

Circadian reactivity rhythms of human skin to histamine or allergen and the adrenal cycle

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

Six adults, apparently healthy subjects, were standardized, for one week on a routine of diurnal activity and nocturnal (2400-0800) rest. Circadian rhythms in cutaneous responses (erythema and wheal areas) to constant intradermal injections of histamine (10 μ g) and of 48/80, a histamine liberator (35 μ g), were found. These rhythms are synchronized roughly in phase; the cutaneous reactions reach their crest at about 2300 and their trough around 0700-1100. Circadian rhythms in immediate cutaneous response to penicillin (scratch tests) were also found in two patients suffering from humoral allergy to that drug. A crest occurred at 2300 and trough around 0700 to 1500, under the conditions studied. Data on such circadian rhythms extend to man the concept of the hours of changing responsiveness documented extensively by F. Halberg. A temporal relationship, though not necessarily a causal one, has been defined between the circadian rhythms in cutaneous responsiveness and those of the integrated 4 hourly urinary excretion of 17-hydroxy-corticosteroids, 17-ketosteroids and potassium. Adrenal hormone excretion showed a trough at about 2300 to 0300 and a crest at about 1100 to 1500. Several theoretical and practical aspects of those results are discussed.

We undertook the studies described in this paper after a consideration of the following experimental facts and hypothesis:

1. Franz Halberg^{8, 9} has shown experimentally circadian* variations of animal resistance or susceptibility to a fixed stimulus. Under conditions standardized for reproducibility, the susceptibility of the total organism changes predictably along a 24 hour scale. These periodic changes in physiologic state

From the Service de Dermato-Allergie of the Fondation A. de Rothschild, 29 rue Manin, Paris, and the Laboratoire de Physiologie Chimique, Faculte des Sciences.

Received for publication July 13, 1964.

*Circadian is derived from *circa* (about) and *dies* (days). 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. 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.^{3, 10} A rhythm with "exact" 24 hours periodicity may be regarded as a "special case" of circadian rhythm.

may tip the scale toward death or survival in the response of mammals to a wide range of potentially noxious agents of biophysical, pharmacologic, endocrinologic, toxicologic, or bacteriologic interest.^{8, 9} It follows that in the analysis of physiologic and physiopathologic processes, the so-called "hours of changing resistance or responsiveness"^{8, 9} must be taken into consideration. Furthermore, study of the phase relations among rhythms and of underlying factors may yield new clues toward a better understanding of a temporal pathology. Attempts to extend experimentally to man this new concept seem interesting to us.

2. For this purpose, we first studied the local effects of histamine and of 48/80, a histamine liberator, injected intradermally in constant doses at fixed times along the 24 hour scale. Two main considerations lead us to elect such a type of experiment: (a) the local skin response (wheal and erythema) that follows an intradermal injection of small doses of these two drugs can be easily and precisely measured, and (b) Cormia,² in studying the itching effect of intradermally injected histamine, showed that the threshold of this response was greatly lowered at night. In two subjects, the doses required to reach the itch threshold were about a hundred times lower at midnight than at 1400.*

In a second exploration, we attempted to investigate the question as to whether or not there is a circadian variation in the skin response to antigens (e.g., the immediate reaction to penicillin) in two selected allergic adults.

3. Corticosteroids seem to intervene in the biosynthesis of histamine^{7, 11, 12} and in the skin reaction⁷ to this substance, among others. Whatever the possible ways in which the adrenal hormones might act,⁷ we may ask whether a physiologic fall (or rise) in their secretion is followed by a rise (or fall) in the skin response. Furthermore, in man, the circadian adrenal cycle involves changes of high amplitude in several functions, including the steroid content of plasma and urine.^{1, 5} Therefore, an added question may be raised: is there a temporal relationship between certain phases, such as the crest or trough of the circadian adrenal cycle and the anticipated circadian periodic variation in skin response to histamine and other substances? Such a temporal relation, if any, does not necessarily mean a causal one,^{8, 9} but it provides a useful basis for further applications of periodicity analysis in order to estimate the extent to which changes in one function might contribute to certain other functional alterations.

SUBJECTS, MATERIALS, AND METHODS

Six apparently healthy adult subjects, physicians and nurses, were studied: two women 23 and 40 years of age and four men, 38, 41, 42, and 51 years of age. None suffered from any known allergic condition. We verified by extensive clinical examinations and routine laboratory procedures that the renal, cardiovascular, and endocrine function of all six subjects could be regarded as normal.

*The local time in this paper is given according to largely adopted international scientific terminology. The period from 0100 to 1200 corresponds to that from 1 A.M. to noon and that from 1300 to 2400 corresponds to that from 1 P.M. to midnight.

The subjects were standardized for one week before each of 3 sets of tests; they stayed in bed at night (2400 to 0800) and followed their usual home and hospital activities during the day. All subjects ate their customary diet, without modification or restriction, except that meals were taken at 0800, 1300, and 2000.

Profiles were obtained on each subject, by sampling at 4 hour intervals during at least three different 24 hour periods, the following three sets of functions: (a) skin reactions to histamine, (b) skin reactions to 48/80, (c) the integrated 4 hourly urinary excretion of 17-hydroxycorticosteroids, 17 ketosteroids, and potassium.

A standard preparation* (Laboratoire Beytout, France) of histamine dihydrochloride, 100 μ g per milliliter, was used. Each injection was of 10 μ g in 0.1 ml. saline solution (trisodium citrate 0.045 mg. sodium chloride, 8.5 mg. per 1 ml. of distilled water). The histamine liberator 48/80, a polyamine compound,† was diluted in saline solution (sodium chloride 9 mg. per 1 ml. of distilled water). Each injection was of 35 μ g in 0.1 ml. solution. Injections were made exclusively on the flexor surfaces of the forearms. The injection site was changed each time, the distance between two different sites being about 2 inches.

Special care was taken to standardize the mode of injection. For any one subject, all injections were made by the same physician. Thus, it was hoped that the depth of injection was maintained as constant as possible. Precision syringes and needles (15/5) were used to insure that the amount of injected drug was uniform. The objective reactions were measured 15 to 20 minutes after the injection of the drug.

To evaluate the exact areas of both wheal and erythema, we used the following procedure: with a ball-point pen, we delineated on the skin the precise limits of both reactions. A large piece of cellulose adhesive tape which entirely covered the area of the reactions was then stuck briefly to the skin. After its removal, the tape was placed on a sheet of paper and saved. The excess of ink which left the skin printed the tape. By this procedure, it was easy to reproduce the two drawings of the areas of reaction made originally on the skin.

In order to quantitate the areas of reaction, the drawings were placed on transfer paper which was then carefully cut and weighed. The quotient, weight in milligrams, over surface in square centimeter of the transfer paper must be constant. Average values for the erythema surface were 20 sq. cm. (limits 7 and 34 sq. cm.) and for the wheal surface 2.5 sq. cm. (limits 1.2 and 5.6 sq. cm.).

At the time of the tests, the subjects stayed either in their homes or in the hospital. Consecutive testing periods of 24 to 48 hours were separated by at least one week.

Intradermal injections were made at fixed hours—0700, 1100, 1500, 1900, 2300, and 0300 for the study of reactions to histamine as well as for the study of responses to 48/80.

*Laboratoire Beytout, France.

†Burroughs-Wellcome & Company, the United States of America.

The subjects emptied their bladders at 0700 (7 A.M.) at the start of a profile, and the collection of urine began thereafter. The specimens were collected during at least six successive 4 hour periods in separate bottles. At any time during the 4 hour period, but also always at the end of each 4 hour period, each subject voided and thereafter immediately refrigerated the urine sample.

The method of Scholler and associates,¹⁸ a modification of the Silber-Porter procedure, was used for the determination of 17-hydroxycorticosteroids, and that of Drekter¹⁹ for 17-ketosteroids. Potassium was evaluated by flame photometry. All samples from a given subject were analyzed at the same time.

Apart from the six normal subjects, we studied two patients suffering from a humoral allergy to penicillin characterized as a so-called anaphylactic or type 1 reaction.⁷ These latter subjects were a woman, 39 years of age, and a man, 61 years of age. In each: (1) Quincke's edema, urticaria, and lipothymy were present from 2 to 4 hours after therapy by injection or oral administration of penicillin, (2) basophil degranulation tests²⁰ were positive for penicillin (1/100 dilution), and (3) skin scratch tests showed strongly positive reactions to penicillin, occurring within 15 minutes.

We verified that the renal, cardiovascular, and endocrine functions of these two patients could be regarded as normal for their age. These patients were hospitalized for 2 weeks; they stayed in bed at night (2000 to 0700) and followed nonstrenuous leisure-time activities during the day.

In these two patients we studied every 4 hours, during a 24 hour period, the skin scratch test reactions to penicillin instead of the intradermal injections of histamine and 48/80. For this purpose, we used freshly prepared solutions (25,000 U. per milliliter, of crystallized sodium benzyl-penicillinate.* Scratches (5 mm. in length) were made by the same physician on the flexor surfaces of the forearms, the site being changed at each 4 hour test time. A constant (0.4 ml.) amount of penicillin solution was applied to the scratches. The reactions were measured according to the method described above.

A week after the skin tests, the urine of these two patients was collected and analyzed, the procedures being those employed for the six healthy subjects.

RESULTS

Data from skin tests and the determination of the urinary excretion of corticoids and potassium, obtained for the six 4 hour periods, were first expressed as a percentage of the series mean. This presentation as a so-called relative change for each function studied allows for a fairer comparison of the changes with time in individuals with different sizes of skin reactions or different totals excreted. The mean relative change in each function for the whole healthy subject group is shown as a function of time in Fig. 1.

It can be seen that, under the conditions of study, all 6 functions revealed a statistically significant 24 hour synchronized circadian rhythm.

*Lab. Specia, France.

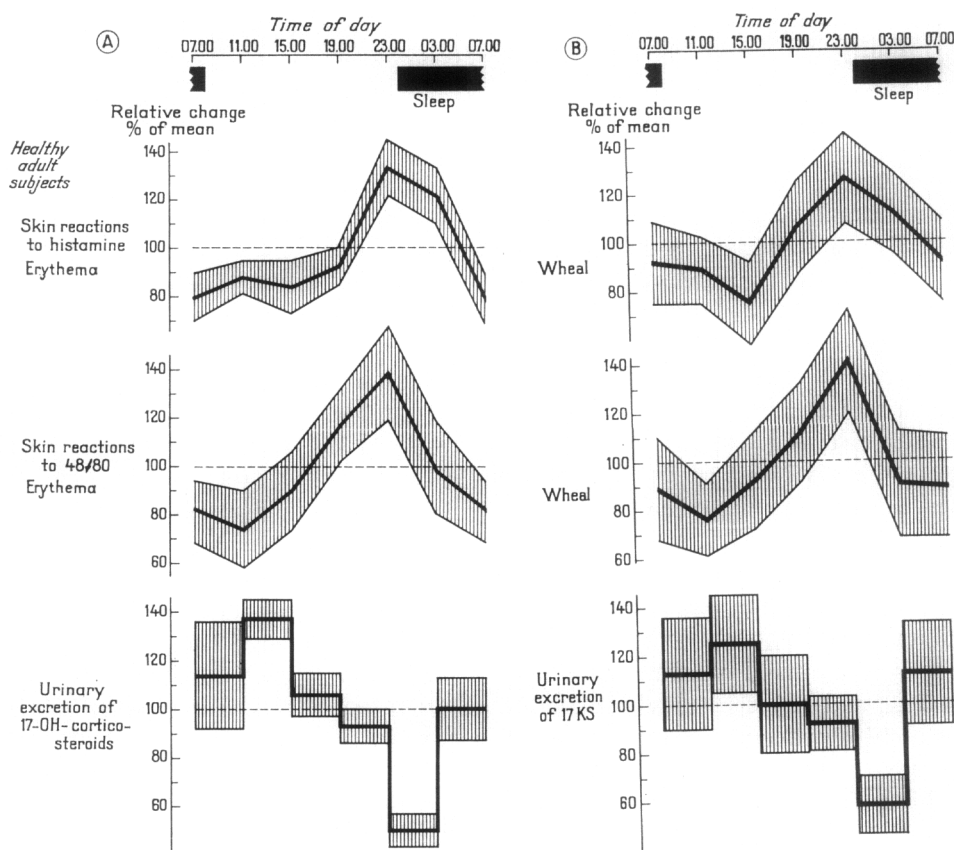


Fig. 1

Circadian rhythms of skin reactions (areas of erythema, *A*, and wheal, *B*) to intradermal injections of histamine (upper curves *A* and *B*) and of 48/80 (middle curves *A* and *B*). Circadian rhythms of urinary excretions of 17-hydroxycorticosteroids and of 17-ketosteroids (lower curves *A* and *B*).

Data for each function are expressed as the mean percentile deviation for a group of 6 healthy subjects. The mean, plus, and minus t times the standard error of the mean are included within the shaded areas, t being Student-Fischer's parameter with 95 per cent confidence.

The erythematous skin reactions to histamine and 48/80 (Fig. 1*A*) as well as the wheal reactions to these two substances (Fig. 1*B*), were roughly synchronized in phase with each other. Skin responses were at their crest at about 2300 and at their trough at about 1100 to 1500.

The question may be raised as to whether a crest in skin response might not result from a cumulative effect of repetitive injections. Conversely, a trough might correspond to an "exhaustion" in skin responses as a result of repeated stimulations. Hence, three of the six subjects were submitted to a 48 hour skin-testing period, with histamine as well as with 48/80. For each subject and each of the drugs, both crest and trough in skin responses occurred with regularity at predictable times. Moreover, the series of 7 to 13 intradermal injections, while they were made at fixed hours, were not started at

the same time. We changed the clock hour of the first injection from subject to subject, and, even for a given subject, from profile to profile, as different drugs were tested. Circadian rhythms in skin responses were consistently apparent. Thus, the rhythm in skin responses described here cannot be attributed to a mere direct cumulative or exhaustive effect of the repeated sampling procedure.

The total 24 hour urinary excretion of 17-hydroxycorticosteroids, 17-ketosteroids, and potassium can be regarded as normal in our six healthy subjects and in the two allergic patients (Table I). Moreover, in all six healthy subjects a statistically significant circadian rhythm characterizes the urinary excretion of the three substances examined. This rhythm corresponded roughly in extent of change and in its timing to similar data published earlier for healthy adult subjects.^{1, 5, 14}

The circadian rhythms in the excretion of 17-hydroxycorticosteroids, Fig. 14, and of 17-ketosteroids, Fig. 1B, and potassium (not presented) are roughly synchronized in phase. Under the conditions of study, all three functions show a trough around 2300 to 0300 and a crest around 1100 to 1500.

If we compare the circadian rhythms of corticosteroid and potassium excretion, on the one hand, and the circadian rhythm of skin reactions to histamine and 48/80, on the other hand, we see that: (a) the crest of the cutaneous responses occurred at about 2300, at a time of low urinary corticosteroid excretion; and (b) the trough of the cutaneous responses and the crest of corticosteroid excretion appeared around 0700 to 1500; (c) the trough of adrenal hormone excretion, between 2300 and 0300, followed the crest in susceptibility to histamine by about 4 hours, suggesting that plasma levels of free corticosteroids were, on the average, lowest when the cutaneous response to histamine

Table I. *17-Hydroxycorticosteroids, 17-ketosteroids, and potassium content in total 24 hour urines*

	<i>17-Hydroxycorticosteroids (mg./24 hr.)</i>	<i>17-Ketosteroids (mg./24 hr.)</i>	<i>Potassium (mEq./24 hr.)</i>
Healthy men (A)* (38, 41, 42, and 51 years of age)	4.6, 4.9, 4.1, 4.4	14.3, 17.6, 15.9, 14.6	55, 52.5, 69, 49
Healthy women (A) (23 and 40 years of age)	3.9, 4.3	11.8, 10.2	48, 51
Allergic patients (A) 61-year-old man 39-year-old woman	3.1 5.2	12.1 11.7	54.5 66
Determination from 87 healthy adults (B)			
Men	4.5 ± 2.4	15.4 ± 3.1	59.5 ± 12.3
Women	4.3 ± 2.1	10.5 ± 2.7	58 ± 13.5

* (A) Values of individual determinations. (B) Average ± t times the standard error of the mean with 95 per cent confidence.

was highest. The curve for urinary corticosteroid excretions lags behind that for plasma levels by a few hours.¹

The data on the functions studied here have been viewed thus far as group phenomena. One may further ask whether coincidence in time of crest and

Table II

<i>Subjects</i>			<i>Functions</i>	<i>Time of day</i>							
<i>No.</i>	<i>Sex</i>	<i>Age</i>		<i>0700</i>	<i>1100</i>	<i>1500</i>	<i>1900</i>	<i>2300</i>	<i>0300</i>	<i>0700</i>	
1	M	38	Histamine	—	—			+		+	
			48/80	—	—			+	+		
			17-OH-CS	x	x	x	x				
2	M	41	Histamine	—				+			
			48/80	—				+			
			17-OH-CS		x	x	x	x			
3	M	42	Histamine			—	—			+	+
			48/80		—	—		+	+		
			17-OH-CS	x	x	x	x				
4	M	51	Histamine			—		+			
			48/80			—		+			
			17-OH-CS		x	x	x	x	x	x	
5	F	23	Histamine	—				+			
			48/80	—				+			
			17-OH-CS		x	x	x	x			
6	F	40	Histamine	—	—	—	—	+		+	
			48/80	—	—	—	—	+	+		
			17-OH-CS		x	x	x	x			

Time of minimum — and of maximum + skin reactions (erythema) to histamine and 48/80. Time of minimum and of maximum x x x x excretions of 17-hydroxycorticosteroids.

Table III

<i>Subjects</i> <i>No.</i>	<i>Skin reactions to</i>			
	<i>Histamine</i>		<i>48/80</i>	
	<i>area of erythema in sq. cm. (time)</i>		<i>area of erythema in sq. cm. (time)</i>	
	<i>Minimum</i>	<i>Maximum</i>	<i>Minimum</i>	<i>Maximum</i>
1	17.2 (0700)	33.3 (2300)	12 (0700)	18.3 (2300)
	19.6 (1100)	28.3 (0300)	7.1 (1100)	19 (2300)
2	13.5 (0700)	27.4 (2300)	12 (0700)	24.7 (1900)
3	15.9 (1500)	26.4 (2300)	11 (1100)	24.9 (2300)
	16.3 (1900)	26.6 (0300)	17.5 (1500)	28.8 (2300)
4	10.6 (1500)	16.8 (2300)	8.2 (1500)	19 (2300)
5	10.4 (0700)	30.8 (2300)	15.2 (1100)	31.3 (2300)
6	15.4 (1100)	23.2 (2300)	16.2 (1100)	25.1 (2300)
	15.4 (1100)	27.6 (0300)	17 (1100)	28.7 (2300)

For each subject and each drug the minimum and maximum skin reactions are reported with the time of their occurrence on the 24 hour scale. Subjects 1, 3, and 6 were submitted to a 48 hour skin-testing period with histamine, as well as with 48/80. Therefore, in such cases, a group of two figures, instead of one, is reported for each minimum and each maximum.

trough may be found in each individual case. Let us point out that (a) with the sampling interval used, the minimal (or the maximal) excretion for a substance may cover periods longer than 4 hours, and (b) among the three subjects submitted to a continuous 48 hour skin-testing period, 4 hour changes may occur for the time of maximal (or minimal) cutaneous response.

In Tables II and III, we indicate the individual peculiarities of three functions. In spite of these qualifications, the fact remains that the time of maximal skin responses (erythema) to histamine and 48/80 coincides, roughly, in all of the 6 cases with the lowest excretion of 17-hydroxycorticosteroids. The reverse is also true (maxima of skin responses, minima of 17-hydroxycorticosteroid urinary excretions), but the coincidence in time spreads over 1 to 3 consecutive 4 hour periods. The same correlations are found with the same qualifications for the other studied functions; that is to say, skin responses (wheal) to histamine and 48/80, on the one hand, and urinary excretion of 17-ketosteroids and potassium, on the other hand, provided that these last two indices are less specific of adrenal activity than 17-hydroxycorticosteroid excretion.

Fig. 2 presents the curves obtained from determinations of the 4 hourly urinary excretion of 17-hydroxycorticosteroids and of the 4 hourly immediate

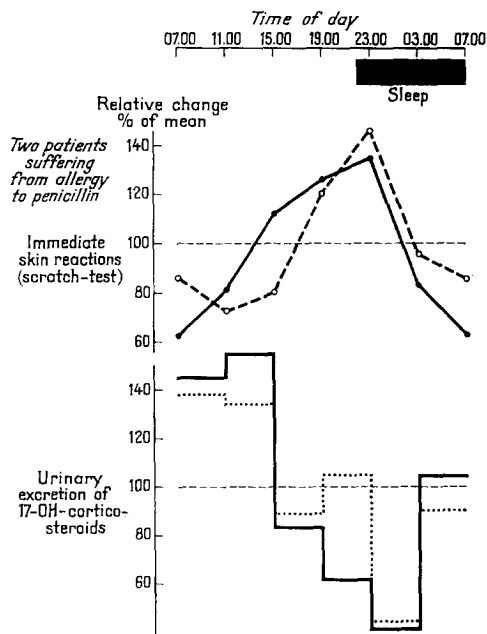


Fig. 2

Circadian changes in skin reactions (erythema areas) noted in standardized penicillin scratch tests (above). Circadian changes in urinary excretion of 17-hydroxycorticosteroids (below). Data from two patients suffering from humoral allergy to penicillin, a 39-year-old woman (solid lines) and a 61-year-old man (dotted lines). Each of the curves represents percentile deviations for each subject regarding his (or her) own mean.

skin responses (erythema) to penicillin scratch tests in two allergic patients. Their circadian rhythm of excreted 17-hydroxycorticosteroids corresponded roughly to that obtained from healthy subjects. Let us remember that in a previous study the circadian rhythms of excreted corticosteroids in 8 asthma patients were found to be comparable to those of healthy subjects.¹⁴

The curves of immediate allergic responses seemed to show a circadian rhythm with a crest at 2300 and a trough at about 0700 to 1500, under the conditions of this study. In both cases, wheal areas that surrounded the scratches reached their crests at about 2300. The series of skin tests began at 1500 in the case of the man and at 1700 in the profile on the woman. The scratch test performed on her at 1900 was followed by a transient flare-up of urticaria located on the forehead and neck. This reaction was not observed at any other test time. The rhythm in skin reactions to penicillin in two allergic patients seems to correspond in extent of change and in its timing to that of skin reactions to histamine in healthy subjects. Let us point out in this connection that the time of the maximal allergic skin responses to penicillin coincides with the lowest excretion of 17-hydroxycorticosteroids.

DISCUSSION

Under the condition of study, we observed several 24 hour synchronized circadian rhythms in skin responses (erythema and wheal) to histamine and 48/80 in six healthy subjects and to penicillin in two allergic patients. These facts may be regarded as an experimental extension to man of Halberg's concept of the hours of changing responsiveness.^{8, 9} Such a possibility has been suspected by Menzel¹² concerning the reaction of the human organism to drugs and by ourselves concerning the predominantly nocturnal occurrence of asthma attacks.¹⁴

We believe that the hours of changing resistance or responsiveness will have to be taken into consideration from a practical as well as a theoretical point of view. Within the scope of this paper, let us remember that circadian rhythms have been described in the mitotic activity of human epidermis⁴ and in cutaneous capillary resistance,⁶ as well as in the study by Cormia² cited earlier. The data of the present study define a temporal relationship, though not necessarily a causal one,^{8, 9} between the circadian rhythms in skin responsiveness to the effects of histamine and to those of 48/80 (penicillin in allergic patients) on the one hand, and the physiologic adrenal cycle on the other. Before assuring a direct effect of corticosteroids on the skin responses studied here, it must be remembered that these hormones, or related compounds, administered in excess, have few inhibitory effects, if any, on immediate skin reactions.^{7, 21}

It is known that under the influence of corticosteroids the level of tissue histamine falls^{7, 11} and the activity of enzymes may be depressed⁷ (i.e., that of histidine decarboxylase,¹³ etc.) but we do not know to what extent these effects are implicated in immediate skin reactions. Among several possible ways in which the corticosteroids might act one merits our attention—these compounds increase the stability of many cell membranes such as those of

capillaries.^{7, 15, 16, 22} According to Döring and Riecke⁶ the circadian rhythm of capillary resistance showed a statistically significant minimum at about 2300 as a group phenomenon. In two of our six healthy subjects, we performed twice the determination of capillary resistance on the flexor surfaces of the forearms, every 4 hours during 24 hours (standard count of petechiae after vacuum depression of 500 mm. Hg during 60 seconds). Like Döring and Riecke, we found a trough in capillary resistance at about 2300.

Such observations suggest that adrenal cortical changes do not determine, but may prepare or facilitate, among other factors, the periodic variations in skin reactivity. What we know of circadian systems and their functional coordination^{8, 9, 10} leads us to think that not one, the adrenal, but many component circadian rhythms, with differences or coincidences in timing (temporal coordination) have to be taken into consideration to elicit the determinism of circadian rhythms in skin responsiveness.

Nevertheless, the adrenal cycle might be one of the circadian periodic and intrinsic mechanisms on which depend the circadian of response rhythms disclosed here.

The authors would like to express their gratitude to Dr. Franz Halberg, Professor of Experimental Pathology, University of Minnesota, for his encouragement and advice. Special thanks are due to Mrs. Laurette Veza for her help in the translation of this text from French into English and to our colleagues and nurses at the Rothschild Foundation (Paris) who cooperated as subjects.

REFERENCES

1. 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, 1962.
2. Cormia, F. E.: Experimental Histamine Pruritus. I. Influence of Physical and Psychological Factors on Threshold Reactivity, *J. Invest. Dermat.* **19**: 21, 1952.
3. Cold Spring Harbor Symposia on Quantitative Biology, Long Island Biological Association, New York, 1960.
4. Cooper, Z. K.: Mitotic Rhythm in Human Epidermis, *J. Invest. Dermat.* **2**: 289, 1939.
5. Doe, R. P., Flinck, E. B., and Goodsell, M. G.: Relationship of Diurnal Variations in 17-OH-Corticosteroid Levels in Blood and Urine to Eosinophils and Electrolyte Excretions, *J. Clin. Endocrinol* **16**: 196, 1956.
6. Döring, G. K., and Riecke, H.: Über tagesperiodische Schwankungen der Kapillarresistenz, *Klin. Wchnschr.* **30**: 1098, 1952.
7. Gell, P. H. G., and Coombs, R. R. A.: *Clinical Aspects of Immunology*, Oxford, 1963, Blackwell Scientific Publications, p. 372.
8. Halberg, F.: The 24-Hour Scale: A Time Dimension of Adaptive Functional Organization, *Perspectives in Biol. & Med.* **3**: 491, 1960.
9. Halberg, F.: Temporal Coordination of Physiologic Function. Cold Spring Harbor Symposia on Quantitative Biology, New York, 1960, Long Island Biological Association, pp. 289-310.
10. Halberg, F.: *In Circadian Systems*, Report of the Thirty-Ninth Ross Conference on Pediatric Research, Columbus, Ohio, 1961, Ross Laboratories, pp. 58-63, 93.
11. Halpern, B. N., and Briot, M.: Action des hormones corticosurrénales sur le métabolisme de l'histamine endogène, *Rev. franç. étud. clin. et biol.* **1**: 151, 1956.
12. Menzel, W.: *Menschliche Tag-Nacht-Rhythmik und Schichtarbeit*, Basel and Stuttgart, 1962, Benno Schwabe u. Co.
13. Parrot, J. L., and Laborde, C.: Inhibition de l'histidine décarboxylase par la cortisone et par le salicylate de sodium, *J. physiol., Paris* **53**: 441, 1961.
14. Reinberg, A., Ghata, J., and Sidi, E.: Nocturnal Asthma Attacks: Their Relationship to the Circadian Adrenal Cycle, *J. ALLERGY* **34**: 323, 1963.
15. Robson, H. N., and Duthie, J. J. R.: Capillary Resistance and Adrenocortical Activity, *Brit. M. J.* **2**: 971, 1950.

16. Robson, H. N., and Duthie, J. J. R.: Further Observations on Capillary Resistance and Adreno-cortical Activity, *Brit. M. J.* **1**: 994, 1952.
17. Rothman, S.: *Physiology and Biochemistry of the Skin*, Chicago, 1954, The University of Chicago Press.
18. Scholler, R., Busigny, M., and Jayle, M. F.: Méthode de dosage des 17-21-déhydroxy-20-cétostéroïdes, *Semaine hôp. Paris (Arch. biol. & méd.)* **33**: 2, 1957.
19. Seligson, D.: Method of Dreker, *in* *Standard Methods of Clinical Chemistry*, New York, 1958, Academic Press, Inc., vol. 2.
20. Shelley, W. B., and Caro, W. A.: Detection of Anaphylactic Sensitivity by the Basophil Degranulation Response, *J. A. M. A.* **182**: 170, 1962.
21. Sidi, E., and Bourgeois-Gavardin, J.: Modifications cutanées consécutives aux applications et aux injections locales d'acétate d'hydrocortisone, *Presse méd.* **61**: 1760, 1953.
22. White, A., Blecher, M., and Jedeikin, L. A.: Mechanism of Action of Adrenal Cortical Hormones, *in* *Mechanism of Action of Steroid Hormones*, London, 1961, Pergamon Press, Ltd.