejecting the

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last hypothesis, and it has also been found that severe cases of Addison's disease may exhibit a well-marked diurnal rhythm of excretion (O. Garrod, personal communication to S.W.S.). Stanbury & Thomson (1951) studied simultaneously the glomerular filtration rate, plasma electrolyte concentrations, changes in urine pH and changes in the excretion of Na^+, K^+, NH_4^+, Cl^-, HCO_3^- and phosphate. They found a close correlation between changes in urine pH and changes in electrolyte excretion; the diurnal rhythm of Na^+, K^+ and Cl^- excretion appeared to be intimately linked with a simultaneously occurring cycle of acid or alkali excretion. It was suggested that although small diurnal changes in glomerular filtration rate might contribute to the production of the rhythm, cyclic changes in tubular function must also take place. Reasons were given for believing that neither adrenocortical nor posterior pituitary hormones were concerned in the production of these cyclic changes.

Mills (1951*a*, *b*), who studied only the diurnal changes in urine flow, used the alternative approach of suspending the customary external periodicity of habit and was able to show that even after 6 days of inversion of the sleep rhythm, the urine flow maintained its original 24 hr rhythmicity. It seemed important to know whether the diurnal rhythm of electrolyte excretion was also resistant to change of habit; and whether, as suggested by Stanbury & Thomson (1951), the urinary flow rhythm was ascribable to the rhythm of electrolyte excretion. In the present investigation we have used the 12 hr routine described by Mills (1951*b*), measuring electrolyte excretion in addition to urine flow. We have also sought evidence of a 24 hr rhythmicity in alveolar carbon dioxide tension since reduction of alveolar carbon dioxide by overbreathing was the only way in which Stanbury & Thomson (1951, 1952) succeeded in simulating the matutinal behaviour of the kidney, and since an evening rise and matutinal fall of alveolar CO_2 tension, independent of sleep, has been recorded by Straub (1915), Bass & Herr (1922), Cohen & Dodds (1924), and Kroetz (1926). A preliminary account of these experiments has already appeared (Mills & Stanbury, 1951).

METHODS

Five subjects, aged 20, 20, 21, 25 and 36 years, with no history of renal disorder, spent 48 hr on a routine exactly repeated every 12 hr as follows:

3.00 – 4.10	prepare, eat, and clear up main meal,
4.20 – 8.10	sleep,
8.15 – 8.45	small meal of biscuits,
8.45–12.00	mainly sedentary occupation, doing analyses, etc.,
12.00 – 3.00	strict rest in easy chair, where they could void urine without rising.

The main meal was of constant nature, with a water content of 380 g, supplemented by a number of biscuits adjusted to individual taste but exactly repeated in each 12 hr period, as was also the small meal and the fluid ingestion of 390–530 ml. milk and water. Except for one accidental omission, this was all drunk before 10.00. No alcohol nor caffeine derivatives were consumed nor was tobacco smoked.

The experiment was performed in a darkened basement room, artificially lit for the waking hours, with an equable temperature showing no diurnal rhythm. External periodicity, both meteorological and social, was thus excluded.

The routine was arranged so that those factors under least perfect control, the occurrence and depth of sleep and the timing of the changes in urine resulting from meals, should be as far separated as possible from the 3 hr after midnight and midday when frequent measurements were to be made.

Rectal temperature was determined with clinical thermometers, inserted until a constant reading was attained, on retiring to sleep, on waking, and every half-hour between 12.00 and 3.00.

Haldane-Priestley end-inspiratory alveolar samples were delivered into evacuated tubes and were analysed in duplicate with the Haldane apparatus.

Urine was passed before and after sleep, about 10.00, at 12.00, and every half hour until 3.00. It was stored in screw-topped bottles to minimize CO_2 loss, and subsequently analysed as follows:

pH, with glass and calomel reference electrodes and 'Cambridge' pH meter, standardized against 'Cambridge' standard pH solutions. These determinations were all completed within 24 hr of the end of the experiment and most were performed within a few hours of voiding.

Chloride, Volhard-Whitehorn without filtration. A few were checked in an independent department by another analyst and agreed within 1 or 2 m.equiv/l.

Sodium and potassium, flame photometer.

Phosphate, Fiske and Subbarow.

Two urinary collections, giving flows of 0·13 and 0·10 ml./min for 75 and 141 min, were presumed to be erroneous and were ignored.

For convenience in referring to corresponding points in successive 12 hr cycles the 12 hr system of time recording has been used in the text. The 24 hr system has been used in the figures to demonstrate the habitual 24 hr rhythms.

RESULTS

Urine flow. In every subject a 24 hr rhythm in urine flow was clearly discernible when corresponding times in successive 12 hr cycles were compared. Thus in Fig. 1 it is seen that flow was low during nocturnal sleep only, and a conspicuous waking diuresis occurred at 8.15 a.m. only. With subjects at complete rest at 12 o'clock the urine flow was always high, but the midday peaks were far above the midnight ones.

For the flow between 3 and 12, the five subjects provide fifty-eight possible comparisons between an a.m. and a preceding or subsequent p.m. flow. With only two exceptions, the flow between 3 and 8.15 was higher in the afternoon, and between 8.15 and 12 was higher in the morning than in the evening. Each period from 12 to 3 provided six figures for urine flow, in consecutive half-hours, so that the significance level of each individual comparison could be assessed. The flow very often fell steadily during this period, and where this was so the standard error of the mean of differences between corresponding a.m. and p.m. samples, 12–12.30, 12.30–1, etc., was calculated; otherwise, the standard error of the difference between the mean of each series of six determinations was used, giving twice the degrees of freedom.

From the five subjects, fifteen comparisons were thus made between consecutive midday and midnight flows. Three of the differences were not significant; for the other twelve, midday always exceeded midnight flow, with $0{\cdot}05 > P > 0{\cdot}01$ in four, and $0{\cdot}01 > P$ in the other eight.

Whatever may be the cause of the variations corresponding to activities within each 12 hr cycle, such as the high flow which is commonly observed on sitting down, it is clear that these are superimposed upon a habitual 24 hr

cycle of urinary flow, which has persisted despite the suspension of any 24 hr rhythm in habit or environment.

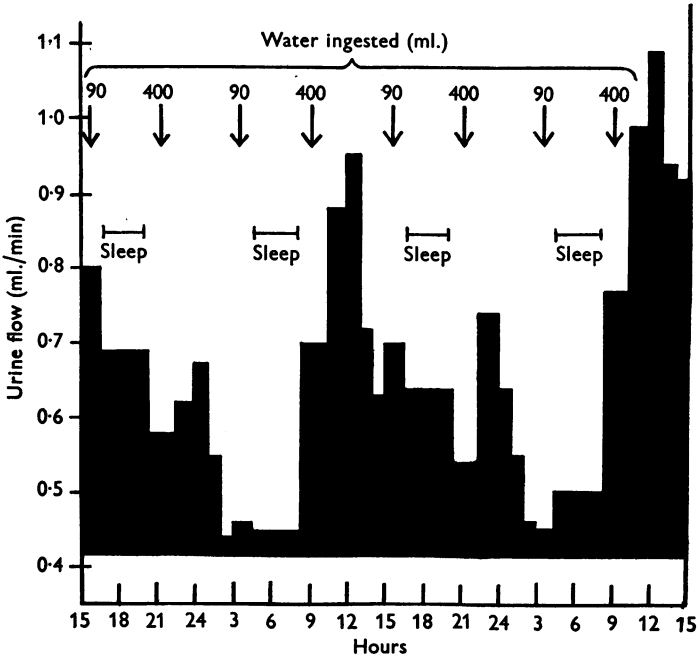

Fig. 1. Urine flow during 48 hr. Mean of all five subjects.

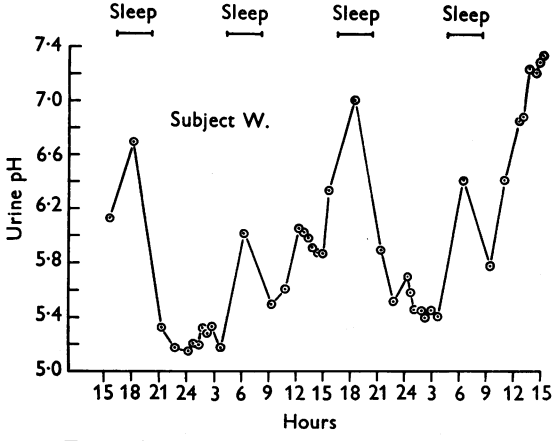

Fig. 2. Subject W. Urinary pH during 48 hr.

Urinary reaction. The pH of the urine showed in all subjects, like the flow, a clear-cut 24 hr rhythm superimposed upon fluctuations occurring in each 12 hr cycle. Of the latter, the most obvious was the alkalinity which always developed during sleep and is well shown in Fig. 2 in which it is also apparent

that the urine flow was much more alkaline during afternoon than during morning sleep. This alkalinity contrasts markedly with the acidity observed in sleep urine during life on an ordinary routine. It has not been further investigated, but it should be noted that the sleep period was the only period when the subjects were completely recumbent, and that it followed the main, and proteinous, meal. It may not therefore be a consequence of sleep *per se*. On two occasions when the subject, though recumbent, failed to sleep, a similar alkaluria developed.

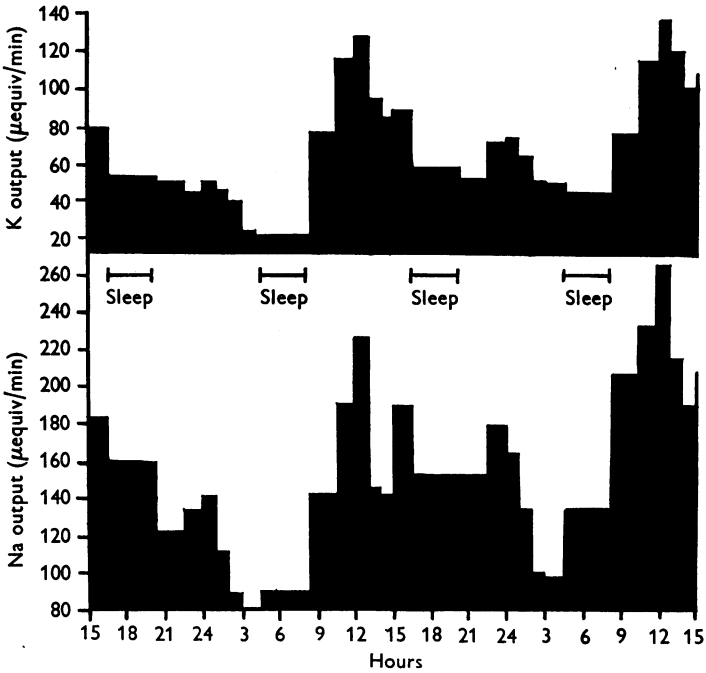

Fig. 3. K (above) and Na (below) output during 48 hr. Mean of all five subjects.

What concerns us here is that, with only three exceptions out of the forty-five possible comparisons between a.m. and p.m. samples in the periods 12–3, 3–4.15 and 4.15–8.15, the morning urine was always more acid than the corresponding afternoon urine—a result which would occur by chance less than once in two thousand million times. Between 8 and 12 this rhythm of urinary pH is less apparent, although now the morning urine tends to be more alkaline.

Excretion of sodium, potassium and chloride. A 24 hr rhythm was discernible in the excretion of all these three electrolytes, being most regular for potassium and least for sodium. Fig. 3 shows the mean outputs of both these electrolytes. The fluctuations are clearly determined by the habitual 24 hr cycle, and almost

uninfluenced by the 12 hr cycle on which the subjects were living. Sodium and potassium excretions were fairly well correlated ($r = 0{\cdot}582$–$0{\cdot}884$), as shown in Fig. 4, so the major diurnal fluctuations in excretion of each are probably expressions of the same variations in renal function.

Although for all subjects the correlation between Na and K excretion rates was highly significant, it was not very high for S., W. and X. The deviations of Na excretion from linear regression on K excretion have therefore been examined for these three subjects, and have been found to be due in part to

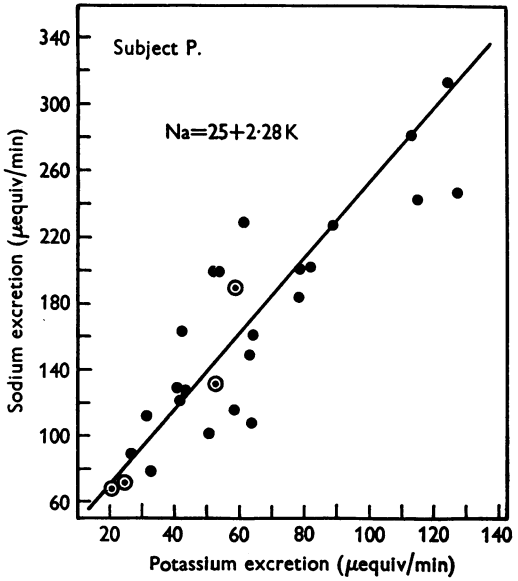

Fig. 4. Subject P. Sodium excretion plotted against potassium excretion measured over periods of 1 hr and over, with regression lines inserted. ●, waking samples; ⊙, sleep samples.

regular variations over each 12 hr cycle. Since Na and K content of the food was not determined, and might well have had a differential effect upon the excretion of the two ions, a full analysis of variance did not appear justified. There was no indication whatever of a 24 hr rhythm in the deviations from regression, which supports our conclusion that, whatever may be the diurnal rhythmic influence upon the kidney, it is one which affects Na and K alike.

Phosphate excretion. Each subject developed a 12 hr cycle of phosphate excretion, and two representative examples of this effect are shown in Fig. 5. While it cannot be denied that some subjects, such as S., showed signs of persistence of the customary 24 hr rhythm (Fiske, 1921), there was no close correspondence between the output of phosphate and of other electrolytes. They varied sometimes together, sometimes in opposite directions. It is hard to believe that there is any close causal connexion between them.

Alveolar carbon dioxide tension. At about 12.40, 1.40 and 2.40 in each period alveolar samples were collected from each subject. Each was obtained by pooling in the same sampling tube three different samples given at intervals of 1 or 2 min. The subjects had thus been seated at rest for over ½ hr before the first sample was given, so that all three, at hourly intervals, should be comparable. It was thought that if the alleged 24 hr. cycle of sleepiness and wakefulness (Kleitman, 1939) caused any decrease and increase in the excitability

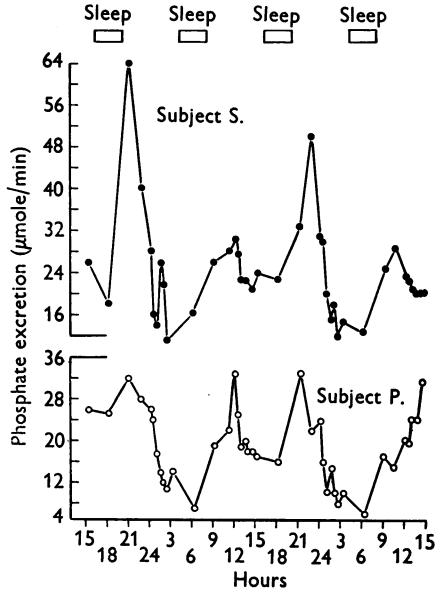

Fig. 5. Phosphate excretion during 48 hr. ●—●, subject S; ○—○, subject P.

of the respiratory centre, this should be manifested by higher alveolar carbon dioxide tensions in samples collected between midnight and 3 a.m. than in those collected between midday and 3 p.m., despite the identical nature of the previous activities of the subjects. This expectation was not fulfilled.

TABLE 1. Variance analysis of alveolar CO_2 tensions. 'Series' indicates a series of determinations at hourly intervals during a 3 hr period between 12 and 3 o'clock. The units are mmHg. Results for all 5 subjects are pooled.

	n	$S(x-\bar{x})^2$	Variance
Variance within series	35	46·065	1·316
Variance between series for one subject	14	27·792	1·985

One subject, S., showed small but regular and significant fluctuations, the mean CO_2 tension of five samples collected shortly after midnight exceeding the mean tension of six samples at midday by $1{\cdot}3 \pm 0{\cdot}4$ mmHg. The other subjects showed no signs of such a rhythm. The variance analysis of Table 1 shows that, taking all five subjects together, the variance within sets of three samples collected over a period of 3 hr is nearly as great as the variance between the four such sets obtained at 12 hr intervals on any one subject.

The P value for the variance ratio exceeds 0·3. All the alveolar samples given by any one subject might therefore be randomly selected members of the same population.

Sleep and rectal temperature. Two subjects failed to sleep during the first period in the afternoon, and in one of these and one other the afternoon sleep was regularly lighter and less continuous than the early morning sleep. Since everyone present was participating in the experiment it was impossible to obtain more definite evidence whether we showed a diurnal rhythm of sleepiness (Kleitman, 1939).

All subjects showed a persistent 24 hr rhythm in rectal temperature similar to that observed by Kleitman, although in some the temperature was also affected by the varying influences within each 12 hr cycle. Two examples are shown in Fig. 6.

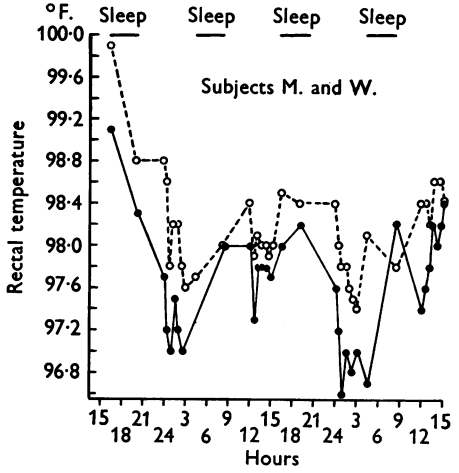

Fig. 6. Rectal temperature during 48 hr. O- - -O, subject M; ●—●, subject W.

Correlation between the functions measured

Inspection of the data showed an obvious correlation between flow and electrolyte excretion. Since Na and K account for the bulk of the cations, whilst one extremely variable anion, bicarbonate, was not determined, a formal demonstration of this correlation was sought by calculating regression lines of flow upon minute output of Na+K. The full results are shown in Fig. 7. The regression functions for all five subjects were almost identical, so a single function, flow in ml./min = 0·155 + 2·27 × cation excretion in m.equiv/min was calculated for the pooled data. This regression function accounts for 79% of the variance of urine flow. It would thus appear likely that flow is largely osmotically determined by the output of electrolytes. A similar increase in flow per increment of loading solute was found by Rapoport, Brodsky, West & Mackler (1949) during osmotic diuresis in hydropenic man.

It might be objected that urine flow enters into the computation of the quantity with which it is correlated, electrolyte output, and that we have introduced a spurious correlation both here and between output of Na and K. This objection is, however, only valid if a major source of variation is experimental error in flow determination. Volume errors due to incomplete emptying

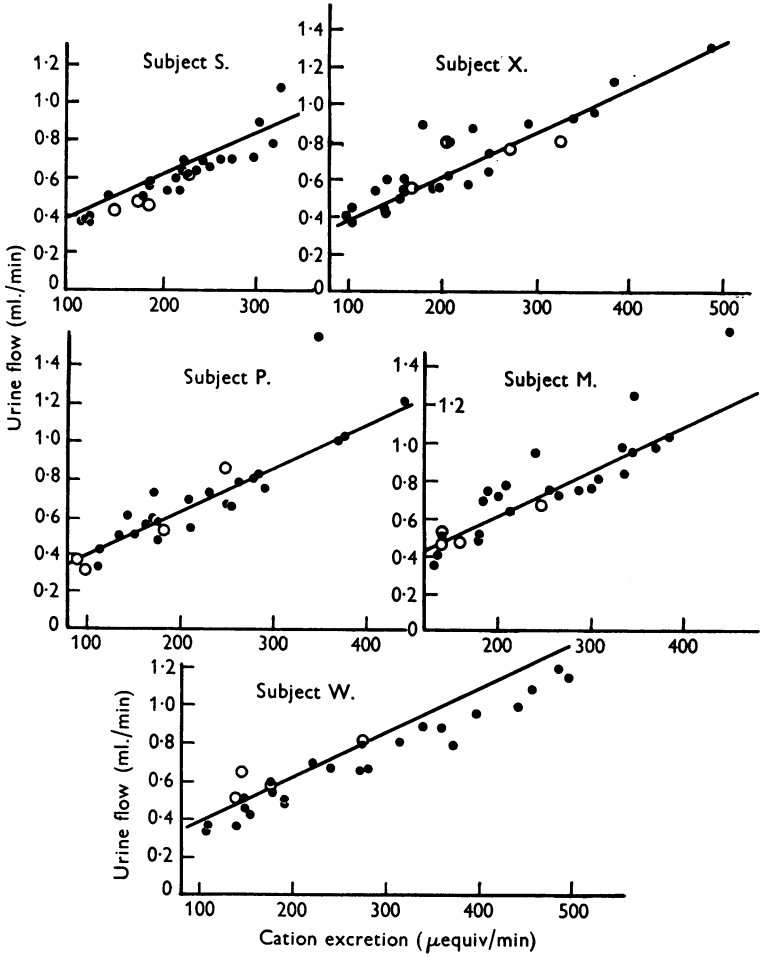

Fig. 7. Urine flow plotted against cation (Na + K) output. For clarity, determinations on each subject are plotted separately, but the inserted regression line is calculated from the pooled data. ●, waking; ○, sleep, samples.

of the bladder will only be significant in the ½ hr collections at low flows, and to minimize any such errors we have correlated outputs derived from at least 1 hr collection, pooling two successive ½ hr when urine was in fact voided half hourly. The correlations are in any case apparent even if all the half-hour collections are ignored.

A mathematician might prefer a correlation between the actual measured quantities, flow and electrolyte concentration, but no simple relationship is here to be expected. McCance (1945) has shown that, in an osmotic diuresis due to salt, the salt concentration in the urine increases with flow up to a maximum at about 1 ml./min and then slowly falls with further increase in flow. Our flows were nearly all below this critical value, and in fact electrolyte concentration, Na + K, was positively correlated with flow. The values for *W* are shown in Fig. 8.

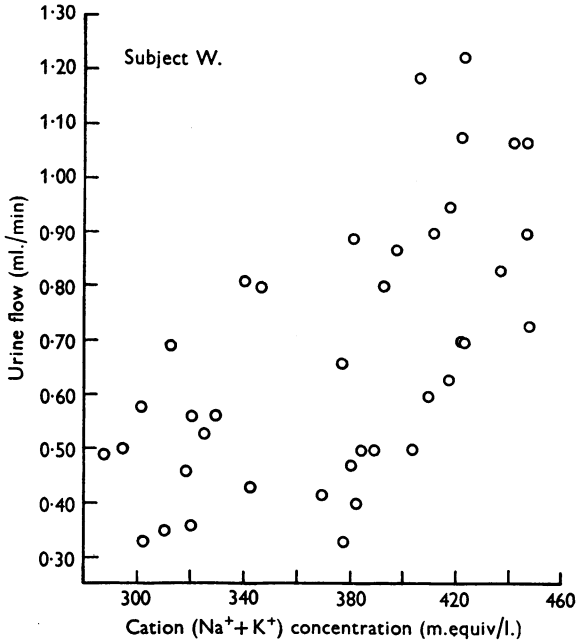

Fig. 8. Subject W. Urine flow plotted against cation (Na + K) concentration. ½ hr urine collections included. 1 point, flow = 0·64 ml./min, cation concentration = 234 m.equiv/l. omitted. $r = 0{\cdot}576$, $P < 0{\cdot}01$.

No regression line has been inserted since a linear regression is not to be expected.

Na and K excretion showed in all subjects a linear regression upon pH (Fig. 9) similar to that described by Stanbury & Thomson (1951), if sleep samples are omitted. These all diverged markedly in the direction of high pH. Sleep samples showed no such divergence in the regression of Na on K excretion (Fig. 4).

A possible determinant of pH changes would be a reciprocal change in the ratios of chloride and of bicarbonate output to the sum of the outputs of Na and K. We have therefore plotted chloride against Na + K outputs, and if sleeping samples are omitted these all fall fairly closely on straight lines through

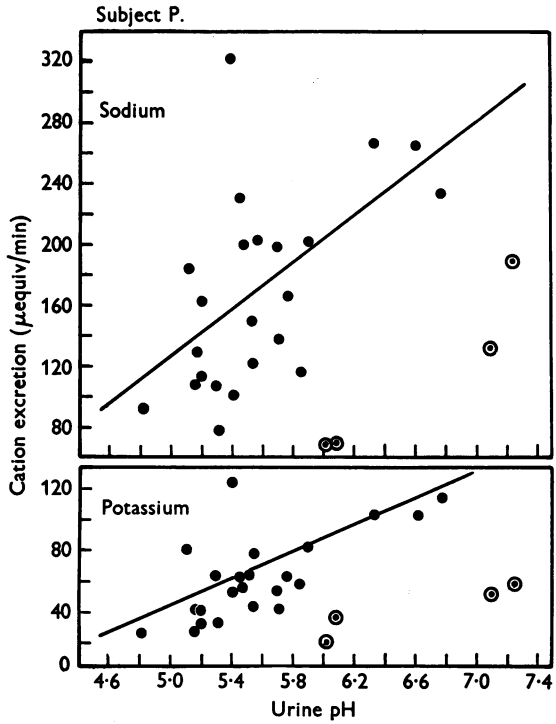

Fig. 9. Subject P. Na (above) and K (below) outputs plotted against urinary pH, with linear regression for waking samples inserted. ●, waking, ⊙, sleep, samples. Urine collections of 1 hr and over.

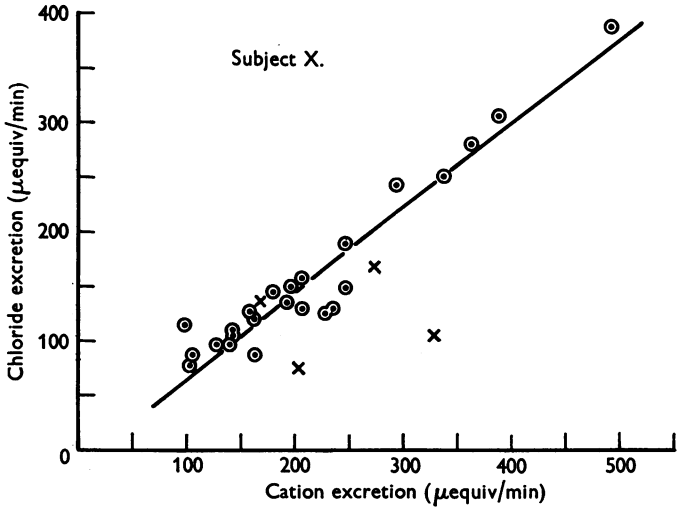

Fig. 10. Subject X. Chloride excretion plotted against cation (Na + K) excretion, with linear regression for waking samples inserted. ⊙, waking; ×, sleep, samples. Urine collections of 1 hr and over.

the origin (Fig. 10). The slopes were 0·73–0·81, suggesting chloride/bicarbonate excretion ratios of 3–4. Seven of the twenty sleep determinations, including two of those in Fig. 10, deviated markedly in the direction of low chloride, and hence perhaps had a high bicarbonate content which might account for the high pH.

DISCUSSION

It is clear from the present experiments that, in healthy adults, the intrinsic 24 hr rhythm in the excretion of water and electrolytes is not immediately dependent upon the diurnal periodicity of habit. Evidence pointing to a similar conclusion was obtained by Stanbury & Thomson (1951) who found a nocturnal fall in urine pH and in electrolyte excretion despite abstention from sleep, and by Kaye (1929) who made similar observations of urine pH alone. Although the 24 hr renal periodicity persisted during the present experiments and throughout the 6 days of inverted routine in Mills's (1951*a*, *b*) experiments, it will not survive indefinite suspension of the customary external periodicity. Norn (1929), in one subject, found that the diurnal rhythm of sodium, potassium and chloride excretion became inverted after 6 weeks of work at night and sleep during the daytime. The 24 hr rhythm of excretion is, therefore, ultimately dependent upon a stabilized routine or pattern of diurnal activity. There is little, either in the present investigation or in previous work, to indicate which of the many repeated diurnal occurrences might be originally responsible for the production of the rhythm. Stanbury & Thomson (1951) drew attention to the similarity between the changes in electrolyte excretion produced by voluntary hyperventilation and those which occur spontaneously with the diurnal excretory rhythm. They suggested that the latter were in some way related to the regular alternation of hypoventilation and relative hyperventilation with the cycle of sleep and wakefulness. Nocturnal sleep is associated with a rise in alveolar CO_2 tension and waking with a fall (Bass & Herr, 1922); and Endres (1922) found that an abrupt fall in alveolar CO_2 tension accompanied the spontaneous matutinal increase in urine pH. Since Stanbury & Thomson (1951) found also a close correlation between the matutinal rise in urine pH and the matutinal increase in sodium, potassium and chloride excretion, a relation between the renal and respiratory changes was strongly suggested. The persistent 24 hr excretory periodicity in the subjects of the present investigation was not, however, associated with a 24 hr rhythm in their alveolar CO_2 tension, and the periodicity of water and electrolyte excretion cannot, therefore, be directly dependent upon diurnal changes in CO_2 tension. The nature of the apparent relationship between the diurnal changes in respiratory and renal function remains obscure, and will be the subject of further investigation.

The correlation between sodium and potassium excretion, and the linear regression of sodium and potassium excretion on urine pH, suggest that a single

change of renal function is responsible for the 24 hr. rhythm in both cation excretion and urine pH. No such change in electrolyte output can be produced in man by the administration of adrenal cortical extracts; and Stanbury & Thomson (1951) have given further reasons for rejecting the contention of Borst & de Vries (1950) that the diurnal renal cycle is due to rhythmic variations in adrenal cortical secretion.

It appears that the 24 hr periodicity of urine flow is determined by the changes in electrolyte excretion. It has been shown that, both under conditions of presumably maximal (McCance, 1945; Rapoport *et al.* 1949) and minimal (Rosenbaum, Nelson & Strauss, 1950; Stanbury & Thomson, 1951) secretion of posterior pituitary antidiuretic hormone, the urine flow varies with total solute load. It is at least plausible that the same should be true in intermediate conditions; and since under normal conditions sodium + potassium, with associated anions, account for a large fraction of total solute load, the close dependence of flow upon electrolyte output is readily explicable upon an osmotic basis.

Increase and decrease of posterior pituitary secretion leads to a large decrease and increase of urine flow with little or no change in electrolyte output (Hare, Hare & Phillips, 1943; Thorn, 1949; Chalmers, Lewis & Pawan, 1951; Lauson, 1951); the urinary electrolyte concentration in such conditions varies widely and inversely with the urine flow. The midday flow peaks in Fig. 1 appear at first sight like water diureses; but this cannot have been so, as they were associated with rising concentrations of sodium and potassium. The absorption of the water ingested with the previous meal must have been too slow to affect Verney's (1947) cranial osmoreceptor mechanism. It is possible that some of the random variation in, for example, Fig. 7, was due to random variation in posterior pituitary activity. Inspection of these regression lines for all subjects shows, however, no consistent deviation by the sleep samples, nor by any other selected set of samples, as should have been observed if posterior pituitary secretion had varied regularly during either the 12 hr or the 24 hr cycle. Mills's (1951*b*) suggestion of such a rhythm must be discarded. The absence of any regular deviations from the relationship between urine flow and electrolyte output within each 12 hr cycle thus suggests that neither sleep, ingestion of food nor any of the other variations in activity has been associated with any regular variation in posterior pituitary secretion. It appears likely that the spontaneous diuresis observed by Hart & Verney (1934) was also due to variations of electrolyte output, and not of posterior pituitary secretion.

Although its intimate nature is unknown, the renal periodicity which underlies the spontaneously occurring excretory rhythm appears also to determine the pattern of renal response to a variety of imposed external stimuli. Stanbury & Thomson (1951) found a diurnal variability in the diuretic response

to a standard dose of water; and the changes in water and electrolyte excretion which followed the intravenous injection of deoxycortone acetate or posterior pituitary antidiuretic hormone were also different at different phases of the diurnal cycle. A similar conditioning of the renal responses was apparent in the present experiments; for identical events within each 12 hr cycle produced effects which were significantly different when adjacent 12 hr cycles were compared. For the most part, the intimate nature of the stimuli recurring in each 12 hr cycle is unknown; they will have resulted from, *inter alia*, meals and changes of posture; but their regular repetition was ensured by the experimental conditions. Mention has been made of the high urine flow when the subjects were at complete rest, and the invariably higher flow during midday than midnight rest. The considerable increase in urine pH which developed during the 4 hr periods of sleep and recumbency was also clearly modified by the prevailing influence of the 24 hr rhythm. This change is of interest since it differs from that which accompanies sleep on a normal routine, when bicarbonate excretion falls and the urine becomes acid; and it led to the superimposition of a 12 hr cycle upon the 24 hr cycle of pH change (Fig. 2). It is possible that the observed change is related more closely to recumbency than to the sleeping state. White *et al.* (1926) showed that on changing from standing to the lying position, there was a threefold increase in chloride excretion and six- or sevenfold increase in the output of bicarbonate, the urine pH invariably increasing. The authors made no measurement of sodium or potassium excretion, but it is obvious that cation excretion must have been considerably augmented. This pattern of response was modified in the present experiments. There was no regular nor comparable change in sodium or potassium excretions, which were usually somewhat diminished. The ratio of chloride output to that of these cations was however often markedly depressed, so that the alkaluria could in many instances be ascribed to a change in the rate of excretion of chloride relative to bicarbonate.

The apparent changes in bicarbonate/chloride excretion ratio must, in the absence of comparable changes in the ratio of these ions in the plasma, have resulted from an enhanced tubular reabsorption of chloride. We find it difficult to believe that a plasma change of the appropriate magnitude could have taken place and presume that this particular renal response was a manifestation of tubular activity. The present investigation throws no light on the nature of the stimulus originally responsible for the 24 hr excretory periodicity, but it has shown that a wide variety of renal responses are modified by this influence. Many of these modified responses involve the active participation of the renal tubules, and the fact of their modification supports the previous contention that the diurnal excretory rhythm is in part ascribable to a rhythmic change of renal tubular behaviour.

Two negative conclusions can be drawn. First, the negative findings on

alveolar air exclude respiratory influences on CO_2 tension as a necessary cause of the renal excretory rhythm; and secondly, the excretion of other electrolytes has no close connexion with the rhythm of phosphate output, which in these experiments assumed a 12 hr cycle.

SUMMARY

1. Five subjects spent 48 hr in a basement room on a routine of sleep, meals, illumination, etc., which was exactly repeated every 12 hr.

2. Rectal temperature maintained a 24 hr rhythm.

3. A persistent 24 hr rhythm was apparent in the urinary output of water, sodium, potassium and chloride, as well as in urinary pH.

4. Urinary volume was osmotically determined by the electrolyte output.

5. The 12 hr cycle of activity had its most obvious effect upon phosphate excretion, but had some effect upon other renal functions. Urine for instance always became alkaline during the period spent in sleep and recumbency, frequently with a fall in the Cl/(Na + K) ratio, but pH was higher in afternoon than in night sleep.

6. It is considered that superimposed upon the various influences of food, posture, etc., which recurred every 12 hr, there was a single variation of renal tubular activity occurring rhythmically over 24 hr and affecting urinary pH and output of all measured electrolytes except phosphate, and secondarily urinary volume. No cyclical change in alveolar carbon dioxide tension was found as a cause of this, nor did it show any apparent connexion with the rhythm of phosphate excretion.

Our thanks are due to M. Moss, R. Postlethwaite, J. E. Scott and A. A. Wagland, who acted as subjects and carried out all pH and many chloride determinations.

REFERENCES

Bass, E. & Herr, K. (1922). *Z. Biol.* **75**, 279.
Bazett, H. C., Thurlow, S., Crowell, C. & Stewart, W. (1924). *Amer. J. Physiol.* **70**, 430.
Borst, J. G. G. & de Vries, L. A. (1950). *Lancet* i. 1.
Brun, C., Knudsen, E. O. E. & Raaschou, F. (1945). *Acta med. scand.* **122**, 315.
Chalmers, T. M., Lewis, A. A. G. & Pawan, G. L. S. (1951). *J. Physiol.* **112**, 238.
Cohen, I. & Dodds, E. C. (1924). *J. Physiol.* **59**, 259.
Endres, G. (1922). *Biochem. Z.* **132**, 220.
Fiske, C. H. (1921). *J. biol. Chem.* **49**, 171.
Hare, R. S., Hare, K. & Phillips, D. M. (1943). *Amer. J. Physiol.* **140**, 334.
Hart, P. D'A. & Verney, E. B. (1934). *Clin. Sci.* **1**, 367.
Kaye, G. (1929). *Aust. J. exp. Biol. med. Sci.* **6**, 187.
Kleitman, N. (1939). *Sleep and Wakefulness.* Chicago: University of Chicago Press.
Kroetz, C. (1926). *Z. ges. exp. Med.* **52**, 770.
Laehr, H. (1890). *Allg. Z. Psychiat.* **46**, 286.
Lauson, H. D. (1951). *Amer. J. med.* **11**, 135.
McCance, R. A. (1945). *J. Physiol.* **104**, 196.
Mills, J. N. (1951*a*). *J. Physiol.* **112**, 53*P*.

Mills, J. N. (1951*b*). *J. Physiol.* **113**, 528.
Mills, J. N. & Stanbury, S. W. (1951). *J. Physiol.* **115**, 18*P*.
Norn, M. (1929). *Skand. Arch. Physiol.* **55**, 184.
Quincke, H. (1877). *Arch. exp. Path. Pharmak.* **7**, 115.
Rapoport, S., Brodsky, W. A., West, C. D. & Mackler, B. (1949). *Amer. J. Physiol.* **156**, 433.
Rosenbaum, J. D., Nelson, W. P. & Strauss, M. B. (1950). *J. clin. Invest.* **29**, 841.
Simpson, G. E. (1924). *J. biol. Chem.* **59**, 107.
Simpson, G. E. (1926). *J. biol. Chem.* **67**, 505.
Simpson, G. E. (1929). *J. biol. Chem.* **84**, 393.
Sirota, H., Baldwin, D. S. & Villarreal, H. (1950). *J. clin. Invest.* **29**, 187.
Stanbury, S. W. & Thomson, A. E. (1951). *Clin. Sci.* **10**, 267.
Stanbury, S. W. & Thomson, A. E. (1952). *Clin. Sci.* (in the Press).
Straub, H. (1915). *Dtsch. Arch. klin. Med.* **117**, 397.
Thorn, G. W. (1949). *Recent Progr. Hormone Res.* **4**, 286. Cited by Chalmers, Lewis & Pawan in *J. Physiol.* 1951, **112**, 238.
Verney, E. B. (1947). *Proc. Roy. Soc.* B, **135**, 25.
Wendt, E. (1876). *Arch. Heilk.* **17**, 527.
Wesson, L. G., Anslow, W. P. & Smith, H. W. (1948). *Bull. N.Y. Acad. Med.* **24**, 586.
White, H. L., Rosen, I. T., Fischer, S. S. & Wood, G. H. (1926). *Amer. J. Physiol.* **78**, 185.