CHAPTER V

CIRCADIAN PERIODICITY OF ADRENOCORTICOIDS

IN EXPERIMENTAL ANIMALS
Adrenocortical response to stress, whether surgical or traumatic, is well-documented (Singh et al, 1972, 1975 a, 1975 b, 1975 c, 1976 a, 1976 b and 1977). Diurnal rhythmicity in adrenocortical secretory activity in normal human subjects, rats and mice is also clearly established (Migeon et al, 1956, Perkoff et al, 1959 and Saba et al, 1963). For the first time a rhythm had been reported in adrenocortical activity in terms of plasma cortisol levels in normal rabbits similar to that in human beings and other animal species (Singh et al, 1975 c). They observed peak plasma cortisol (17-OHCS) concentrations in the morning at 8 A.M., followed by a gradual fall during the day and reaching the minimum around midnight. Arora and Singh, 1977 and Singh et al 1977 reported deranged circadian periodicity of adrenocortical secretory activity following bone injury in rabbits.

Therefore, the present study was planned to examine whether the same degree of trauma produced during maximum and minimum cortical activity periods of day-night cycles plays a role in the disruption of the circadian periodicity and in the response of the gland to meet the challenge produced by the stress of fracture or whether the derangement of the cortical rhythmicity is inevitable, irrespective to the time the trauma is produced.
MATERIAL AND METHODS

Forty healthy rabbits of both sexes each weighing approximately 1.5 kg were used in the present study. These animals were divided into two groups. Group I was comprised of 10 rabbits and served as control group. Ten millilitres of blood from each animal belonging to group I was collected at 4-hour intervals from the ear vein over a 24-h period. The remaining 30 animals were kept for the trauma group. This group was further divided into three subgroups, each consisting of 10 rabbits. Following collection of blood at 08°0, closed fracture of right femur was produced manually at mid-thigh level in all the animals of first subgroup. Thereafter blood was collected at 4-h intervals up to 24 hours. Similarly, following collection of blood at 16°0 and 00°0, the same degree of trauma was produced in rabbits belonging to subgroups II and III. Thereafter the blood was again collected at 4-h intervals for 24 hours in both subgroups. All these animals were anaesthetized by administering urethane (30 mg/kg body weight) intraperitoneally prior to fracture.

All the blood samples were collected in heparinized tubes. Plasma was separated by centrifugation and stored at 0-4°C until steroid estimation was done. Plasma 17-hydroxycorticosteroids (17-OHCS) was estimated, employing the procedure described by Peterson et al. 1957. The natural dark and light schedule followed the day-night cycles and was kept constant throughout the experiment.
RESULTS

Control group: Mean plasma 17-OHCS level at 08° was 52.2 µg% (SE ± 3.2) which declined gradually during the rest of the day, reaching minimum at 00°. The mean values were 16.0 µg% (SE ± 2.9; p < 0.01). The levels increased noticeably at 04° reaching 51.3 µg% (SE ± 3.6) at 08° the next day (Table, Fig.1).

Trauma Group: Plasma 17-OHCS level at 08° in animals belonging to subgroup I was 49.6 µg% (SE ± 4.1). However, following fracture the level increased markedly with maximum elevation at 16°, the average value being 102.6 µg% (SE ± 9.6; p < 0.01). Thereafter the levels declined gradually, raching 60.4 µg% (SE ± 3.8) at 08° the next day (Table I, Fig.1).

Similarly, mean plasma 17-OHCS level at 16° and 00° were 31.3 µg% (SE ± 4.2) and 15.2 µg% (SE ± 3.8) which also rose markedly following injury. The maximum elevation in the levels occurred at 00° and 12° following 8 and 12 hours of injury in both the subgroups. The mean values at 00° and 12° were 121.4 µg% (SE ± 5.6) and 98.2 µg% (SE ± 8.6) respectively. Thereafter, a gradual fall in the levels was observed, but, the levels remained significantly elevated up to 24 hours after fracture (p < 0.01; p < 0.01) (Table 1, Fig.1).

Plasma 17-OHCS levels were markedly elevated in all the traumatized animals when compared with the initial values at the same time intervals in normal rabbits (p < 0.01) (Table 1, Fig.1).
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<th>TIME INTERVAL</th>
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<th>08°</th>
<th>12°</th>
<th>16°</th>
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p = When compared with 8 A.M. values in normal rabbits.

p₁ = When compared with 8 A.M. levels in subgroup I.

p₂ = When compared with 4 P.M. values in subgroup II.

p₃ = When compared with 12 midnight concentrations in subgroup III.

*p₄ = When compared with initial values at same time intervals in normal rabbits and traumatized animals.
FIG 1. PLASMA 17-OHCS IN NORMAL AND TRAUMATIZED RABBITS
DISCUSSION

We observed a definite circadian rhythm of plasma 17-OHCS levels in normal rabbits (control group). Peak plasma 17-OHCS levels were recorded in the morning at 08° which were followed by consistent fall during the day time reaching the minimum around mid-night. Earlier we also reported a similar rhythm in plasma 17-OHCS levels in normal rabbits (Singh et al. 1975c). However, in response to trauma by fracture of femur, the rabbits (trauma group) exhibited a marked increase in plasma 17-OHCS levels and the circadian periodicity was deranged up to 24 hours. We noticed a two-fold increase in plasma 17-OHCS levels at 12° when the trauma was produced at 08°, however, the same degree of trauma produced at 16° and 00° resulted in a three-fold and a five-fold increase respectively in level as compared with the initial values at the time of injury. In contrast to normal rabbits, the traumatized animals showed very high plasma 17-OHCS levels at mid-night and the levels remained elevated up to 24 hours. Maximum increase in plasma 17-OHCS levels was observed at 00° when the trauma was produced at 16°, and minimum response in the level occurred at 08° when trauma was produced at mid-night. Similar increases in plasma 17-OHCS levels have been reported in response to trauma and other stressful stimuli (Singh et al. 1972, 1975a, 1975b, 1976a, 1976b). Surgical operations were followed by a prompt rise in plasma cortisol levels which reached a maximum at 4 to 12 hours after surgery and returned to normal levels within 36 to 48 hours (Steenburg et al. 1956 and Thomasson 1959).
In this study we also noticed maximum increase in plasma 17-OHCS levels at 8 and 12 hours following injury; however, here we observed significantly elevated levels of plasma 17-OHCS up to 24 hours after fracture in all the traumatized animals. Singh et al. 1977 reported a similar pattern in the levels with a second peak at 32 hours and the levels remained elevated up to 64 hours in traumatized animals.

However, in this experimental study we recorded the changes in plasma 17-OHCS levels up to only 24 hours. Elevated levels of plasma corticoids up to 24 hours following injury could be due to painful stimuli lasting for a comparatively longer period in such a situation. Moreover, these rabbits were not mobilized and this might also have provided a stimulus in elevating plasma 17-OHCS levels up to 24 hours as observed in the present study. Elevated levels of plasma 17-OHCS up to 24 hours following injury might also be due to the long-lasting effect of stress as produced by the fracture of femur.

Normal circadian periodicity in plasma cortisol levels has been reported to be lost following surgical operations and head injuries in human beings (King et al. 1970). Similarly, normal rhythm of adrenal activity has been found to be deranged in diseases of the central nervous system (Kreiger, 1961), as well as in altered states of consciousness, abnormal sleep pattern, delirium, semicoma or coma (Perkoff et al. 1959, and Eik-Nes and Clark, 1958). In Cushing's syndrome, the loss of normal rhythm is probably due to deranged ACTH production from pituitary
Electrolytic lesions of the hypothalamus have been found to change the normal circadian rhythms of plasma cortisol levels (Doe et al. 1960). The above observations tend to suggest the possible control by the central nervous system of the normal rhythms. In normal circumstances, stress, plasma cortisol levels and circadian rhythm regulate to ACTH release (Cushman, 1968). Following stress the normal feed-back mechanism influencing the ACTH release has been reported to be lost (Carroll et al. 1969). As a result, the plasma cortisol levels remain elevated without affecting the ACTH release. This abnormally high level of plasma cortisol might be responsible for altering the normal pattern of circadian rhythms of adrenal activity as seen in this study. Wise et al. 1972 have suggested three possible mechanisms responsible for elevated levels of plasma corticoids in response to injury. These are: increased numbers of secretory episodes per day, increased duration of secretion per day and increased rate of secretion during secretory episodes per day. Thus, certain of the above mechanisms might also have played a role in disrupting the diurnal periodicity of adrenocortical secretory activity in traumatized rabbits.

We noticed different degrees of response in plasma 17-OHCS levels when the same degree of trauma was produced at maximum and minimum hours of adrenocortical secretory activity. Maximum response of the gland was observed at 00° when the injury was inflicted at 16° and minimum at 08° when the trauma was produced at 00° in traumatized animals in contrast to normal ones where the plasma 17-OHCS levels
were maximum at 08\textdegree0 and minimum at 00\textdegree0. Thus, it seems that the response in plasma 17-OHCS levels not only depends on the magnitude and severity of trauma or injury but also on the time during the 24-hour day-night cycle when the trauma is produced.

Furthermore, the derangement of plasma 17-OHCS levels was found to be inevitable in response to trauma and injury since we observed deranged circadian periodicity of plasma 17-OHCS levels in all the traumatized animals irrespective of the time the trauma was produced. Thus, adequate adrenocortical response to trauma and derangement of the adrenocortical secretory activity are essential and inevitable, regardless of the time the trauma is produced. However, the response in the gland also depends on the time the trauma is produced along with magnitude and severity of trauma.
CONCLUSIONS

Effects of bone injury at different intervals of the 24-hour day-night cycle on adrenocortical secretory circadian periodicity in rabbits have been reported in the present study. We observed significant increase in plasma 17-OHCS levels following fracture in all the traumatized animals and the levels remained elevated up to 24 hours after trauma. However, the same degree of injury produced at different hours of day-night cycles exhibited different degrees of response in plasma 17-OHCS levels. We noticed a deranged circadian periodicity of plasma 17-OHCS levels in all the animals belonging to the trauma group. Thus, adequate adrenocortical response to trauma and derangement of the cortical secretory activity is inevitable, irrespective of the time the trauma is produced. However, the response in the gland also depends on the time the trauma is inflicted as well as the magnitude and severity of injury.