Basis verschiedener Quecksilber-Verbindungen, jedoch nicht Lindan, wirken, berichteten mehrere Autoren[20–24]. Die in den eigenen Untersuchungen verwendeten Beizmittel mit organischen Hg-Verbindungen als Wirksubstanzen ohne Lindan-Zusatz zeigten keine polyploidieauslösende Wirkung.

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*Summary.* After treatment of *Hordeum vulgare, Secale cereale, Triticum aestivum* and *Avena sativa* with Lindan-containing seed dressings, the somatic chromosomes in the root tip mitoses were analyzed. Most of the chromosome sets in the root tip cells have been polyploidized. Using pure Lindan ($\gamma$-hexachlorocyclohexane), it could be shown that this insecticide is responsible for the induction of polyploidy.

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## Daily Fluctuations in Adrenal Catecholamine Concentration[1]

The unique arrangement of the mammalian adrenal gland as two concentric organs of different embryonic origins has provoked considerable interest in the possible functional interactions between the cortex and medulla. Until the last few years the significance of their juxtaposition was not understood. However, it now generally is accepted that glucocorticoids, acting via an intra-adrenal vascular system, induce the synthesis of the medullary enzyme which catalyzes the terminal step in epinephrine synthesis[2]. In light of the latter finding, the present study was undertaken to determine whether the catecholamines, like the glucocorticoids, fluctuate with a circadian periodicity.

*Materials and methods.* Animals used in the present study were adult (250–350 g), male, Sprague-Dawley (Charles River) rats. Each study was limited to animals of the same shipment that had been housed 2 rats per cage for at least 2 weeks under conditions of controlled lighting (fluorescent illumination from 04.00–18.00 h and temperature (24 ± 1 °C). Purina laboratory chow and tap water were available ad libitum. In the first study, rats were transferred to individual cages 3 days prior to the experiment. In the second study rats were transferred to individual cages 3 days prior to the experiment and handled daily in order to familiarize the animals with entry to the animal quarters, cage opening and removal from cage. To further standardize conditions, the animal quarters were locked prior to both experiments and not enterred during the 18 h preceding the experiment. All treatment and collection procedures were done outside the animal quarters and were conducted according to a completely randomized design. In all cases rats were removed individually from the animal quarters to an adjacent preparation room where they were rapidly decapitated (< 20 sec after cage opening). The adrenals were quickly removed, cleaned and weighed individually to the nearest 0.1 mg. Immediately thereafter, they were frozen and placed in small polyethylene capsules for storage (−20 °C).

Adrenals were fluorometrically analyzed for norepinephrine (NE) and epinephrine (E) content following extraction with 0.4 *N* perchloric acid. Differential determination of the amines was performed using a procedure similar to that described by WEIL-MALHERBER[3] for estimation of total catecholamine content in human urine. Statistical probabilities were determined by analysis of variance and Student's *t*-test.

*Results.* Experiment 1. As shown in Figure 1 and Table I, significant periodicity was observed in adrenal E concentration ($F = 3.37$, d.f. $= 7/55$, $P < 0.05$). Peak values occurred at 01.00 h and trough values were observed at 07.00 h. The 24-h mean equaled 314 ± 63 µg/g.

The corresponding NE concentrations are illustrated in Figure 2 and Table I. Although the 24-h fluctuations were

[1] Supported by U.S.P.H.S. Grant No. NS-08929.
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Table I. 24-h changes in adrenal norepinephrine (NE) and epinephrine (E) in male rats (Exp. 1)

| Clock time (h) | NE | E |
|---|---|---|
| 10.00 | 11.3 ± 8.7[a] (8)[b] | 202.2 ± 67.1 (8) |
| 13.00 | 64.9 ± 23.8 (8) | 168.1 ± 74.6 (8) |
| 16.00 | 73.0 ± 35.2 (8) | 274.8 ± 53.7 (8) |
| 19.00 | 36.1 ± 18.8 (7) | 174.9 ± 50.5 (7) |
| 22.00 | 15.6 ± 3.4 (8) | 310.6 ± 124.4 (8) |
| 01.00 | 20.3 ± 8.5 (8) | 1012.7 ± 396.6 (8) |
| 04.00 | 40.8 ± 10.8 (8) | 207.0 ± 71.2 (8) |
| 07.00 | 27.2 ± 5.7 (8) | 140.4 ± 33.3 (8) |
| 24-h Mean | 42.5 ± 9.0 | 313.5 ± 63.0 |

[a] Mean ± S.E.; µg/g. [b] Number of animals/time point.

Table II. 24-h changes in adrenal norepinephrine (NE) and epinephrine (E) in male rats (Exp. 2)

| Clocktime (h) | NE | E |
|---|---|---|
| 08.00 | 18.1 ± 4.1[a] (7)[b] | 158.2 ± 29.2 (7) |
| 11.00 | 31.9 ± 12.8 (8) | 282.8 ± 68.8 (8) |
| 14.00 | 35.9 ± 7.7 (7) | 264.8 ± 43.2 (7) |
| 17.00 | 67.9 ± 32.8 (8) | 117.9 ± 21.6 (8) |
| 20.00 | 28.7 ± 10.9 (8) | 305.4 ± 79.9 (8) |
| 23.00 | 27.2 ± 7.2 (8) | 419.2 ± 109.8 (8) |
| 02.00 | 20.7 ± 3.0 (8) | 251.8 ± 75.1 (8) |
| 05.00 | 20.4 ± 4.9 (8) | 282.9 ± 71.6 (8) |
| 08.00 | 14.6 ± 4.2 (7) | 233.8 ± 76.5 (7) |
| 24-h Mean | 29.8 ± 4.6 | 259.1 ± 24.4 |

[a] Mean ± S.E.; µg/g. [b] Number of animals/time point.

not significant according to analysis of variance ($F = 1.34$, d.f. = 7/56, $P > 0.05$), comparison of the highest (73.0 µg/g at 16.00 h) and lowest (11.3 µg/g at 10.00 h) means showed a 6½ fold increase.

Experiment 2. Adrenal E concentration showed a periodicity during the 24-h light-dark cycle consistent with that observed in the first experiment (Figure 1 and Table II); peak values occurred at 23.00 h and trough values occurred at 17.00 h. However, in contrast to the first experiment, statistical significance was not demonstrated for the fluctuations in adrenal E concentration ($F = 1.53$, d.f. = 8/60, $P > 0.05$). Comparison of the highest catecholamine mean (419 µg/g) during the 24-h period with the lowest (118 µg/g) showed a $3^1/_2$ fold increase in epinephrine concentration, and this difference was significant at the $P < 0.05$ level.

Consistent with the first experiment, adrenal NE levels showed peak values in the afternoon and through values in the morning (Figure 2 and Table II).

*Discussion.* Because of the wide range of catecholamine concentrations, it is difficult to statistically demonstrate circadian periodicity with routine procedures. However, the reproducibility of the non-stress curves in the present study, along with that previously reported for the rat[4] and hamster[5], offers considerable evidence that adrenal NE and E fluctuate markedly during a 24–h period. In the present study, the periodicity in NE and E concentration was characteristically circadian. In both experiments NE concentration was highest during the afternoon and E showed peak values during the late evening and early morning hours.

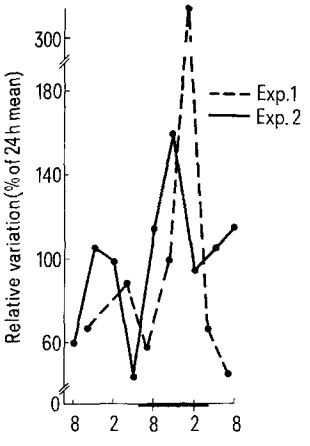


Fig. 1. Daily variation in adrenal E concentration. In this and subsequent figure, the mean values are expressed as % of the 24-h mean, i.e., relative variation.

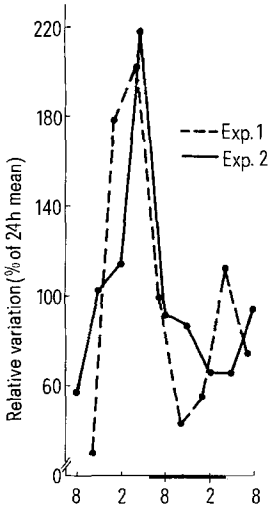


Fig. 2. Daily variation in adrenal NE concentration.

These data are consistent with that recently reported for the hamster[5] but contrasts somewhat with that previously reported for the rat[4]. In the non-hibernating hamster, adrenal NE levels were highest during the morning hours and peak E values occurred during the lights-off period of the light-dark cycle. In contrast, SCHEVING et al.[4] observed peak E levels in the rat during the late morning hours, approximately 12 h earlier than that observed in the present study. The latter discrepancy may in part reflect the differences in environmental and experimental conditions.

In light of the fact that the enzyme necessary for methylation of NE is activated by glucocorticoids, the relationship between the catecholamine curves and that previously reported for plasma corticosterone levels is of considerable interest. Peak plasma corticosterone levels in male rats housed under the controlled environmental conditions of this laboratory occur at 17.00 h[6], just after the crest of NE and 5 h prior to the peak in E. Although, evidence exists which suggests that glucocorticoid availability is not an important factor in the minute-to-minute changes in the rate of E synthesis[2], it is tempting to suggest that the late evening rise in adrenal E concentration may, in part reflect the daily rhythm in adrenocortical secretion.

Regardless of the mechanisms involved, the results of the present study offer further evidence that the concentration of adrenal NE and E vary during a 24-h period with a reproducible frequency. The physiological significance of the catecholamine fluctuations is not readily apparent. However, the need for recognizing the rhythmic nature of the medullary hormones in subsequent research is obvious.

*Zusammenfassung.* Feststellung eines Tag-Nacht-Rhythmus der Konzentration von Adrenalin und Noradrenalin in den Nebennieren von Ratten.

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