24-HOUR PERIODICITY OF ADRENAL CORTEX

TOSHIO TORII, SATOSHI SASAKI, SEIJI MURANAKA,
TAKESHI MORINAGA, SHOJIRO KUSAHANA AND
HISAYOSHI YOSHIZAWA

Institute of Physical Therapy and Internal Medicine, Department of
Medicine, Tokyo University, Tokyo, Japan

SIX FIGURES

The diurnal rhythm in the urinary excretion of 17-ketosteroids and neutral
reducing lipids has been reported by Pincus. On the other hand the diurnal
variation of circulating eosinophils in normal subjects has been studied by Rud, Bonner and others. The characteristic feature in diurnal rhythm of urinary
steroids is an increase in the morning especially after awakening. Meanwhile
the morning fall in circulating eosinophils such as termed “endogenous eosino-
penia” and high level during the night time were established. Halberg has studied eagerly on 24-hour periodicity in various bodily functions with special re-
ference to adrenal cortical function.

In this paper a quantitative study on 24-hour periodicity in circulating
eosinophils and urinary steroids will be presented.

MATERIALS AND METHODS

Materials: Four healthy adults and nine patients with pulmonary tuber-
culosis in after-care ward aged from 21—51 years were subjected to this study,
who had no clinical sign of adrenal cortical abnormality and kept almost bed
rest except meals during the examination.

Counting of eosinophils: Capillary blood was taken from ear lobe at every
3 hours interval from morning (06:00) to the same time of the following day. For staining and dilution of blood Hinkleman’s direct method was used.

Urine specimens: Urine was collected at every 3 hours interval over 24
hours.

Estimation of urinary 17-ketosteroids: Original Drekter’s method was used.

Estimation of urinary total 17-hydroxycorticoids: Reddy’s original method
was modified by use of highly purified n-butanol and longer incubation period
(30 minutes) with 17 N sulfric acid for Porter-Silber’s reaction.

RESULTS

Relative eosinophil levels expressing each count as a percentage of the day
mean have been computed and are presented in Fig. 1-a. Larger differences exist

Received for publication April 10, 1955.
Fig. 1-a. 24-hour periodicity of circulating eosinophils
— Mean value of each time section.

Fig. 1-b. Significance of differences between eosinophils in varying time sections. Figures designate the time sections presented in Fig. 1-a.

** below 1% level.
* below 5% level.
in the eosinophil levels of different subjects as well as in the levels of the same subject on different days of sampling.

An analysis of variances was therefore undertaken to segregate source of variation and to examine the significance of the "hour of day" effect. The

![Fig. 2-a. 24-hour periodicity of urinary 17-ketosteroids.](image)

Mean value of each time section.

![Fig. 2-b. Significance of differences between percent output of urinary 17-ketosteroids in varying time sections. Figures designate the time sections presented in Fig. 2-a.](image)

** below 1% level.
* below 5% level.
summary of analysis is presented in Fig. 1-b, in which eosinophil levels at various
time of day can be compared with statistical significance. The maximal eosino-
phil level appeared at midnight and the minimal level at noon.

In Fig. 2-a, the 24-hour periodicity of urinary 17-ketosteroids excretion is
shown. Data represent percentage of daily total excretion (3 hours output/24
hours output × 100).

![Chart showing 24-hour periodicity of urinary total 17-hydroxycorticoids.](image)

Fig. 3-a. 24-hour periodicity of urinary total 17-hydroxycorticoids.

Mean value of each time section.
The maximal excretion of urinary 17-ketosteroids was observed in the morning after awakening (06:00—09:00) and minimal value was obtained at the time period just before awakening (03:00—06:00).

We present the 24-hour periodicity of urinary total 17-hydroxycorticoids in Fig. 3. The feature of the rhythm is similar to that of 17-ketosteroids. The maximal value was observed at the awakening time (06:00—09:00) and minimal one at midnight (00:00—03:00).

In Fig. 4, the 24-hour periodicity of eosinophils and urinary steroids are summarized as to elucidate the mutual relationship.

A correlation between eosinophils and 17-ketosteroids is shown in Fig. 5. Each point represents percentage value of eosinophil level and that of 17-ketosteroids.

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**Fig. 3-b.** Significance of differences between percent output of urinary total 17-hydroxycorticoids in varying time sections. Figures designate the time sections presented in Fig. 3-a.

** below 1% level.
* below 5% level.

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**Eosinophils**

17-KS

17-OH-CS

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Fig. 4. 24-hour periodicity of eosinophils and urinary steroids.
Fig. 5. Correlation between circulating eosinophils and urinary 17-ketosteroids.

Fig. 6. Correlation between circulating eosinophils and urinary total 17-hydroxycorticoids.
out put in each individual at the same time. The correlation coefficient is -0.343 and statistically significant below 1% level.

In Fig. 6 the correlation between eosinophils and urinary total 17-hydroxycorticoids excretion is shown. The correlation coefficient is -0.307 and also statistically significant below 1% level.

**DISCUSSION**

Many papers concerning 24-hour periodicity of bodily functions have been published up to the present time. Among these phenomena, endogenous eosinopenia shows synchronization with change in variable, some of which are currently accepted as indices of adrenal cortical function. 24-hour periodicity in the glycogen content of the liver has been observed in mice and in other mammals, including man. On the average, glycogen levels decrease during the ascending phase of eosinophil rhythm. A higher relative specific activity of phospholipid phosphorus and of pentosenucleic acid phosphorus from liver microsomes was noted in materials obtained during a period associated with a low eosinophil level. It is also noted that a difference has been reported for lipid content of the adrenal cortex in white mice killed at two different time of day. In mice killed at 09:00 (high eosinophil phase) the zona fasciculata was filled with lipid in tuto. In mice killed at midnight (low eosinophil phase) the lipid did not extend the outer third of the fasciculata.

Increase during the morning hours in the urinary excretion of 17-ketosteroids and 17-hydroxycorticoids, where the endogenous morning eosinopenia occurs, and decrease during the night in the out put of urinary steroids, where the eosinophil level increases, are presented in this paper. Furthermore, a negative correlation between the percentage changes in eosinophil counts and that of 17-ketosteroids or 17-hydroxycorticoids is accertained with statistical significance. It is conceivable therefore, that the 24-hour periodicity in number of eosinophils and possibly some of the associated 24-hour periodicities in man are controlled by periodic changes in the rate of secretion of adrenal cortical hormones. It has been demonstrated that patients with surgical adrenalectomy, Addison's disease and panhypopituitalism do not exhibit a 24-hour rhythm in number of circulating eosinophils.

Halberg and other authors reported that temporal placement of eosinophil rhythm in mice could be shifted under various experimental conditions. The endogenous eosinopenia in man also modulated by initiation of daily activities and is markedly impaired in blind subjects and deaf children.

Recently a significant seasonal rhythm in adrenal cortical function in man has been demonstrated by our research group. Higher excretion of urinary 17-ketosteroids and larger response of adrenal cortex in normal subjects against intravenous ACTH were observed during cold seasons than during warmer seasons. Some of the bodily functions, especially electrolytes and nitrogen metabolism was accepted fairly synchronized with the adrenal cortical function. Subsequently, bronchial asthma, as an example of “weather or season sensitive disease,” was investigated with special reference to adrenocortical function. The maximal response of the adrenal cortex to intravenous drip of ACTH by Renold's method.
was in lesser degree than that of normal controls. The significant seasonal variation of adrenal cortical response to in tavenous ACTH in asthmatic patients could not be demonstrated. Furthermore, the difference between maximal and minimal percentage value of circulating eosinophils in 24-hour rhythm was significantly smaller than that of normal subjects.

From theoretical and also clinical point of view 24-hour periodicity of bodily functions, especially, adrenal cortical cycle deserves a new methodological approach to the investigation of "stress" phenomena as Halberg already suggested.

**SUMMARY**

24-hour periodicity in circulating eosinophils and urinary excretion of 17-ketosteroids and total 17-hydroxycorticoids in normal subjects were studied.

1) The maximal level of circulating eosinophils was observed at midnight and minimal one at noon.

2) The maximal excretion of urinary 17-ketosteroids was observed during the morning hour (06:00—09:00) and minimal one at the time period just before awakening (03:00—06:00).

3) The feature of the rhythm of urinary total 17-hydroxycorticoids was similar to that of 17-ketosteroids. The maximal value was observed at the same time as 17-ketosteroids (06:00—09:00), but minimal one at midnight (00:00—03:00).

4) The correlation coefficient between relative value of circulating eosinphils and that of 17-ketosteroids or 17-hydroxycorticoids are -0.343 and -0.307 respectively. These figures are not large, but statistically significant below 1% level.

**REFERENCES**