

materials bedingt sein, in unserem Fall frisches Gewebe, im anderen tiefgefrorenes.

Es war uns hingegen nicht möglich, selbst im 30-kg-Ansatz 11-Dehydrocorticosteron und Aldosteron nachzuweisen. Obwohl aus Tabelle I hervorgeht, dass die Werte von Cortisol und Cortison von Placenta zu Placenta sehr stark schwanken, so können wir vorläufig für die Diskrepanz hinsichtlich des 11-Dehydrocorticosteron und Aldosteron keinen Grund angeben. Möglicherweise spielt hier eine variable Konzentrations- oder Retentionsfähigkeit des placentaren Gewebes für Corticosteroide eine entscheidende Rolle^{15,42,43}. Wir konnten auch keinen Hinweis dafür finden, dass anstelle von Aldosteron etwa 11-Dehydroaldosteron⁴⁴ vorlag.

Nach Beendigung unserer Versuche ist eine ausführliche Arbeit von SIBULSKY und VENNING⁴⁵ über die Bildungsmöglichkeit von Corticosteroiden in menschlicher Placenta erschienen, welche in Übereinstimmung mit unseren Befunden steht. Da entwässertes Gewebe verwendet wurde, sind die angegebenen Werte in $\gamma/100$ g Gewebe bedeutend höher als unsere; trotzdem konnte von diesen Autoren weder 11-Dehydrocorticosteron noch Aldosteron nachgewiesen werden, und übrigens auch keine *in-vitro*-Bildung eines Corticosteroids durch menschliche Placenta.

SIBULSKY und VENNING beobachteten ebenfalls das Auftreten eines UV-absorbierenden, aber nicht reduzierenden Stoffes (X_1) von ähnlichen chromatographischen Eigenschaften wie Aldosteron. Soweit ihre Angaben einen Schluss erlauben, könnte es sich ebenfalls um 16α -Hydroxytestosteron handeln.

Im Fall unseres 30-kg-Ansatzes war die Konzentration des 16α -Hydroxytestosteron sogar grösser als diejenige des Cortison. Soweit bisher bekannt, scheint dieses C_{19} -Steroid selbst keine bemerkenswerten biologischen Wirkungen zu besitzen; es kann aber nach den Untersuchungen von RYAN⁴⁶ als placentarer Precursor von Östriol aufgefasst werden.

Summary. The occurrence of cortisol, cortisone, 20-dihydrometabolites of cortisone and several other steroid-like compounds in fresh placental tissue is described. Neither aldosterone, corticosterone nor 11-dehydrocorticosterone has been found. One of the most abundant compounds present has been identified as 16α -hydroxy-testosterone.

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⁴² H. A. SALHANICK, J. E. JONES und D. BERLINER, Fed. Proc. Abstr. Nr. 1356 (1956).

⁴³ CL. MIGEON, J. BERTRAND und P. E. WALL, J. clin. Invest. 36, 1350 (1957).

⁴⁴ J. SCHMIDLIN und A. WETTSTEIN, in Vorbereitung.

⁴⁵ S. SYBULSKI und E. H. VENNING, Canad. J. Biochem. Physiol. 39, 203 (1961).

⁴⁶ K. J. RYAN, J. biol. Chem. 234, 2006 (1959).

Diurnal Rhythm in the Adrenal Ascorbic Acid Concentration of the Rat

It has been observed that a certain diurnal variation occurs in adrenal cortical function. In man, the corticoid content of urine and blood varies as a function of the time of day¹⁻³. It has recently been suggested that the mouse and the rat also display a rhythmic variation, both in the content of plasma corticosteroids⁴⁻⁶ and white cell count^{4,5,7}, and in the mitoses of the adrenal cortex^{4,7}.

The concentration of adrenal ascorbic acid is widely used as an indicator of adrenocortical activity in test animal studies^{8,9}. No reports, however, have been found in the literature of possible diurnal variation in adrenal ascorbic acid concentration, and for this reason the present study was undertaken.

The test animals used were 70 male albino rats. These were divided into seven groups of 10 animals, taking care to ensure that the mean weight of the animals in each group was equal. The experiment was carried out in June, and no artificial illumination was therefore necessary. Ten animals were killed, by rapid decapitation with sharp scissors, every 4 h, starting at 0800 h. The adrenal glands were immediately removed from the surrounding tissue; they were weighed on a torsion balance, and then homogenized in 4% trichloroacetic acid. The content of total ascorbic acid in the homogenate was determined by the method of SCHAFFERT and KINGSLEY¹⁰. Student's 't'-test was used in the statistical treatment of the results.

The results are summarized in the Table, from which it can be seen that the adrenal ascorbic acid was at its lowest at 1600 h. The difference is statistically significant compared with both the 1200 group ($P < 0.01$) and the 1800 group ($P < 0.05$). The difference between the other groups is not statistically significant. These results are plotted in the graph of the Figure.

The Table also shows that the absolute weight of the adrenals was greatest at 2400 h, i.e. at midnight. The difference is significant both against the 2000 group ($P < 0.01$) and the 0400 group ($P < 0.05$).

Body weight, weight and ascorbic acid concentration of the adrenal glands at various times of day

Hour of day	Number of animals	Body weight g		Adrenal glands weight mg		ascorbic acid $\mu\text{g}/100$ mg fresh tissue	
		Mean	S. D.	Mean	S. D.	Mean	S. D.
0800	10	223	20.5	37.1	7.5	410	50.7
1200	10	223	18.2	40.0	6.9	377	85.2
1600	10	223	17.7	37.6	7.0	251	87.3
2000	10	223	22.0	36.4	4.0	356	91.8
2400	10	223	37.4	45.4	8.5	349	88.3
0400	10	223	30.8	37.9	6.5	356	99.9
0800	10	223	17.5	36.5	6.1	398	66.3

¹ R. P. DOE, E. B. FLINK, and M. G. GOODSSELL, J. clin. Endocr. 16, 196 (1956).

² R. E. PETERSON, J. clin. Endocr. 17, 1150 (1957).

³ B. THOMASSON, Scand. J. clin. Lab. Invest. 11, Suppl. 42 (1959).

⁴ F. HALBERG, R. E. PETERSON, and R. H. SILBER, Endocrinology 64, 222 (1959).

⁵ R. GUILLEMIN, W. E. DEAR, and R. A. LIEBELT, Proc. Soc. exp. Biol. Med. 101, 394 (1959).

⁶ J. L. MCCARTHY, R. C. CORLEY, and M. X. ZARROW, Proc. Soc. exp. Biol. Med. 104, 787 (1960).

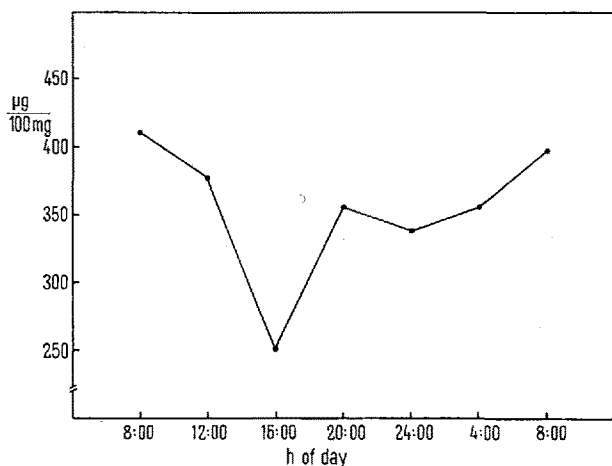
⁷ F. HALBERG, M. J. FRANTZ, and J. J. BITTNER, Anat. Rec. 129, 349 (1957).

⁸ G. SAYERS, Physiol. Rev. 30, 241 (1950).

⁹ M. A. SLUSHER and S. ROBERTS, Endocrinology 67, 873 (1960).

¹⁰ R. R. SCHAFFERT and R. KINGSLEY, J. biol. Chem. 212, 59 (1955).

These results indicate that there is a diurnal variation in the adrenal ascorbic acid concentration. It is interesting to note that the depletion of adrenal ascorbic acid occurred at 1600 h, since other investigations have shown that the corticoid level in the blood of the rat⁶, and also of the mouse⁴, is at its highest at this time. This tends to support the theory that the adrenal ascorbic acid concentration is a good indicator of adrenocortical activity and that it may be of importance in the synthesis and/or release of corticoids^{8,11}. From the practical point of view, too, it is useful to know that a reduction of this order occurs in the concentration of adrenal ascorbic acid in the afternoon. As a result, critical experiments by which depletion of adrenal ascorbic acid is induced should, wherever possible, be carried out during the morning.



Daily change in adrenal ascorbic acid concentration of the rat.

The increase in the weight of the adrenals noted at midnight is in conformity with observations that the number of mitoses in the adrenal cortex is highest at this hour^{4,7}. Evidently there may be a time lag between adrenocortical activity (indicated by increased corticoid level of the blood and by adrenal ascorbic acid depletion) and cortical hyperplasia (indicated by increased number of mitoses in the cortex and increased adrenal weight).

As for the similarity between the diurnal rhythm of the adrenal cortical function in man and in the rat, a temporal difference is noted. The human adrenal function is at its highest in the morning¹⁻³. The fact that the rat is a nocturnal animal has been put forward as an explanation for this difference^{4,5}.

Previous reports suggest that the nervous system is the site of the regulator primarily responsible for the diurnal cyclicity of adrenocortical activity^{12,13}.

Zusammenfassung. Der tägliche Rhythmus des Ascorbinsäuregehalts in den Nebennieren von Ratten wurde untersucht. Es wurde beobachtet, dass der Ascorbinsäuregehalt um 16 h nachmittags am geringsten ist. Das absolute Gewicht der Nebennieren wiederum ist in der Nacht gegen 24 h am höchsten. Bei praktischen Arbeiten ist dieser Abfall im Ascorbinsäuregehalt der Nebennieren am Nachmittag zu berücksichtigen.

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Department of Anatomy, University of Turku (Finland), August 7, 1961.

¹¹ H. S. LIPSCOMB and D. H. NELSON, *Endocrinology* 66, 144 (1960).

¹² J. W. MASON, Intern. Symposium on Reticular Formation of the Brain (Little, Brown & Company 1958), p. 645.

¹³ K. B. EIK-NES and L. D. CLARK, *J. clin. Endocr.* 18, 764 (1958).

¹⁴ Aided by a grant from the SIGRID JUSÉLIUS Stiftelse.

Magnetic and Photic Responses in Snails¹

Since the first report of a magnetic response in the mud snail *Nassarius obsoleta*² a number of remarkable characteristics of this orientation reaction have been disclosed³⁻⁵ and demonstrations of its occurrence extended to include *Paramecium*⁶, *Planaria*⁷, and *Drosophila*⁸.

In the snail studies animals were permitted to emerge from a narrow corridor into a symmetrical field which was constant for factors normally regarded as capable of influencing their orientation. Under these conditions mean angle of orientation of the snail paths displayed both solar and lunar variations. This angle could be altered quantitatively by a weak magnetic field in a predictable manner dependent upon direction and magnitude of the horizontal component, hour-angle of sun and moon, and lunar elongation.

More recently the relationship between magnetic and photic responses in snails has been examined. The present paper reports further analysis of some preliminary results which have already been described briefly⁹.

Materials and Methods. The apparatus and procedure for obtaining data have been recounted elsewhere³. In brief, the amount of turning of snails was measured after 3 cm of free movement following their emergence from a narrow corridor directed magnetic south. The orientation apparatus was situated within a white box which provided the constant light field. This field was made asymmetrical by lining the inside right or left half of the box with mat

black cardboard. When the cardboard liner was in place incident illumination in all cases was 150 lux from above, 20 lux from the black side, and 60 lux from the white side.

Every experiment comprised two series; a series constituted ten snail emergencies in each condition of the following sequence: (1) in the earth's magnetic field into symmetrical illumination, (2) in the earth's magnetic field into asymmetrical illumination black to left and white to right, (3) same illumination as (2) but in a 5-gauss horizontal magnetic field oriented in reverse to the earth's, (4) same as in (1), (5) in the earth's magnetic field with asymmetrical illumination black to right and white to left,

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² F. A. BROWN, M. F. BENNETT, and W. J. BRETT, *Biol. Bull.* 117, 406 (1959).

³ F. A. BROWN JR., W. J. BRETT, M. F. BENNETT, and F. H. BARNWELL, *Biol. Bull.* 118, 367 (1960).

⁴ F. A. BROWN JR., H. M. WEBB, and W. J. BRETT, *Biol. Bull.* 118, 382 (1960).

⁵ F. A. BROWN JR., M. F. BENNETT, and H. M. WEBB, *Biol. Bull.* 119, 65 (1960).

⁶ F. A. BROWN JR., *Paramecium*, unpublished manuscript (1961).

⁷ F. A. BROWN JR., *Dugesia*, unpublished manuscript (1961).

⁸ F. A. BROWN JR., unpublished experiments.

⁹ F. A. BROWN JR. and A. HUTTNER, *Biol. Bull.* 119, 306 (1960).