PHOTOPERIODICITY AND THE FUNCTION OF THE HYPOPHYSIS AND THE ADRENAL CORTEX

I. A. Eskin and N. V. Mikhailova

From the Department of Experimental Biology (Head – Prof. I. A. Eskin), All-Union Institute of Experimental Endocrinology (Director – Prof. E. A. Vasiukova), Moscow

(Received January 21, 1958. Presented by Active Member AMN SSSR V. V. Parin)

It was shown in an earlier paper [2] that a considerable fall (to 64% on the average) takes place in the eosinophil count of the peripheral blood of rats during the evening hours, as compared with the values found for blood samples taken in the morning. These rhythmic diurnal variations of eosinophil count were observed at all seasons of the year, but the highest counts were found at 2 p. m. in January, and at 6 p. m. in June. Disturbance of the normal day and night rhythm (24-hour illumination or darkness) leads to abolition of the eosinophil rhythm of peripheral blood. Inversion of normal photoperiodicity (illumination by night and darkness by day) leads to inversion of the eosinophil count rhythm. A correlation was thus found between the eosinophil count of the peripheral blood of rats and the environmental photoperiodicity. The diurnal eosinophil rhythm of rats was found to be disturbed within 8-13 days of bilateral adrenalectomy, pointing to the role of the adrenals in effecting the rhythm. The eosinophil rhythm is parallelled by a similar rhythm of urinary 17-ketosteroid excretion. Analogous effects were found by Brown and Dougherty in mice (1956). These authors reported rhythmic changes affecting not only the eosinophil count, but also the total leucocyte, lymphocyte, and neutrophil counts. The leucocyte count of blood taken from the tail vein during daylight hours was found by these authors to be 3-4 times higher than at night. Fourteen days after adrenalectomy mice no longer showed the diurnal rhythmic changes in eosinophil, lymphocyte, neutrophil, and total leucocyte counts.

Doe, Flink and Godsell (1956) examined the eosinophil count of healthy men, as well as the 17-hydroxycorticosteroid content of their blood and urine. They found that the 17-hydroxycorticosteroid content began to rise at 6 a.m. to a maximum at 9 a.m., falling thereafter to a minimum at 9 p. m. The eosinophil count began to fall at 6 a.m., reaching a minumum at 9 a.m., and then rising abruptly toward the evening.

Radnot, Wallner and Török (1956) found that the morning fall in eosinophil count of a series of patients took place only under normal conditions, but was abolished when the patients were kept in the dark.

Similar findings were reported by Migeon, Tyler, Mahoney, Florentin, Castle, Bliss and Samules (1956). These authors also found a considerable fall in the amplitude of the rhythmic fluctuations of corticosteroid hormones in blind persons.

There is, therefore, a diurnal rhythmic change in the eosinophil count of peripheral blood of humans, rats, and mice, as well as in the 17-keto- and 17-hydroxycorticosteroid contents of the blood and urine.

The object of our research was to find whether the photoperiodic fluctuations in eosinophil count of the blood of rats depend on the diurnal rhythm of the adrenocorticotrophic function of the hypophysis and of the endocrine activity of the adrenal cortex.

EXPERIMENTAL METHODS

We examined the content of adrenocorticotrophic hormone (ACTH) in the hypophyses of rats in the morning and the evening, i.e., at times corresponding with those of pronounced changes in eosinophil count.

The rats were killed by decapitation at 9 a.m. and in the evening (at 9 p.m. in June, at 6 p.m. in August and September, and at 4-2 p.m. in October to December). Extraction of ACTH from the hypophyses was effected by the method of Sydnor and Sayers (1954). The hypophyses were weighed and placed in 0.1 N hydrochloric acid (0.5 ml per hypophysis), and left in the refrigerator for a week. They were then ground up with glass powder, and the suspension was centrifuged; the supernate was then diluted to the desired volume with 0.01 N hydrochloric acid. The adrenocorticotrophic activity of the hypophyses was derived from the fall in ascorbic acid content of the adrenals of 4-7-day-old rats following intraperitoneal injection of the extracts, at a rate of 2.0-2.5 mg of hypophysis per rat (in 0.1-0.2 ml of solution). The rats were killed $1\frac{1}{2} - 2$ hours after the injection, and the adrenals were removed. Their ascorbic acid content was determined by the method of Roe and Kuether (1943). Each experimental group consisted of not less than 10 animals. One group served as a control, and was not injected, while two other groups were given injections of extracts of hypophyses of rats killed in the morning or evening.

TABLE 1

Effect of Extracts of Hypophyses of Rats Killed in the Morning or the Evening on the Ascorbic Acid Content of the Adrenals of Newborn Rats

Experimental conditions	Ascorbic acid content of the adrenals of baby rats (mg-%)		
	control group	given extracts of the hypophyses of rats killed in the	
		morning	evening
Normal illumination	247	173	154
	147	67	52
	185	70	41
	305	174	147
	—	93	31
	81	38	11
	183	126	89
24-hour illumination	195	128	133
24-hour darkness	—	88	80
	173	130	122

EXPERIMENTAL RESULTS

The results of our determinations of the ACTH contents of the hypophyses of 75 rats killed during the morning and the evening hours of the day are shown in Table and Fig. 1. The data of Table 1 show that the fall in ascorbic acid content of the adrenals of 7 groups of baby rats, following injection of extracts of rat hypophyses, was invariably greater (by 15-62 mg-%) when the hypophyses were taken from rats killed in the evening than in the morning. This finding shows that the ACTH of the hypophysis was higher in the evening than in the morning.

In order to find out whether the diurnal fluctuations in ACTH content of the hypophysis depends on the alternation of nights and days, viz., on photoperiodicity, a group of rats was maintained for 30 days under conditions of continual illumination, while a second group was kept in darkness for the same time. The ACTH content of the hypophyses of the animals of these two groups was then determined, for the morning (9 a.m.) and evening hours. As appears from Table 1 and Fig. 1, the diurnal fluctuations in ACTH content of the hypophysis are abolished or smoothed out when the animals are kept for a long time under conditions excluding the normal alternation of light and darkness. We have thus found experimental evidence of the dependence of the diurnal rhythm of the ACTH content of the hypophysis of rats on photoperiodicity of the environment.

It is known that the ascorbic acid content of the adrenals varies according to the functional state of the gland. The effect of ACTH in enhancing adrenal function is associated with a fall in its ascorbic acid content. It was therefore thought to be of interest to find out whether there existed a diurnal rhythm in the ascorbic acid content of the adrenals of rats. We determined the ascorbic acid content of the adrenals of adult rats in the morning and the evening, by the method of Roe and Kuether. The times of killing the animals were the same as in the experiments for determination of ACTH content.

Table 2 and Fig. 2 present the results of such determinations in 9 groups of rats (120 animals in all). All groups showed considerable differences between the ascorbic acid content of the adrenals of rats killed in the morning or the evening. Higher contents (by 43-116 mg %) were always found in the evening. The ascorbic acid content of the adrenals thus undergoes regular diurnal fluctuations.

In order to correlate this rhythm with the photoperiodicity of the environment, we took 3 groups of 10 rats each, and exposed them to continual illumination, while another 3 groups were kept in continual darkness.

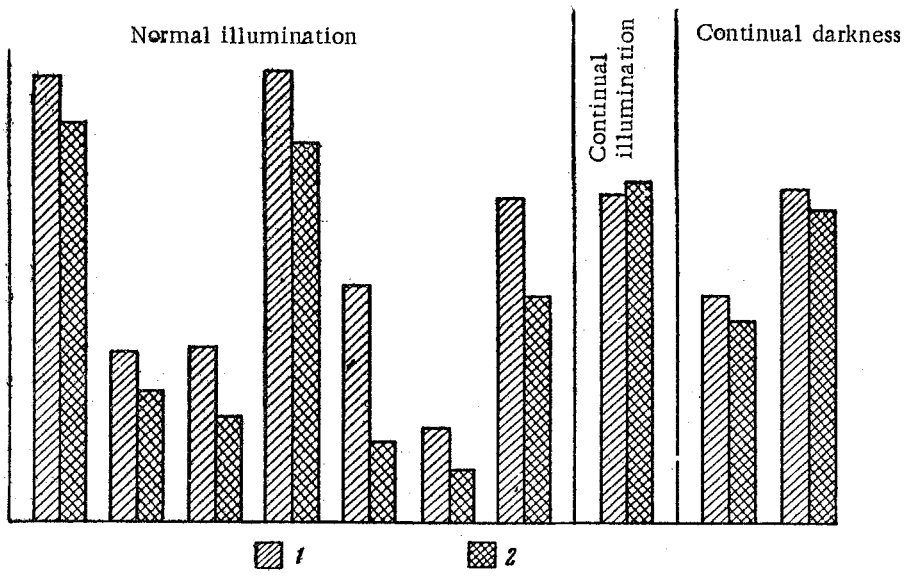

Fig. 1. Ascorbic acid content (in milligrams per 100 g) of the adrenals of newborn rats following injection of extracts of hypophyses of rats killed in the morning or evening. 1) Morning, 2) evening.

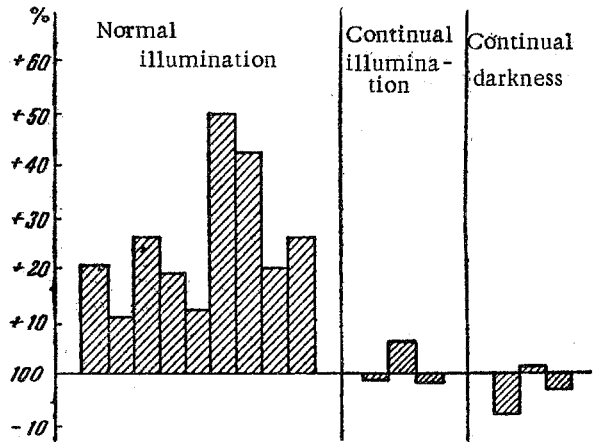

Fig. 2. Ascorbic acid content of the adrenals during the evening hours (as percentages of the values found during the morning).

After the animals had been maintained under these abnormal conditions of illumination for 30 days the rhythmic fluctuations in ascorbic acid content were found to have been abolished. It may be concluded that the diurnal rhythm in ascorbic acid content of the adrenals of rats is also dependent on regular photoperiodicity.

TABLE 2

Ascorbic Acid Content of the Adrenals of Adult Rats at Different Times of the Day

Experimental conditions	Ascorbic acid content (mg-%)		Change in ascorbic acid content (mg-%)
	morning	evening	
Normal illumination	338	409	+71
	425	472	+47
	368	465	+97
	279	331	+52
	352	395	+43
	232	348	+116
	232	329	+97
	388	466	+78
	336	422	+86
Continual illumination	383	379	—4
	394	418	+24
	366	358	—8
Continual darkness	385	356	—29
	403	409	+6
	440	429	—11

It is of interest that the ascorbic acid content of the adrenals was found to be higher in the evening than in the morning. This finding would at first sight seem not be in agreement with the enhanced adrenocorticotrophic function of the hypophysis during the evening hours, and with the earlier evidence of the fall in eosinophil count of peripheral blood, and of the rise in urinary 17-ketosteroid excretion in the evening. This discordance is, however, only an apparent one. The lowering of the ascorbic acid content of the adrenals in a

short-term response to a single dose of ACTH, or to the action of a stress-producing agent. The transient fall in ascorbic acid content is followed by its regeneration. Prolonged administration of ACTH (Comsa and Leroux, 1955), or maintenance of chronic stress conditions (Endröczi, Lassak, and Szereday, 1956), are associated with elevation of the ascorbic acid content of the adrenals. The same is the case in the evening, when the adrenals have been exposed to prolonged stimulation by ACTH secreted during the day. As with multiple doses of ACTH, one would expect to find an increase, rather than a fall, in the ascorbic acid content of the adrenals in these circumstances, and this is in fact what takes place.

Our results permit of the conclusion that there is a definite diurnal rhythmicity of adrenocorticotrophic function of the hypophysis, and of corticoadrenal function, regulated by the effect of light, i.e., of photoperiodicity. Expressions of these rhythms are to be seen in the diurnal fluctuations of ACTH content of the hypophysis, of ascorbic acid content of the adrenals, of 17-ketosteroids in the urine, of the 17-keto- and 17-hydroxycorticosteroids contents of the blood and urine, and of the total leucocyte, eosinophil , and lymphocyte counts of peripheral blood. It is probable that, as with the relations between the gonads and the gonadotrophic function of the hypophysis (Bissonette, 1936; Benoit, 1938; Ia. M. Kabak and S. I. Tereza, 1939, and others), or with the relation of thyroid activity to thyrotrophic activity of the hypophysis (Voitkevich, 1945, and others), light acts through photoreceptors on the hypothalamic region to enhance the adrenocorticotrophic activity of the hypophysis, and thus to stimulate the function of the adrenal cortex.

SUMMARY

The ACTH content of the hypophysis of rats rises from a minimum in the morning to a maximum in the evening hours. These fluctuations are associated with the diurnal rhythm of light and darkness, and are abolished under conditions of continual light or darkness for 30 days. Rhythmic fluctuations of the ascorbic acid content of the adrenals are also found, with maximum values in the evening, and these are likewise abolished by maintenance under conditions of constant illumination.

LITERATURE CITED

[1] A. A. Voitkevich, Izvest. Akad. Nauk SSSR, Ser. Biol. 4, 285-395 (1945).

[2] I. A. Eskin and G. A. Vidavskaia, Problemy Endokrinol. i Gormonoterap. No. 1, 82-87 (1956).

[3] J. Benoit, Bull. Biol. France, Belg. 1937, v. 71, pp. 393-497.

[4] T. Bissonnette, Quart. Rev. Biol. 1936, v. 11, pp. 371-386.

[5] R. P. Doe, E. B. Flink and M. G. Goodsell, J. Clin. Endocrinol. 1956, v. 16, pp. 196-206.

[6] M. Radnot, E. Wallner and E. Török, Acta med. Acad. Sci. Hung. 1956, v. 9, pp. 231-236.

[7] H. E. Brown and T. F. Dougherty, Endocrinology, 1956, v. 58, pp. 265-275.

[8] C. Migeon, F. H. Tyler and J. Mahoney and others, J. Clin. Endocrinol. 1956, v. 16, pp. 622-633.

[9] J. H. Roe and C. O. Kuether, J. Biol. Chem. 1943, v. 147, pp. 399-407.

[10] K. L. Sydnor and G. Sayers, Endocrinology, 1954, v. 55, pp. 621-636.