

ism and discharge of the substance. The accumulation of the labelled material at the time of ecdysis is very remarkable. The examination of the limited number of permanent larvae available indicates that this accumulation is due to a special physiological condition, rather than to the duration of exposure to the treated substrate. This accumulation may be connected with the limited excretion caused by the anti-diuretic hormone, and the sudden decrease after the ecdysis may be brought about by the increased discharge triggered by the diuretic hormone as described by Mills and Whitehead<sup>4</sup> in the American cockroach. The fact that most of this accumulated material is not the original substance but some metabolite, mostly present in the gut, seems to support this interpretation.

The total amounts of the IGR of 1 and 0.3 µg found in the 8-day-old larvae exposed to 100 and 10 µg/cm<sup>2</sup>, respectively, are relatively low, when compared with the doses usually applied by topical treatment. The high proportion of the unchanged active substance in the total amount of the labelled material in larvae exposed to the higher dose indicates the limits of the enzymatic deactivation in the

insect. In the larvae exposed to the lower dose, the daily intake and discharge is nearly in equilibrium because the amount of the active compound present in the body equals the amount of the metabolites found in the daily discharged faeces. The presence of unaltered IGR together with its degradation products in the faeces demonstrates that both metabolism and excretion are responsible for the loss of active compound. This is consistent with the results of a study by Erley et al.<sup>5</sup> on the distribution and metabolism of JH-3 in the adult locust.

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## Effect of lesions of the locus coeruleus complex on the circadian rhythm of plasma corticosterone in the mouse<sup>1</sup>

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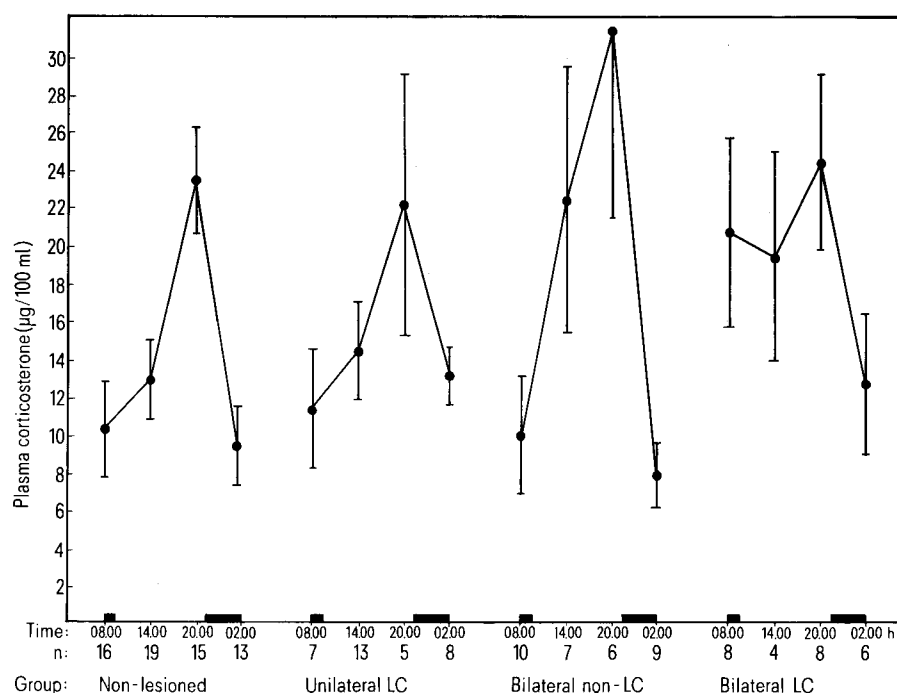
**Summary.** An apparently transient elevation of basal morning (08.00 h) plasma corticosterone levels in male mice was found 48 h after bilateral electrolytic lesions of the brainstem locus coeruleus complex but was not observed 6 weeks after lesioning.

Brainstem noradrenergic systems ascending to the hypothalamus can exert an inhibitory effect on corticotropin (ACTH) secretion<sup>2</sup>. For example, electrical stimulation in the locus subcoeruleus (LSC), or in its efferent projection, the ventral noradrenergic bundle, effectively inhibits stress-induced ACTH release in dogs<sup>3</sup>. Similarly, electrical stimulation in the LSC or in the anteroventral locus coeruleus (LC) rapidly and profoundly depresses ACTH release in chloralose-treated cats<sup>4</sup>. The present study examines the effect of lesions in LC cell groups on the basal (non-stressed) pituitary-adrenal diurnal rhythm in mice.

200 male Swiss ICR mice (Flow Research Labs) were housed individually with food and water ad libitum and a 12-h light/12-h dark lighting cycle (lights on 09.00–21.00 h). Mice were anaesthetized with Nembutal (50 mg/kg) and implanted bilaterally with Teflon-insulated platinum-iridium electrodes (125 µm diameter) aimed at the LC nuclear complex as previously described<sup>5</sup> (coordinates: anterior-posterior = -1.3 mm from lambda; medial-lateral = 0.7 mm; ventral-dorsal = 3.5 mm below the brain surface). 7 days after implantation and either 48 h (n = 91) or 6 weeks (n = 46) prior to decapitation the mice were anaesthetized with ether and lesioned (500 µA anodal current for 10 sec) bilaterally in groups of 12 at 1 of 4 different times of day (6-h intervals). Similarly implanted control groups of 12 mice each were also anaesthetized at 6-h intervals but were not lesioned. Following another 48 h (acute) or 6 weeks (chronic) in the controlled environment, the lesioned and sham lesioned mice were killed at (±30 min) 08.00, 14.00, 20.00 or 02.00 h (acute group) or at 08.00 h only (chronic group) by rapid decapitation within 90 sec of removal from the home cage. Trunk blood was collected and assayed in duplicate for plasma corticosterone<sup>6</sup>. Brains were removed for histological verification of electrode

placement and lesion damage. Lesioned mice were divided into 3 groups for each of the 2 lesion-decapitation intervals: unilateral LC-lesioned, bilateral LC-lesioned and bilateral non-LC lesioned mice. Substantial bilateral damage to the LC complex was required to qualify an animal for the designation as bilateral LC-lesioned.

Corticosterone values for mice killed 48 h after lesion or sham-lesion manipulations are shown in the figure 1. Analysis of variance (ANOVA) revealed a significant (p < 0.01) diurnal variation in the sham-lesioned control animals similar to that previously reported<sup>7</sup>. The bilateral non-LC lesioned mice also showed a significant daily rhythm (p < 0.025, ANOVA). The diurnal rhythm of the unilateral LC-lesioned mice appears normal but was not significant (p > 0.05) by either ANOVA or by a Student's t-test comparing morning (08.00 h) and evening (20.00 h) corticosterone values. The rhythm within the bilateral LC-lesioned group, however, may be disrupted. A diurnal variation within this group was not apparent by ANOVA, due primarily to the fact that steroid concentrations at 08.00 h were approximately double those of the bilateral non-LC lesioned and sham-lesioned controls at the same time of the day (p < 0.05, Student's t-test). Furthermore, when bilateral LC-lesioned and unilateral LC-lesioned groups were combined to increase the error degrees of freedom in the ANOVA, there was no significant diurnal variation (p > 0.05). Plasma corticosterone concentrations for the mice killed at 08.00 h 6 weeks after lesioning were as follows (not shown in the figure): unilateral LC = 6.6 ± 1.5 (n = 14), bilateral non-LC = 8.7 ± 2.4 (n = 14) and bilateral LC = 8.9 ± 1.4 µg/100 ml (n = 18). Thus, the possible acute elevation of the basal morning (08.00 h) corticosterone levels 48 h after bilateral LC-lesions was not found in the chronic lesion groups. We suggest that a transient disrupt-



Circadian rhythms of plasma corticosterone in male Swiss mice implanted bilaterally and either lesioned or sham lesioned 48 h before decapitation. Times of day and lesion groups are shown on the abscissa, accompanied by the numbers of animals in each time lesion category. The vertical lines represent  $\pm$ SEM. The dark bars along the abscissa indicate the periods of darkness (21.00–09.00 h).

tion of the corticosterone diurnal rhythm produced by bilateral LC-lesions is consistent with an inhibitory role for an ascending system impinging directly upon the basal hypothalamus. It is also conceivable that the lesions could influence neural input to the adrenals without altering ACTH secretion.

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## PRO EXPERIMENTIS

### An 'ecobox' with a discontinuous temperature gradient and a continuous light intensity gradient

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**Summary.** An apparatus is described for culturing micro-algae in a discontinuous temperature gradient and a continuous light intensity gradient. The apparatus provides 100 different combinations of these abiotic factors at 1 time. The cross-gradient culture apparatus is called 'ecobox'.

In studies on the cyanobacterium *Spirulina platensis*<sup>1</sup>, cultures grown at different temperatures and light intensities were compared. To save time and space and to avoid variations in experimental results due to variable conditions over long time intervals, it was considered appropriate to culture the cyanobacterium at various combinations of temperature and light intensity at one time.

Edwards and Van Baalen<sup>2</sup> described an apparatus for the culture of benthic marine algae under varying regimes of

temperature and light intensity. The apparatus described here involves an improvement, especially concerning heat transport and temperature regulation.

**Materials and methods.** Description of the apparatus (figure 1). A colourless anodized aluminium plate (15 mm thick) with 2 longitudinal borings, through which water of different temperatures is pumped, is used to achieve a temperature gradient. Aluminium is used because it is light, strong, non-toxic, easily machined, non-magnetic and