DIURNAL VARIATION IN THE URINARY EXCRETION OF NEUTRAL LIPID-SOLUBLE REDUCING STEROIDS IN CONGESTIVE CARDIAC FAILURE AND CIRRHOSIS OF THE LIVER WITH ASCITES [1, 2]

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Pincus and his collaborators (1–4) have demonstrated that there is a normal cycle of excretion of neutral, lipid-soluble reducing steroids which has a maximum between 7 a.m. and 11 a.m., decreases during the afternoon, and is at a minimum during the night. This corresponds to the normal excretory cycle of other urinary constituents (5–13). It has also been shown that in congestive cardiac failure (14, 15) and in cirrhosis of the liver with ascites (16) there is a reversed diurnal cycle of water and sodium excretion, with maximum values during the night in a high proportion of the individuals studied. This investigation was designed to determine if the excretion of steroids was also altered by these conditions.

MATERIAL AND METHODS

Ten normal male adults were used as controls. Fourteen male patients with congestive heart failure, of various etiologies and in differing degrees of decompensation, and 12 male patients with cirrhosis of the liver and ascites comprised the abnormal groups. Almost all of the patients were on a standard hospital diet containing a calculated 1,000 milligrams of sodium daily. Diuretics were not used within 48 hours of the urine collection periods, and no clinical evidence of urinary obstruction was present. Three consecutive, eight-hour, spontaneously voided urine specimens were collected from each individual. The one collected between 6 a.m. and 2 p.m. was designated the morning specimen, the one collected between 2 p.m. and 10 p.m. was designated the afternoon specimen, and the one collected between 10 p.m. and 6 a.m. was designated the night specimen. The time of the night specimen was chosen so that it would coincide most closely with the hours of sleep. The volume of each urine was measured and the quantity of neutral

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TABLE I

Normal subjects

Pt.	Age	Date	Steroids*				Water			
			Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night
			mg.	%	%	%	*ml.*	%	%	%
C. D.	30	Feb. '51	2.95	52	22	26	1,165	54	14	32
W. A.	31	Feb. '51	3.25	45	25	30	1,550	61	16	23
S. B.	54	Mar. '51	3.23	38	37	25	1,190	45	34	21
E. M.	30	Mar. '51	3.43	45	29	26	1,070	65	20	15
W. M.	30	Mar. '51	2.62	39	30	31	1,425	50	19	31
R. G.	31	Sept. '50	2.51	40	29	31	1,822	37	30	33
W. F.	29	Sept. '50	2.60	50	30	20	1,230	54	32	14
G. M.	60	Apr. '51	2.63	38	33	29	1,065	40	30	30
M. L.	30	Mar. '51	4.35	37	33	30	1,370	33	31	36
W. B.	30	May '51	2.47	35	35	30	1,153	40	30	30
Standard deviation			0.59	5.8	4.5	3.5	240	10.6	7.5	7.7
Mean			3.00	**42**	30	28	1,304	**48**	26	26

* Neutral lipid-soluble reducing steroids.
Boldface value for mean indicates that difference from mean night value exceeds 0.05 level of confidence.

TABLE II

Congestive cardiac failure

Pt.	Age	Date	Steroids*				Water				Sodium				Creatinine				Remarks
			Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night	
			mg.	%	%	%	*ml.*	%	%	%	*mg.*	%	%	%	*mg.*	%	%	%	
E. P.	58	Sept. '50	2.28	41	27	32	1,034	31	41	28	730	36	29	35	1,190	33	34	33	Old myocardial infarction. Compensated.
		May '51	3.15	25	34	41	1,390	24	40	36	524	13	8	79	795	26	41	33	Decompensated. Died June '51 of new infarct.
E. J.	62	Sept. '50	3.51	48	27	25	1,225	21	39	40	524	16	28	56	1,126	30	32	38	Hypertensive and arteriosclerotic heart disease. Old myocardial infarction. Aortic aneurysm. Compensated.
W. K.	57	Mar. '51	3.95	30	38	32	2,530	24	23	53	2,213	29	3	68	1,392	34	36	30	Arteriosclerotic heart disease. Old myocardial infarction. Decompensated. Improving.
J. T.	51	Mar. '51	3.95	27	32	41	1,495	23	19	58	19	21	26	53	1,063	29	33	38	Rheumatic heart disease. Mitral stenosis, severely and recurrently decompensated. Improving. Died suddenly July '51 of pulmonary embolism.
E. L.	73	Mar. '51	1.79	18	41	41	1,036	12	33	55	1,350	10	35	55	808	16	39	45	Hypertensive and arteriosclerotic heart disease. Decompensated. Improving.
G. W.	32	Apr. '51	3.41	38	31	31	925	35	31	34									Mitral and aortic valvulitis with healed subacute bacterial endocarditis. Severely decompensated, pulmonary emboli. Died April '51.
E. D.	28	Apr. '51	3.59	29	32	39	1,515	32	38	30									Rheumatic heart disease, aortic and mitral valvulitis, subacute bacterial endocarditis. Moderately and recurrently decompensated. Improving.
J. H.	61	Apr. '51	2.24	27	44	29	1,375	21	46	33									Rheumatic heart disease, mitral stenosis and insufficiency, auricular fibrillation, recurrently decompensated. Improving.
C. T.		Apr. '51	1.41	30	31	39	995	30	23	47	1,363	32	17	51	830	32	25	43	Arteriosclerotic heart disease, decompensated, improving.
C. W.	58	May '51	3.51	39	39	22	1,445	28	52	20	1,289	25	52	23	1,106	39	41	20	Pulmonary fibrosis and emphysema, cor pulmonale, chronic congestive failure, improving.
J. C.	63	May '51	2.43	28	28	44	625	23	33	44	214	17	41	42	1,407	25	35	40	Hypertensive and arteriosclerotic heart disease. Decompensated, improving.
A. Z.	50	May '51	3.11	30	28	42	1,295	22	33	45	1,704	18	37	45	2,260	26	30	44	Rheumatic heart disease with mitral stenosis. First time decompensated. Improving.
H. P.	57	May '51	3.21	28	32	40	1,220	23	32	45	815	16	30	54	1,449	23	37	40	Luetic heart disease. Mildly decompensated. Improving.
J. P.	57	Jun. '51	2.80	30	46	24	1,280	27	43	30	223	25	45	30	1,517	27	48	25	Arteriosclerotic heart disease. Old myocardial infarction. Decompensated. Improving. Died suddenly a few days after study.
Standard deviation			0.79	7.4	6.2	7.3	420	5.6	9.0	11.0	†	8.0	14.5	15.6	408	6.1	6.0	7.8	
Mean			2.96	31!	34	35!	1,292	25!	35!	40!	914	22	29	49	1,245	28	36	36	

* Neutral lipid-soluble reducing steroids.

† Standard deviation not determined; several patients not on 1,000 milligram sodium diet.

Boldface value for mean indicates that difference from mean night value exceeds 0.05 level of confidence.

Exclamation point after mean value indicates that difference from corresponding normal value exceeds 0.05 level of confidence.

TABLE III

Cirrhosis of the liver with ascites

Pt.	Age	Date	Steroids*				Water				Sodium				Creatinine				Remarks
			Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night	Total	Morning	Afternoon	Night	
			mg.	%	%	%	*ml.*	%	%	%	*mg.*	%	%	%	*mg.*	%	%	%	
E. L.	61	Jul. '50	2.39	23	43	34	891	10	60	30	340	1	31	68	1,529	30	35	35	Moderately severe ascites, improving, ambulatory.
O. R.	55	Jul. '50	1.61	23	48	29	1,138	17	63	20	164	18	35	47	1,372	30	34	36	Moderate, increasing ascites, ambulatory.
O. L.	52	Oct. '50	2.07	36	26	38	723	23	38	39	457	20	66	14	1,155	28	28	44	Severe, increasing ascites, ambulatory.
A. R.	55	Oct. '50	2.87	34	37	29	1,120	26	36	38	400	26	16	58	1,231	28	40	32	Severe ascites, ambulatory.
R. M.	58	Aug. '50	2.12	12	36	52	1,900	45	21	34	30	9	30	61					Moderate ascites, ambulatory. Died Sept. '50 of hemorrhage.
H. G.	57	Mar. '51	4.56	22	29	49	1,575	15	39	46	123	6	8	86	1,298	23	35	42	Severe, progressive ascites, ambulatory. Died May '51 of hemorrhage.
E. H.	60	May '51	1.79	26	20	54	172	29	19	52	15	30	15	55	278	31	19	50	Severe ascites, absolute bed rest.
J. V.	41	May '51	6.40	29	37	34	1,320	19	47	34	2,821	20	54	26	1,045	26	38	36	Ascites cleared, ambulatory.
W. L.	47	May '51	4.35	48	27	25	935	70	16	14	154	45	26	29	957	42	33	25	Severe ascites, absolute bed rest.
A. H.	54	Jun. '51	3.88	30	39	31	1,080	20	28	52	680	11	31	58	1,335	27	36	37	Decreasing ascites, slightly ambulatory.
S. H.	41	Jun. '51	3.99	43	32	25	1,100	41	37	22	69	38	38	24	1,231	41	35	24	Severe ascites, lavatory privileges only. Died July '51 of peritonitis.
H. H.	43	Jun. '51	3.83	35	22	43	1,140	25	12	63	349	15	15	70	979	33	16	51	Ascites subsiding. Ambulatory.
Standard deviation			1.40	9.9	8.4	10.3	425	16.6	16.4	14.6	†	13.0	16.8	21.9	331	5.9	7.7	8.9	
Mean			3.32	31!	33	37!	1,091	28!	35	39!	467	**20**	**30**	50	1,128	31	32	37	

*Neutral lipid-soluble reducing steroids.
† Standard deviation not determined; several patients not on 1,000 milligram sodium diet.
Boldface value for mean indicates that difference from mean night value exceeds 0.05 level of confidence.
Exclamation point after mean value indicates that difference from corresponding normal value exceeds 0.05 level of confidence.

lipid-soluble reducing steroids [3] determined by the method of Heard, Sobel and Venning (17). In almost all of the specimens obtained from the cardiac and cirrhotic patients, the quantity of excreted sodium was determined by means of the Beckman flame photometer and the quantity of excreted creatinine was determined by the Bonsnes and Taussky (18) modification of the Jaffe reaction.

RESULTS

The tables summarize the clinical data and the results. The total amount of the excreted substance is indicated in the first column of each group, and the succeeding three columns indicate the amount excreted in the morning, afternoon and night periods, respectively, expressed as per cent of the total amount excreted.

Table I indicates the results obtained in the normal subjects. All demonstrated a morning maximum steroid excretion, and most had a morning maximum water excretion. The average 24-hour excretion of steroids was 3.00 milligrams.

The pattern of excretion of steroids, water, sodium and creatinine in 14 patients with congestive cardiac failure is seen in Table II. The average 24-hour excretion of steroids was 2.96 milligrams, which was remarkably close to the normal value. However, the distribution of the excreted steroids showed a definite alteration: in only four studies was the maximum excretion in the morning, and in two of these, the patients (E. P. and E. J.) were very well compensated by intensive therapy. The steroid pattern resembled the excretory cycles of the water, sodium and creatinine. It can be seen that the fluctuations in excretion of steroids, water and creatinine from period to period are of the same order, but that excretion of sodium shows more marked variations.

The observations made on 12 patients with cirrhosis of the liver and ascites are seen in Table III. The results compare closely to those of the cardiac group. The average 24-hour excretion of steroids, 3.32 milligrams, is slightly higher than for the other two groups. However, these differences are not statistically significant. It can be seen that the excretory cycle is abnormal in most instances, that the fluctuation in the water, creatinine and steroid cycles is less than normal, while fluctuation in the sodium cycle is proportionately greater, and that most of the variations in excretion of the observed substances from period to period are in the same direction. Despite the apparent trend, the differences in the creatinine values are not significant, and cannot be interpreted to demonstrate a nocturnal increase in the glomerular filtration rate.

[3] Hereafter referred to as steroids.

DISCUSSION

Most studies on diurnal variation in urinary excretion have concerned the normal patterns. These patterns have been well documented, and there is general agreement among investigators that the maximum output of water, sodium, potassium, chloride, urea and creatinine is during the day, with a peak either just before or just after noon (5–13). Recently, a number of studies on abnormal variations have appeared, especially pertaining to the nocturnal diuresis found in patients with congestive cardiac failure (14, 15). We have recently shown that there is a similar nocturnal diuresis in cirrhosis of the liver with ascites (16). Although a nocturnal increase in renal plasma flow has been demonstrated in congestive cardiac failure (14, 15), which is associated with an increased glomerular filtration rate, this does not necessarily explain the entire mechanism of the nocturia. To our knowledge no similar studies of renal plasma flow and glomerular filtration rate have been performed on patients with cirrhosis of the liver. In both of these conditions a nocturnal diuresis of water and sodium has been observed, but in our series (16) most of the patients exhibited no consistent associated diuresis of potassium.

The work of Pincus and his collaborators demonstrated that normally there was a maximum excretion of steroids during the morning, a decreased excretion during the rest of the day, and a minimum during the night. They believed that the morning increase was not due to increased filtration alone, since the excretion of steroids showed a greater proportionate increase than did the excretion of creatinine. The variation was considered to be the result of the increased stress due to resumption of activity after sleep. They noted that specific stress situations were associated with an increase in adrenal cortical steroid excretion occurring within a short time following the application of the stress.

The present studies confirm the nature of the steroid excretory cycle in normal individuals. However, there is a fairly consistent relationship between the excretion of steroids, water and creatinine, suggesting that there may be a similar excretory mechanism for each, and that under these conditions excretion may be related to the filtration rate. A similar relationship is seen in congestive failure and cirrhosis, although there is displacement in the time of peak excretion. This shift in the cycle cannot be attributed to periods of excessive stress, first because the total steroid excretion is no greater than in the normals, and second because there is no evidence of an altered stress cycle. It may be suggested that in some of the patients with congestive cardiac failure nocturnal episodes of paroxysmal dyspnea and insomnia may be related to increased stress; however, patients who slept soundly during the collection period also demonstrated increased nocturnal steroid excretion. None of the patients with cirrhosis showed evidence of disturbed sleep other than that caused by the inconvenience of the nocturia itself. It is apparent either that more steroids are being presented to the kidney during the night, or that the kidney is relatively more efficient in excreting the steroids at that time.

From the data it appears that the amount of steroid excreted in 24 hours is relatively constant, and that the values for patients with congestive cardiac failure and cirrhosis of the liver are not significantly different from the normals. Abnormal patterns of diurnal urinary excretion of water, sodium, and creatinine are associated with similarly abnormal patterns of steroid excretion. It does not seem possible to determine from the data whether this abnormal excretory pattern for water, sodium and creatinine results from the pattern of steroid excretion, or whether the rate of steroid excretion is itself governed by the same alternate factors. In addition to the absence of evidence of increased adrenocortical activity, there was no evidence of an alteration in the stress cycle of these patients. Gaunt, Birnie and Eversole (19) have reviewed data demonstrating that an adequate amount of cortical hormone is necessary for water diuresis. However, they point out that at the same time as there is increased cortico-steroid there is usually an increased retention of sodium. While this may be true on an overall balance, fluctuations in sodium excretion during the day are in the same direction as fluctuations in steroid excretion, rather than in the opposite direction as would be expected.

CONCLUSIONS

1. The average 24-hour excretion of neutral lipid-soluble reducing steroids was 3.00 milligrams in ten normal adults, 2.96 milligrams in 14 patients with decompensated or recompensated cardiac failure, and 3.32 milligrams in 12 patients with cirrhosis of the liver and ascites.

2. In all of the normal controls there was a significant morning maximum excretion of steroids. In both of the abnormal conditions studied the period of maximum excretion was most commonly observed in the afternoon or during the night.

3. With few exceptions, the excretion of steroids in cardiac failure and cirrhosis was proportional to the excretion of creatinine within the limits of error of the methods. This suggests that the excretion of steroids may be proportional to the glomerular filtration rate.

4. Since the total steroid excretion is within normal range, there is no evidence of increased or abnormal stress as a cause of the abnormal steroid excretory pattern. It is possible that the glomerular filtration rate is proportional to adrenocortical activity as measured by steroid excretion. However, it seems more probable that the urinary content of the steroid is proportional to the excretory rate.

5. In the normal and abnormal states studied, the water excretion generally paralleled that of creatinine and steroids, while the sodium excretion showed more marked variations, usually, but not invariably, in the same direction.

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REFERENCES

1. Pincus, G., A diurnal rhythm in the excretion of urinary ketosteroids by young men. J. Clin. Endocrinol., 1943, 3, 195.
2. Pincus, G., Studies of the role of the adrenal cortex in the stress of human subjects. Recent Progr. Hormone Research, 1947, 1, 123.

3. Pincus, G., Romanoff, L. P., and Carlo, J., A diurnal rhythm in the excretion of neutral reducing lipids by man and its relation to 17-ketosteroid rhythm. J. Clin. Endocrinol., 1948, **8**, 221.
4. Romanoff, L. P., Plager, J., and Pincus, G., The determination of adrenocortical steroids in human urine. Endocrinology, 1949, **45**, 10.
5. Cathcart, E. P., Kennaway, E. L., and Leathes, J. B., On the origin of endogenous uric acid. Quart. J. Med., 1907–1908, **1**, 416.
6. Gerritzen, F., The rhythmic function of the human liver. Acta med. Scand. Suppl., 1940, **108**, 121.
7. Kleitman, N., Studies on the physiology of sleep. I. The effects of prolonged sleeplessness on man. Am. J. Physiol., 1923, **66**, 67.
8. Kleitman, N., Sleep and Wakefulness as Alternating Phases in the Cycle of Existence. Univ. Chicago Press, Chicago, 1939.
9. MacKay, E. M., Studies of urea excretion. V. The diurnal variation of urea excretion in normal individuals and patients with Bright's disease. J. Clin. Invest., 1928, **6**, 505.
10. Manchester, R. C., The diurnal rhythm in water and mineral exchange. J. Clin. Invest., 1933, **12**, 995.
11. Simpson, G. E., Diurnal variation in the rate of urine excretion for two hour intervals: some associated factors. J. Biol. Chem., 1924, **59**, 107.
12. Simpson, G. E., Changes in the composition of urine brought about by sleep and other factors. J. Biol. Chem., 1929, **84**, 393.
13. Sirota, J. H., Baldwin, D. S., and Villarreal, H., Diurnal variations of renal function in man. J. Clin. Invest., 1950, **29**, 187.
14. Baldwin, D. S., Sirota, J. H., and Villarreal, H., Diurnal variations of renal function in congestive heart failure. Proc. Soc. Exper. Biol. & Med., 1950, **74**, 578.
15. Fejfar, Z., and Brod, J., The excretion of chlorides in patients with heart failure. Quart. J. Med., 1950, **19**, 221.
16. Goldman, R., Studies in diurnal variation of water and electrolyte excretion: nocturnal diuresis of water and sodium in congestive cardiac failure and cirrhosis of the liver. J. Clin. Invest., 1951, **30**, 1191.
17. Heard, R. D. H., Sobel, H., and Venning, E. H., The neutral lipide-soluble reducing substances of urine as an index of adrenal cortical function. J. Biol. Chem., 1946, **165**, 699.
18. Bonsnes, R. W., and Taussky, H. H., On colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem., 1945, **158**, 581.
19. Gaunt, R., Birnie, J. H., and Eversole, W. J., Adrenal cortex and water metabolism. Physiol. Rev., 1949, **29**, 281.