

## NOCTURNAL ASTHMA ATTACKS: THEIR RELATIONSHIP TO THE CIRCADIAN† ADRENAL CYCLE

Alain Reinberg, M.D., Ph.D.,\* Jean Ghata, M.D.,\*\* and Edwin Sidi, M.D.,\*\*\* Paris, France

SEVERAL lines of information bear upon the problem of a possible role played by adrenal cortical function in the asthmatic attack. Experimental asthma in the guinea pig is followed by adrenal cortical hyperactivity, at least as far as this may be judged histologically.<sup>3</sup>

Furthermore, an attack of asthma in a patient may be suppressed, more or less completely, by the administration of cortisone, cortisol, and some of their derivatives, as well as by ACTH.<sup>4, 9, 10</sup> In these cases there is a therapeutic excess of corticoids. Hence, the latter act via their pharmacodynamic properties and almost certainly not by compensating for an as-yet-unproved hormonal deficiency. In keeping with the latter interpretation are reports that the 24-hour urinary excretion of adrenal steroids is not demonstrably abnormal in patients suffering from asthma, vasomotor rhinitis, mild urticaria, or hay fever.<sup>33, 34</sup> These findings, in turn, led several authors to suggest that pituitary-adrenal function is not usually involved in the physiopathology of asthma. If one's attention is restricted to gross deviations from a so-called normal range of values—in this case the range for urinary steroids—this conclusion does seem to be justified. The question then is whether, in a disease such as asthma, adrenal secretions are within the limits of the normal range of values.

It is true that the determination of steroid content in 24-hour urines provides a useful global source of information. Often, this urinary datum is diagnostically more valuable than a determination made on a single plasma sample—which may be too high or too low as a function of the high amplitude changes in plasma steroids along the 24-hour scale. This advantage of the 24-hour urine sample notwithstanding, a shortcoming of this type of information also must be noted. The combined 24-hour urine cannot reflect important and predictable physiological or physiopathological changes during the 24-hour period and their time relation to certain pathologic events.<sup>14</sup>

From the Department of Dermato-Allergie of the Foundation A. de Rothschild, 29 rue Manin, Paris, France, and the Laboratoire de Physiologie Chimique, Université de Paris.

Received for publication Feb. 1, 1963.

\*Maitre de Recherches, Centre National de la Recherche Scientifique, Paris, France.

\*\*Attaché de Recherches, Institut National d'Hygiène, Paris, France.

\*\*\*Chief of Service, Foundation Adolphe de Rothschild, Paris, France.

†Circadian is derived from *circa*—about and *dies*—day. Use of the adjective circadian, in lieu of "diurnal," removes the ambiguities associated with the latter, which denotes both part of the day (day-time versus night-time) and the entire day. Apart from semantics, however, circadian is used to emphasize that following certain manipulations of the organism or of its environment, many physiologic rhythms show periods that deviate from exactly 24 hours by a few minutes or hours.<sup>7-14</sup>

Thus, in man, a circadian adrenal cycle involves high amplitude changes in several functions, including the steroid content of both plasma and urine.<sup>2, 8, 15, 16, 17, 19, 23, 24, 28, 29, 30, 32, 37</sup> In human beings studied under conditions of diurnal activity and nocturnal rest, low levels of plasma steroids are usually reached in the evening hours. The curve for urinary excretions lags behind that for plasma levels by a few hours.

In many patients with asthma, attacks or dyspnea may be accentuated more often by night than by day.<sup>25, 26, 27, 39</sup> It is of interest, therefore, to raise the question whether patients with asthma have a rhythm in urinary steroid excretion such as that known for healthy man. In the present study, patients were selected whose attacks occurred mostly at night. We explored concomitantly the external timing<sup>18</sup> of asthmatic paroxysms and steroid and potassium excretion rhythms. Such a study, in itself, does not bear directly upon causal relations, but it provides a necessary basis for further applications of periodicity analysis—by phase-shifts and other means—for estimating the extent to which changes in one function, if any, might contribute to certain other functional alternations.<sup>13</sup> With this qualification, it will be reported, first, that patients with asthma show significant circadian rhythm in urinary steroid and potassium excretion. Second, the time relation of the asthmatic attacks and of minimal steroid excretion also will be described for an exploratory series of patients.

#### SUBJECTS, MATERIALS, AND METHODS

We have studied circadian variations in urinary excretion of 17-hydroxycorticosteroids, 17-ketosteroids, and potassium in 8 adult asthmatic subjects (4 of each sex) and in 4 healthy subjects (2 of each sex) (see Table I).

The 8 patients were all suffering from typical allergic asthma of exogenous origin. The clinical history, intradermal tests, and a decrease in expiratory

TABLE. I

| CASE NO.           | SEX | AGE | TIME* OF ASTHMA ATTACKS | TIME* OF LOWEST URINARY EXCRETIONS IN: |                 |                 |
|--------------------|-----|-----|-------------------------|----------------------------------------|-----------------|-----------------|
|                    |     |     |                         | 17-HYDROXY-CORTICOSTEROIDS             | 17-KETOSTEROIDS | POTASSIUM       |
| Asthmatic Subjects |     |     |                         |                                        |                 |                 |
| 1                  | F   | 26  | 01.00-04.00             | (23-03)                                | (23-03)         | (23-03)         |
| 2                  | F   | 44  | 02.00-04.00             | (23-03)                                | (19-23) (23-03) | (19-23) (23-03) |
| 3                  | F   | 63  | 01.15-04.00             | (23-03)                                | (23-03) (03-07) | (23-03)         |
| 4                  | F   | 43  | 24.00-02.00             | (19-23) (23-03)                        | (15-19) (19-23) | (23-03)         |
| 5                  | M   | 55  | 23.00-02.00             | (23-03)                                | (23-03)         | (23-03) (03-07) |
| 6                  | M   | 31  | 24.00-01.30             | (11-15) (23-03)                        | (11-15) (23-03) | (11-15) (23-03) |
| 7                  | M   | 48  | 23.00-02.00             | (19-23) (23-03)                        | (15-19) (19-23) | (19-23) (23-03) |
| 8                  | M   | 29  | 01.00-04.00             | (19-23) (23-03)                        | (23-03) (07-11) | (19-23) (07-11) |
| Control Subjects   |     |     |                         |                                        |                 |                 |
| 9                  | F   | 33  | —                       | (23-03)                                | (23-03)         | (23-03)         |
| 10                 | F   | 25  | —                       | (23-03)                                | (23-03)         | (23-03)         |
| 11                 | M   | 45  | —                       | (23-03) (03-07)                        | (23-03) (03-07) | (23-03) (03-07) |
| 12                 | M   | 36  | —                       | (23-03) (15-19)                        | (23-03) (15-19) | (23-03)         |

\*The time in the table represents international scientific terminology. 01 hr. to 12 hr. corresponds to 1 A.M. to noon and 13 hr. to 24 hr. to 1 P.M. to midnight.

flow rate on inhalation of antigens showed allergic reactions to dust, either isolated (Cases 1 and 4) or associated with other sensitizations (feathers, pollen, micro-organisms).

Attacks of asthma occurred only at night in 6 of the patients (Cases 1, 2, 4, 6, 7, 8); in the other 2 patients (3 and 5) some dyspnea was sometimes present during the day, but its accentuation during a certain part of the night was pronounced. We asked each patient to note very precisely, for several weeks and, of course, during the test, the exact time when an attack began and when it ended. Those data appear in Table I and Fig. I.

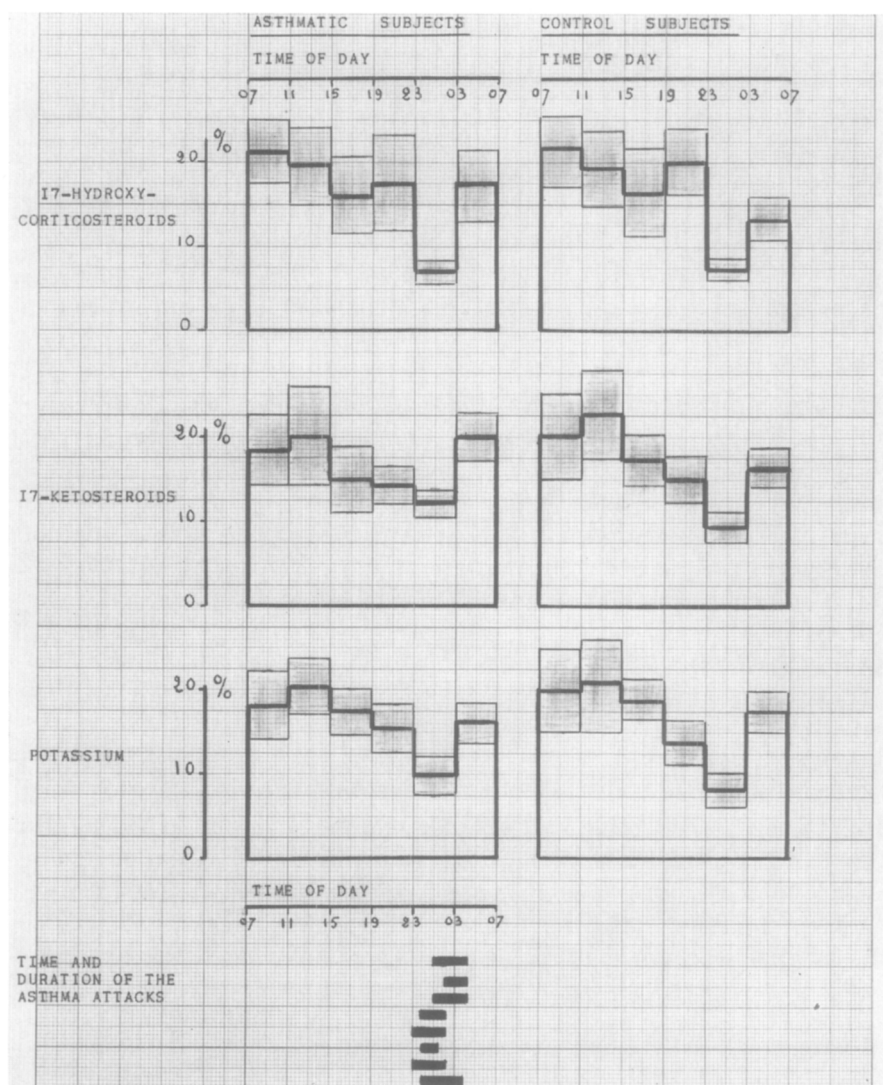


Fig. 1.—Urinary excretions for 17-hydroxycorticosteroids, 17-ketosteroids, and potassium are given in percentage per day, every 4 hours. The upper and lower shadowed rectangles represent  $t$  times the standard error of the mean,  $t$  being Student Fisher's parameter with 95 per cent security.

The control subjects, physicians or nurses, appeared to be in good health and were suffering neither from asthma nor from any other allergic condition. We verified further by extensive clinical examinations and routine laboratory procedures that the renal, cardiovascular, and endocrine functions of all 12 subjects could be regarded as normal.

At the time of the test the patients were either hospitalized or stayed in their homes; the normal subjects also remained at their homes for the period of study. The subjects stayed in bed at night (9 P.M. to 7 A.M.) and followed nonstrenuous leisure-time activities during the day. All subjects ate their customary diet, without modification or restriction, except that meals were taken at 8 A.M., 12 noon, and 7 P.M.

The subjects emptied their bladders at 7 A.M. and the collection of urines began thereafter. The urines were collected in six different vessels labeled with the six successive 4-hour periods. At any time during this period, but always also at the end of each 4-hour period, each subject voided and thereafter immediately refrigerated the urine sample.

The method of Jayle and associates,<sup>35</sup> a modification of the Silber-Porter procedure, was used for the determination of 17-hydroxycorticosteroids, and that of Drekter for 17-ketosteroids.<sup>36</sup> Potassium was evaluated by flame photometry. All samples from a given subject were analyzed at the same time.

## RESULTS

Results on 17-hydroxycorticosteroids, 17-ketosteroids, and potassium, obtained for the six 4-hour periods, are expressed as a percentage of the total 24-hour excretion (Fig. 1). This presentation allows for a fairer comparison of the changes with time in individuals with different total excretions. An average of these data expressed as per cent of the total and the standard errors of these means also are shown in Fig. 1.

1. The total 24-hour urinary excretion of adrenal steroids and potassium can be regarded as normal in our asthmatic patients as well as in the healthy subjects (Table II).

Our results agree well with the corresponding data on patients with asthma reported earlier by Rose<sup>33</sup> and Roskam<sup>34</sup> and their colleagues.

In all 8 patients, a circadian rhythm characterized the urinary excretion of the three substances examined. This rhythm corresponded roughly in extent of change and in its timing to similar data obtained earlier for healthy adult man. Let us remember that the rhythm in potassium excretion and several other circadian rhythms—though not all of them<sup>13</sup>—are obliterated, as group phenomena, in adrenal insufficiency, whether as a result of a primary deficiency of the adrenal or secondarily from a pituitary deficiency.<sup>2, 5, 6, 19, 21, 32</sup>

2. Fig. 1 shows that the circadian rhythms of excretion of 17-hydroxycorticosteroids, 17-ketosteroids, and potassium are roughly synchronized: all three curves show high points between 7 A.M. and 3 P.M. and low points between 11 P.M. and 3 A.M. These findings demonstrate that patients with asthma, as well as normals, have a circadian adrenal cycle.

3. We now raise the question as to whether these periodic changes in adreno-

TABLE II. 17-HYDROXYCORTICOSTEROIDS, 17-KETOSTEROIDS, AND POTASSIUM CONTENT  
IN TOTAL 24-HOUR URINES\*

|                                          | 17-HYDROXYCORTICO-<br>STEROIDS<br>(MG./24 HR.) | 17-KETOSTEROIDS<br>(MG./24 HR.) | POTASSIUM<br>(MEQ./24 HR.) |
|------------------------------------------|------------------------------------------------|---------------------------------|----------------------------|
| <i>Asthmatic Subjects</i>                |                                                |                                 |                            |
| Females (Nos. 1-2-3-4)                   | 3.5 ± 1.1                                      | 10.1 ± 2.4                      | 55 ± 7.4                   |
| Males (Nos. 5-6-7-8)                     | 5 ± 1.6                                        | 15.8 ± 1.7                      | 53.8 ± 8.4                 |
| <i>Control Subjects (A)</i>              |                                                |                                 |                            |
| Females (Nos. 9-10)                      | 3.2 - 3.8                                      | 8.4 - 11.8                      | 55 - 52.5                  |
| Males (Nos. 11-12)                       | 5.6 - 4.6                                      | 16.6 - 14.7                     | 60 - 52                    |
| <i>Control Subjects (B)</i>              |                                                |                                 |                            |
| Determinations from<br>60 healthy adults |                                                |                                 |                            |
| Females (28)                             | 4.2 ± 2.2                                      | 10.4 ± 2.8                      | 58 ± 12                    |
| Males (32)                               | 4.5 ± 2.6                                      | 15.3 ± 3.1                      | 56.5 ± 11.3                |

\*Figures given in Table II for asthmatic and control subjects (B) are expressed as average ± t times the standard error of the mean with 95 per cent of security. Figures for control subjects (A) are the values of individual determinations.

cortical function contribute to the timing of asthmatic paroxysms. The times of attacks of asthma also are shown at the bottom of Fig. 1. It can be seen that such paroxysms occur with surprising regularity during the hours of lowest adrenal steroid and potassium excretion.

One may ask as to whether this coincidence in time may be found in each individual case. Let us point out in this connection, however, that, with the sampling interval used, the minimal excretion for a substance may cover periods longer than 4 hours. In Case 4, for example, the 17-hydroxycorticosteroid excretion was almost as low between 7 P.M. and 11 P.M. as between 11 P.M. and 3 A.M. Furthermore, on several occasions, a second low excretion period for one or several substances also was found at times other than the period from 11 P.M. to 3 A.M. In Table I we indicate those individual peculiarities, such as a long minimum or several minima.

These qualifications notwithstanding, the fact remains that the time of an attack of asthma coincides in all of our cases with the lowest excretion of 17-hydroxycorticosteroids. This same correlation is found in only 7 cases for potassium excretion, and in only 6 cases for the 17-ketosteroids.

Attacks of asthma in our series thus occurred when adrenal activity was at its trough. The good correlation in time of paroxysms of asthma with low 17-hydroxycorticosteroid excretion is not surprising. This index is more specific as compared to the other two. The reverse of the same correlation obviously is not true. There were indeed periods of low adrenal secretion without dyspnea in our series of patients (Cases 4, 6, 7, 8), just as in healthy subjects.

Finally, it is noteworthy that from 7 A.M. to 3 P.M.—a period during which adrenal secretions are relatively high—none of our 8 patients suffered an asthmatic paroxysm. In the series here studied all such paroxysms occurred at night.

#### DISCUSSION

It seems possible to establish a temporal relationship—though not necessarily a causal one—between the manifestation of asthmatic paroxysms at

night and the circadian periodic decrease in adrenocortical activity, occurring at the same time, with the sampling schedule used herein. This aspect of the timing of two events, one physiologic (the adrenal cycle), the other pathologic (the asthmatic paroxysm), is to be qualified, however, by probable lags between the trough in plasma levels of adrenal hormone and the trough in urinary excretion of steroid, with the former leading the latter.

For the sake of completeness, it may be recorded that, in Cases 3 and 5, dyspnea, though not the paroxysm, persisted into the morning or afternoon during periods associated with relatively high steroid values, while in several other patients already noted there were periods of low adrenal steroid excretion in the absence of asthmatic attacks. In man living on a schedule of diurnal activity and nocturnal rest, the circadian periodic nocturnal reduction of adrenal function does not determine, of course, an asthmatic paroxysm, but it may favor or facilitate the induction or realization of an attack by other mechanisms.

Adrenal cortical changes need not be the sole facilitators of an asthmatic paroxysm. The nocturnal timing of the latter has been discussed by W. Menzel<sup>25, 26, 27</sup> in the light of a decrease at night in the pulmonary vital capacity and a postulated rise in vagal tone. This author found that asthmatic patients show a circadian rhythm in vital capacity and that the nocturnal decrease therein is much more pronounced than in healthy subjects.

Such observations are both interesting and pertinent to this discussion because they suggest that more than a single set of physiologic factors must be considered before one accounts fully for the nocturnal timing of asthmatic paroxysms.

There are indeed many circadian periodic changes<sup>1, 11, 17, 22, 32</sup> with differences in timing.<sup>12, 13, 18</sup> These changes characterize functional coordination at several levels of mammalian organization—as demonstrated under conditions standardized for reproducibility by F. Halberg and as viewed in the context of a circadian system physiopathology.<sup>11, 13</sup> The adrenal cycle, in particular, has been viewed by Halberg as an endocrine entity in its own right<sup>20, 38</sup> and it has been aligned by him with other superimposed and juxtaposed humoral and neural controls.<sup>12, 13</sup>

It seems unlikely that the nocturnal occurrence of asthmatic paroxysms of our patients was related solely to periodic changes in external factors. Thus, in most patients similar to ours, with allergy to factors such as dust, the amount of antigens is not likely to be higher at night than during the day.<sup>39</sup> It would then seem logical to suppose that the usually nocturnal occurrence of the asthmatic attack is a result, at least in part, of periodic changes in the patient's reactivity.

This explanation for the predominantly nocturnal occurrence of attacks by periodic intrinsic mechanisms may be further associated with the concept of the hours of changing resistance<sup>12, 13</sup> documented extensively from the results of exposure to a variety of agents.<sup>13</sup> It has indeed been experimentally shown that the periodic changes in physiologic state may tip the scale toward death or survival in the response of a mammal to a fixed stimulus.

We intend to continue our studies involving the periodicity analysis of

asthmatic physiopathology. The antigenic stimulus will have to be applied repeatedly along a 24-hour scale at a constant level under standardized conditions of environment and sampling. In any event, the data here reported, in themselves, are compatible with the assumption that the stage of the circadian adrenal cycle might be one of the circadian periodic mechanisms facilitating the asthmatic attack.

#### SUMMARY

Circadian rhythms of excretion of 17-hydroxycorticosteroids, 17-ketosteroids, and potassium have been studied in 8 asthmatics.

Adrenal functions were normal as revealed by urinary adrenal steroids and potassium excreted during 24 hours. The circadian rhythms of excretions of these substances corresponded to physiologic variations, with a minimum at night and a maximum in the morning.

The asthma attacks appeared in all 8 instances at a time when adrenal and potassium excretions were low. However, some subjects showed periods of low adrenal activity without dyspneic manifestations.

It is suggested that the physiologic hypoactivity of the adrenal system may only facilitate the occurrence of asthma attacks at night, but is not the only factor concerned with this phenomenon.

The authors are greatly indebted to Dr. Franz Halberg, Professor of Experimental Pathology, University of Minnesota, for his friendly encouragement, advice, and help.

#### REFERENCES

1. Aschoff, J.: Spontane lokomotorische Aktivität in Handbuch der Zoologie, Berlin, 1962, Walter de Gruyter & Co., vol. 8, pp. 1-74.
2. Azerad, E., Ghata, J., and Reinberg, A.: Disparition du rythme nyctéméral de la diurèse et de la kaliurie dans 8 cas d'insuffisance surrénale, *Ann. endocrinol.* **18**: 84, 1957.
3. Bachmann, A., Mancini, G., Bur, G., and Pedace, E.: Histochemical Variations of the Lipids of the Adrenals in Guinea Pigs With Experimental Asthma, *J. ALLERGY* **27**: 159, 1956.
4. Baldwin, H. S.: A Clinical Evaluation of Steroid Treatment of Asthma Since 1950, Third International Congress of Allergology, Paris, October, 1958.
5. Bartter, F. C., Delea, C. S., and Halberg, F.: A Map of Blood and Urinary Changes Related to Circadian Variations in Adrenal Cortical Function in Normal Subjects, *Ann. New York Acad. Sc.* **98**: 969-983, 1962.
6. Brown, H. E., and Dougherty, T. F.: The Diurnal Variation of Blood Leucocytes in Normal and Adrenalectomized Mice, *Endocrinology* **58**: 365-375, 1956.
7. Cold Spring Harbor Symposia On Quantitative Biology, Long Island Biological Association, New York, 1960, pp. 524.
8. Doe, R. P., Flink, E. B., and Goodsell, M. G.: Relationship of Diurnal Variations in 17-OH Corticosteroid Levels in Blood and Urine to Eosinophils and Electrolytes Excretions, *J. Clin. Endocrinol.* **16**: 196, 1956.
9. Feinberg, A. R., and Feinberg, S. M.: The Anti-inflammatory Steroid Hormone in Allergy. A Summary of Several Years' Experience, Third International Congress of Allergology, Paris, October, 1958.
10. Forsham, P. H., and Di Raimondo, V. C.: Therapeutics of Allergic Disease With Hormones, Third International Congress of Allergology, (Report) Paris, October, 1958.
11. Halberg, F.: Symposium on Some Current Research Methods and Results With Special Reference to the Central Nervous System, *Am. J. Ment. Deficiency* **65**: 156, 1960.
12. Halberg, F.: In Cold Spring Harbor Symposia on Quantitative Biology, Long Island Biological Association, New York, 1960, pp. 289-310.
13. Halberg, F.: The 24-Hour Scale: A Time Dimension of Adaptive Functional Organization, *Perspect. Biol. Med.* **3**: 491, 1960.

14. Halberg, F.: *In* Circadian System, Report of the Thirty-Ninth Ross Conference on Pediatric Research, Ross Laboratories, Columbus, Ohio, 1961, pp. 58, 63, and 93.
15. Halberg, F., Frank, G., Harner, R., Matthews, J., Aaker, H., Graven, H., and Melby, J.: The Adrenal Cycle in Men on Different Schedules of Motor and Mental Activity, *Experimentia* 17: 282, 1961.
16. Halberg, F., and Howard, R. B.: 24-Hour Periodicity and Experimental Medicine: Examples and Interpretations, *Postgrad. Med.* 24: 349, 1958.
17. Halberg, F., and Kaiser, I. H.: Lack of Physiologic Eosinophil Rhythm During Advanced Pregnancy of a Patient With Addison's Disease, *Acta endocrinol.* 16: 227, 1954.
18. Halberg, F., Loewenson, R., Winter, R., Bearman, J., and Adkins, G. H.: Differences in Period of Circadian Rhythms or in Their Component Frequencies, *Minn. Acad. Sc.* 28: 53, 1960.
19. Halberg, F., Visscher, M. B., Flink, E. B., Berge, K., and Bock, F.: Diurnal Rhythmic Changes in Blood Eosinophil Levels in Health and in Certain Diseases, *Journal-Lancet*, 71: 312, 1951.
20. Haus, E., and Halberg, F.: Stage of Adrenal Cycle Determining Different Corticosterone Responses of C Mice to Unspecific Stimulation and ACTH, *Communications of the First International Congress of Endocrinology, Copenhagen, 1960*, p. 219.
21. Kaine, H. D., Selzer, H. S., and Conn, J. W.: Mechanisms of Diurnal Eosinophil Rhythms in Man, *J. Lab. & Clin. Med.*, 45: 247-252, 1955.
22. Kayser, Ch.: Le sommeil, *J. physiol. Paris* 41: 1A-60A, 1949.
23. Kayser, Ch.: Le rythme nyctéméral des mouvements d'énergie, *Revue Scientif.* 90: 173, 1952.
24. Kleitman, N.: Biological Rhythms and Cycles, *Physiol. Rev.* 29: 1, 1949.
25. Menzel, W.: Der 24-Stunden-Rhythmus des menschlichen Blutkreislaufes, *Ergebnd. inn. Med. u. Kinderh.* 61: 43, 1941.
26. Menzel, W.: Der Tagesrhythmus in seiner Bedeutung für Pathologie und Klinik, *in* Klinische Fortbildung, *Neue Deutsche Klinik*, 8: 410, *Ergänzungsband*, 1944.
27. Menzel, W.: Krankheit und biologische Rhythmen, *Arzt. Mitteilungen-Deutsches arztebl.*, 41: 1201, 1958.
28. Metz, B., and Schwartz, J.: Rapports des cycles nyctéméraux de la température de la diurèse et de l'excrétion urinaire des 17-cétostéroïdes chez l'homme, *J. physiol. Paris* 41: 236 A, 1949.
29. Muller, A. E., Riondel, A. M., and Manning, E. L.: L'excrétion de l'aldostérone au cours du nyctémère, *Helvet. med. acta.* 24: 463, 1957.
30. Pincus, G.: Studies of the Role of the Adrenal Cortex in the Stress of Human Subjects. *Recent Prog. Hormone Res.* 1: 123, 1947.
31. Pittendrigh, C. S., Bruce, V. G., Rosensweig, N. S., and Rubin, M. L.: Growth Patterns in Neurospora: A Biological Clock in Neurospora, *Nature* 184: 169, 1959.
32. Reinberg, A., and Ghata, J.: Rythmes et cycles biologiques, Paris, 1957, Presses Universitaires de France.
33. Rose, B., Fyles, T., and Venning, E.: Corticotrophine, Cortisone, and Hydrocortisone in Diseases of Hypersensitivity. I. Biological Corticoid Excretion During Acute Symptoms, *J. ALLERGY* 26: 1, 1955.
34. Roskam, J., Van Cauwenberge, H., and Lecomte, J.: Excrétion urinaire des corticostéroïdes au cours de certaines réactions et maladies allergiques, *Internat. Arch. Allergy* 9: 102, 1956.
35. Scholler, R., Busigny, M., and Jayle, M. F.: Méthode de dosage des 17-21 déhydroxy-20-cétostéroïdes, *Semaine hôp. Paris (Arch. Biol. Med.)* 33: 2, 1957.
36. Seligson, D.: *Standard Methods of Clinical Chemistry*, New York, 1960, Academic Press, Inc., vol. 2.
37. Tyler, F. H., Migeon, C., Florentin, A. A., and Samuels, L. T.: The Diurnal Variation of 17-OH Corticosteroids Levels in Plasma, *J. Clin. Endocrinol.* 14: 774, 1954.
38. Ungar, F., and Halberg, F.: Circadian Rhythm in the in vitro Response of Mouse Adrenal to Adrenocorticotrophic Hormone, *Science* 137: 1058, 1962.
39. Urbach, E., and Gottlieb, P. M.: *Allergy*, New York, 1946, Grune & Stratton, Inc.