XLV. NOTE ON URINARY TIDES AND EXCRETORY RHYTHM.

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Introduction.

In previous papers [1921, 1922] we gave the results of observations on the composition of the day and night urine of the same subject under four different routines, each of five days duration. We showed that the differences between day and night urine were constant under a routine of complete rest in bed, a laboratory routine, a light muscular work routine and a severe muscular work routine. We found, in each case, that the total N, urea N, water and chloride were excreted in much greater amounts during the day than at night; that the amino-acids and uric acid were excreted in slightly greater amounts during the day than at night; that the acid, ammonia N and phosphate were excreted in much greater amounts during the night than during the day; and that creatinine and sulphate were more or less evenly distributed between day and night. In other words, there was an excretory rhythm which was not altered by any of the conditions of the four routines examined. In this rhythm there were definite tides during the day of water, chloride, total N and urea N, whilst at night there were phosphate, ammonia N and acid tides. It was considered that the greater excretion of acid, ammonia N and phosphate at night was due to delayed excretion of "certain fixed acids" formed in the cells during the day and that the phosphate tide at night was connected with the greater acidity at night.

To investigate further these various tides and this excretory rhythm we employed the same subject under further changes of routine. In the present paper we refer to differences between day and night urine, (1) during a routine of day starvation for 48 hours with food only at night, (2) during a routine of complete starvation for 24 hours and (3) during a reversed routine, for 96 hours, in which work was performed and food was taken during the night whilst the subject slept during the day.

Methods.

We have kept the methods constant throughout all routines.

Our subject took his usual diet but it was controlled to exclude articles which are known to influence greatly the composition of the urine and also to keep the average daily diet for each routine as similar in substance as possible. We employed his usual diet as we were undertaking a prolonged research in which the subject would at times be exposed to somewhat strenuous conditions. By making each routine cover several—and the same—consecutive days and by keeping the quality of the average daily diet constant, we thought that errors would be lessened. Moreover, preliminary observations under his normal daily diet showed that the differences between day and night urines were constant.

We collected the urine between 7 a.m. and 5 p.m. for the day and between 5 p.m. and 7 a.m. for the night. It was not considered that these periods were the best. They were chosen as being most convenient for our research. For a large part of this research the subject lived at the laboratory.

There is no accurate way to decide whether a waste product formed in a cell is excreted as soon as it is formed by the cell, or for how long the cell retains it. All periods for collecting urine have their advantages and disadvantages. The best results will probably be attained by a comparison of figures obtained during various periods of collection from each of many subjects under various conditions.

In estimating the acidity of the urine, we employed four methods—p_H, Folin's titration method, titration to p_H 7·4, and Leathes' acidity percentage method [1919]. As other observers have pointed out we found that the agreement between these methods as regards detail was not marked, but nevertheless the broad significance of the conclusion as regards acidity was similar with each method. Thus we were able to demonstrate the presence of the so-called alkaline tide in our subjects and to determine the difference in acidity between the day and night urines by any of these methods.

Starvation Routines.

Our subject starved all day taking food only between 5 p.m. and 10 p.m., the object of this experiment being to determine whether taking food at night would alter any of the tides, particularly the acidity and total N tides. For two days this routine was followed, and on the next day the subject starved for 24 hours. Table I shows the results for both these routines, namely, "day starvation" and "complete starvation." On comparing these results with our previous work [1921, 1922] it will be noticed that practically no change from our previous figures occurred as regards the differences between day and night urine. That is total N, urea N, water and chloride were still excreted in greater quantities during the day than at night, and acidity, phosphate and ammonia N were still higher at night than during the day.

Lusk [1917] records that the total N excretion during starvation was higher during the day than at night. From our results during starvation, it was obvious that the urine, under normal conditions, was not more acid during the night than during the day because taking of food during the day produced so-called alkaline tides. We have carried out many observations on our subject with regard to the so-called alkaline tide and found that any normal meal was followed by such a tide, in many cases the tide being well marked.

During the "day starvation" routine, our subject carried out sedentary laboratory work, whilst during the "complete starvation" routine he rested in bed reading most of the day.

We found about 20 mg. of creatinine N per hour in our subject, as seen in Table I. In our previous papers [1921, 1922] owing to an error we gave about 30 mg. per hour as the figure for this subject; all the figures in these previous papers for creatinine N should be reduced by one-third and the figures for undetermined N correspondingly increased. The general conclusions, however, are not disturbed by this correction, as the error was constant.

Reversed Routine.

In the "reversed routine" our subject worked and had most meals and fluid at night whilst he slept during the day from about 10.30 a.m. to 5.30 p.m. Meals were taken at 8 p.m., 2 a.m., 5 a.m. and 8 a.m. instead of the usual times 7.30 a.m., 1 p.m., 4 p.m. and 7.30 p.m. respectively. Four consecutive days of this "reversed routine" were spent by our subject C.P. and one of us (J.A.C.). Neither subject had become accustomed to the new routine by the fourth day; although both subjects had slept well during the day they felt very sleepy at night. Table I shows C.P.'s average results for the last 72 hours of this routine and Table II A gives J.A.C.'s average results. Average figures are given because the results for any one day were very similar to these. Subject C.P.'s results show that no change from the normal rhythm was obtained except that the phosphate for the day was much increased, the night phosphate remaining about the usual figure.

In the case of J.A.C. a similar increase of phosphate during the day was noted but also there were other changes; now the acidity and ammonia N were higher during the day when sleep was taken, so that the reversal of routine produced some reversal in composition in J.A.C.'s urine (see Table II A).

It is interesting to note that after four days of complete reversal of routine and meals (including fluid) the day and night partition of water and nitrogen and of practically all other excretions remained unchanged. This points towards a fixed physiological rhythm of the cells which cannot be altered easily. It is not likely that the kidney was responsible for this rhythm. All the body cells were probably responsible. The rhythm may be due to following closely a fixed daily routine—of work, meals and sleep—for a long period. Allowance for this phenomenon must, therefore, be made where necessary when carrying out experiments of this nature.

Table I. *Average hourly results. Subject C.P.*

	48 hours' day starvation (food between 5 p.m. and 10 p.m.)		24 hours' complete starvation		72 hours' reversed routine	
	DAY (Sedentary work, no food)	NIGHT (Food and sleep)	DAY (Rest in bed)	NIGHT (Sleep)	DAY (Sleep)	NIGHT (Work and food)
Amount cc.	56·0	25·0	88·0	17·9	71·3	34·5
Acidity %	46·4	78·6	33·0	58·3	42·4	70·4
Titratable acidity (Folin) cc. $N/10$	6·0	15·7	6·0	10·0	8·0	12·4
Total acidity, cc. $N/10$...	15·0	30·7	14·0	24·3	17·3	29·5
Total N g.	·379 (100)*	·307 (100)*	·392 (100)*	·195 (100)*	·373 (100)*	·337 (100)*
Urea N g.	·315 (83·00)	·248 (80·80)	·305 (77·80)	·142 (72·80)	·313 (84·00)	·250 (74·20)
Ammonia N (A) g. ...	·010 (2·64)	·019 (6·19)	·010 (2·55)	·018 (9·23)	·010 (2·68)	·018 (5·34)
Ammonia N (B) g. ...	·012	·021	·011	·020	·013	·024
Amino-acid N g. ...	·002 (0·52)	·002 (0·65)	·001 (0·25)	·002 (1·02)	·003 (0·80)	·006 (1·78)
Creatinine N g.	·020 (5·28)	·019 (6·19)	·019 (4·85)	·020 (10·25)	·021 (5·63)	·022 (6·53)
Uric acid N g.	·006 (1·58)	·004 (1·30)	·006 (1·53)	·003 (1·54)	·006 (1·61)	·007 (2·07)
Undetermined N g. ...	·026 (6·98)	·015 (4·87)	·050 (13·02)	·010 (5·16)	·020 (5·28)	·036 (10·08)
Chloride (NaCl) g. ...	·472	·213	·497	·134	·335	·261
Phosphate (P_2O_5) g. ...	·068	·082	·068	·078	·093	·081
Total S (SO_3) g. ...	·055 (100)	·068 (100)	·056 (100)	·041 (100)	·063 (100)	·072 (100)
Inorganic S (SO_3) g. ...	·039 (70·9)	·052 (76·5)	·044 (78·6)	·035 (85·4)	·054 (85·7)	·057 (79·2)
Ethereal S (SO_3) g. ...	·006 (10·9)	·009 (13·2)	·005 (8·9)	·001 (2·4)	·002 (3·2)	·006 (8·3)
Neutral S (SO_3) g. ...	·010 (18·2)	·008 (10·3)	·007 (12·5)	·005 (12·2)	·007 (11·1)	·009 (12·5)
Purine N g.	·0015	·0026	—	—	—	—
Calcium (CaO) g. ...	—	—	—	—	·011	·014

(A) Van Slyke's method. (B) Malfatti's method. Total acidity is ammonia (B) + titratable acidity.

* Figures in brackets are percentages.

Table II A. *Hourly averages. Subject J.A.C.*

	Reversed routine		Ordinary routine	
	Night Work and food	Day Sleep	Day Work and food	Night Sleep
Amount cc.	43·1	76·0	60·7	31·5
Acidity %	53·8	57·3	42·8	72·3
Titratable acidity, Folin cc. $N/10$	11·2	13·6	8·2	10·7
Total acidity cc. $N/10$	28·8	35·3	26·5	30·6
Total N g.	·408	·596	·533	·451
Ammonia N (B) g.	·025	·030	·026	·028
Phosphate (P_2O_5) g.	·074	·097	·055	·075

Table II B. *Hourly averages. Subject C.P.*

Experiment no.	Acidity %	Phosphate (P_2O_5) g.	Titratable acidity cc. $N/10$	Total acidity cc. $N/10$	Ammonia N (A) g.	Total N g.	Time	Sleep	Routine
1	20·6	·045	5·4	18·8	·014	·386	day	−	Rest in bed
2	70·2	·080	18·2	36·7	·022	·296	night	+	
3	44·8	·065	10·4	25·4	·015	·428	day	−	Ordinary
4	64·6	·080	16·8	33·0	·019	·332	night	+	
5	50·0	·066	11·6	28·1	·011	·447	day	−	Light muscular work
6	64·0	·075	15·4	31·6	·019	·357	night	+	
7	60·0	·070	14·0	29·0	·015	·446	day	−	Severe muscular work
8	70·0	·080	14·4	30·7	·019	·414	night	+	
9	46·4	·068	6·0	15·0	·010	·379	day	−	Day starvation
10	78·6	·082	15·7	30·7	·019	·307	night	+	
11	33·3	·068	6·0	14·0	·010	·392	day	−	Complete starvation
12	58·3	·078	10·0	24·3	·018	·195	night	+	
13	42·4	·093	8·0	17·3	·010	·373	day	+	Reversed
14	70·4	·081	12·4	29·5	·018	·337	night	−	

In both subjects the phosphate for the day and therefore for the whole 24 hours was distinctly increased. This increase was probably due to some special nervous metabolism connected either with sleep itself or with the desire to sleep. In Table II B, we have drawn up some results from all our experiments including those previously published [1921, 1922]. These show that a high phosphate excretion always accompanied sleep and that the higher of the day and night figures for phosphate excretion in each routine could be separated from every other factor in our experiments but sleep (see Table II B).

Sleep and Acidity of Urine.

In our previous papers we found that sleep was accompanied by a definite increase of ammonia, acidity and phosphate and a relative increase of sulphate. We suggested that there was a delayed excretion of "certain fixed acids" formed in the cells during the day, and that when formed in certain quantities, they were responsible for sleep and fatigue. In our present paper the higher figures for phosphate whether found by day or by night always accompanied sleep and sleep was independent of every other factor including acidity (see Table II B). Therefore, although these "certain fixed acids" might have been responsible for sleep and although higher acidity usually accompanied sleep it was not a necessary accompaniment of sleep (see experiment 13, Table II B). This is contrary to the finding of Leathes [1919], who considered that the CO_2 tension of alveolar air was increased at night above that for the day, as a result of depression of the respiratory centre during sleep. Collip [1920] also considered that an increase of C_H of the blood at night was due to sleep.

We see no reason to abandon the suggestion that sleep might be due to "certain fixed acids" since there was in the case of subject C.P. in the "reversed routine" an increased acidity of the urine previous to sleep although not accompanying it. He was obviously sleepy during the time of excretion of the larger amount of acid, but kept awake purposely. If the high acidity was due to "certain fixed acids" formed by the activity of the cells and capable of causing sleep, the cells would only recover after sleep itself and not after excretion of the "certain fixed acids." However, it was obvious that the subject had not become accustomed to the "reversed routine," so that our results really belonged to a transitional period and it is at present difficult to draw conclusions. We have carried out some observations on a subject who was somewhat more accustomed to work and food at night, with sleep during the day. He worked on a night shift every third week. The results we have obtained from him are in complete agreement with the results here described. Results from subjects accustomed to long periods of night work are required.

Phosphate Excretion.

We conclude from all our results regarding the higher figure for day and night phosphate excretion in each routine that it was intimately related to metabolism either connected with sleep or occurring at the same time as sleep. We were able to separate the higher phosphate excretion in each routine from every factor, but sleep (see Table II B). Although, as a rule, the higher phosphate excretion accompanied the higher acid and the higher ammonia N excretion there was a marked exception to this rule (see experiment 13, Table II B).

Broadhurst and Leathes [1920] have suggested that the phosphate tide at night may be connected with some special muscular or nervous metabolism. Our previous results indicated that there was no connection with muscular metabolism and less clearly that there was none with nervous metabolism. Broadhurst and Leathes also found that the phosphate tide was not dependent upon food. Our results during the starvation routines confirm this finding. Fiske [1921], who considered that the phosphate tide was due in part to "retention of phosphorus," also showed that the phosphate curve was not affected by the taking of sodium bicarbonate so that the phosphate curve was not connected with excretion of acid. This may be the case, but there is much evidence from other observers that phosphate may have, as one of its functions, the removal of acid. Haldane [1921] found that acid was removed by phosphate if there was any phosphate available. In our subject C.P. a high acidity, as interpreted by a high titratable acidity and a high ammonia N excretion, was always accompanied by a high phosphate excretion.

We estimated the calcium in the urine and found that it was not connected with the increase of phosphate, the calcium being low when the phosphate was high and *vice versa*. When in the "reversed routine" the phosphate was absolutely increased, no such marked change was noted in the amount of calcium excreted.

According to text books [Hawk, 1919], some investigators hold that during extensive decomposition of nervous tissue the phosphate is increased. Mendel found that phosphate was increased after sleep produced by potassium bromide or chloral hydrate, so that it is possible that special metabolism connected with sleep was the main factor for the higher of the day and night phosphate excretions in our subject. Nervous metabolism may have been concerned.

It is interesting to note that in children we have found no such correlation; in them the phosphate followed closely the total N. In children, the condition is rendered more complex by the presence of growth metabolism together with the maintenance metabolism. Children did not show the same rhythm, probably because of different conditions with regard to sleep.

SUMMARY.

1. Observations on day and night urine during a routine of day starvation, a routine of complete starvation and a reversed routine—that is with work and meals at night and with sleep during the day—are recorded.

2. Evidence was obtained that the urine was more acid at night, neither because the taking of food during the day produced alkaline tides, nor because the respiratory centre was depressed during sleep.

3. After four days of complete reversal of habit most of the differences between day and night urine still remained as before, so that there must be a fixed physiological rhythm connected with excretion by the body cells. Thus, although in the "reversed routine," most of the fluid was swallowed at night, the greater amount of urine was still excreted during the day which now included the sleep period. Also, although most of the food taken was eaten at night, the total N excretion still remained higher during the day than at night.

4. In the excretory rhythm referred to, the total N, urea N, water and chloride were excreted in greater amount during the day whilst the ammonia N, acidity and phosphate were higher during the night than during the day.

5. The higher figures for phosphate whether found by day or by night always accompanied sleep and could be separated from every other factor except sleep; and, with one exception, the higher phosphate excretion accompanied both the higher acidity and the higher ammonia N excretion in all routines examined. An absolute increase in phosphate occurred in the "reversed routine."

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