Monoamine oxidase (MAO) is known to inactivate the biologically active catecholamines liberated at the adrenergic nerve endings. Catecholamines are actively involved in the regulation of endocrine function[2,3]. Although, MAO activity in the hypothalamus has been studied in a variety of vertebrates[4,5] the areas of ependyma and cerebrospinal fluid (CSF) are less explored. In 2 species of teleosts, weak MAO activity was reported in the ependymal lining of the infundibular recess[5]. Monoamine containing liquor contact neurons have been reported in several animals, including fishes[6-8]. Subsequent to the review on the importance of CSF by HELLER[9], substantial evidence has accumulated in favour of hormones, releasing factors (RF) and monoamines being secreted or blood-borne into the CSF and later transported by the ependyma through the median eminence to the portal vasculature[10,11]. KNOWLES[12] has suggested a short and a long loop feedback pathway by which these active principles get in and released out of the CSF. However, very little is known about these phenomena in the lower vertebrates.

In this study, 65 catfish *Clarias batrachus* were used. MAO activity was demonstrated by the tryptamine tetrazolium method of GLENNER et al.[13]. Specificity of the enzyme reaction was verified by incubating the sections in substrate-free medium and by pretreating them with niamid which is a known MAO inhibitor. In *C. batrachus* subependymal region and the CSF exhibited strong MAO activity in comparison to ependyma (Figure 1). However, MAO positive tracts were seen running between the subependyma and CSF through the ependyma (Figure 2), suggesting a conducting role for the latter. MAO activity is also not uniform throughout the ependyma. The anterodorsal lining of the infundibular recess exhibits more activity than the other areas (Figure 3). These observations tend to support the view that in fishes also the CSF might form an essential link in the process of neuroendocrine control of hypohysial function and ependyma is capable of transporting active principles in the fishes also.

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Circadian Variation of Serum Testosterone in the Adult Male Rat with a Late Morning Acrophase[1]

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Summary. Serum testosterone concentrations were measured in adult male Sprague-Dawley rats. A significant circadian testosterone rhythm ($p < 0.01$) was found with peak values at 10.00 and 13.00 h.

The study and knowledge of circadian rhythms is important in elucidating the control of endocrine systems and in designing or interpreting studies which may be influenced by alterations in circulating hormone concentrations. A diurnal serum testosterone rhythm in the male human has been described and confirmed by a number of investigators (see review by RUBIN et al.[4]). Circadian serum testosterone rhythms have also been described in a number of other species including the bull, cock and monkey[5-7]; however, data concerning commonly used laboratory animals such as the rat are scanty. KINSON and LIU[8] have reported a circadian serum testosterone rhythm in the rat with peak values found at 03.00 and 06.00 h. In their study blood samples were obtained every 3 h from

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the same animals following ether anesthesia. Since it has been shown that stress, such as exposure to ether, can elevate the serum gonadotropin levels and abolish the rhythm of serum LH concentrations[9], the present work was undertaken.

Materials and methods. Adult male Sprague-Dawley rats (ARS/Sprague-Dawley, Madison, Wis.), 320–350 g, were maintained under conditions of constant temperature and lighting (lights on from 06.30 to 19.00 h) for 3 weeks prior to study. They were fed standard laboratory chow and water ad libitum. The studies were carried out in the month of September at the Minneapolis V.A. Hospital research facility. Animals were rapidly decapitated and trunk blood was collected, allowed to clot at room temperature, and separated by centrifugation. The serum was stored at −20 °C until assayed for testosterone by radioimmunoassay[10]. The between assay variability of this method, as estimated by the coefficient of variation of a serum pool containing 344 ng/100 ml, is 7% ($N = 20$). There was no effect of the order of killing of the animals on the testosterone levels within each group.

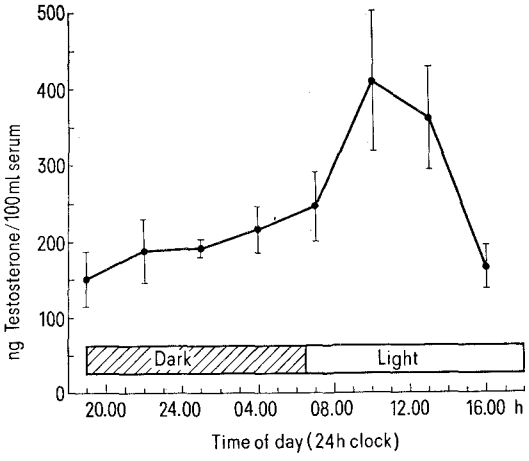
Results and discussion. The results are expressed graphically in the Figure. They show a circadian serum testosterone rhythm with peak values found in the late morning and early afternoon (10.00 and 13.00 h). The mean serum testosterone concentrations ranged from a peak of 410 ng/dl at 10.00 to 118 ng/dl at 19.00. The testosterone concentrations at the peak were nearly 3.5 times those of the low point of the day and demonstrated a greater variability. The mean testosterone concentrations at 10.00 and 13.00 were significantly elevated ($p < 0.02$) above those at 16.00 h. Analysis of variance using a one tailed test revealed an F value of 3.81 with 7,40 degrees of freedom ($p < 0.01$).

These studies confirm the presence of a circadian serum testosterone rhythm in the adult male rat; however, the timing of this rhythm differs significantly from previous reports. Howland[11] measured serum concentrations of testosterone in Sprague-Dawley rats (06.00 to 18.00 lighting schedule) at 3 times of day (04.00, 12.00 and 20.00) following decapitation. Serum testosterone concentrations were significantly higher at 04.00 than at 12.00 h, which, although the time points samples are few, is essentially in agreement with those of Kinson and Liu[8].

Diurnal cyclicity in plasma testosterone concentrations appears to result from changes in the rate of testicular secretion of this hormone[12] and increasing evidence suggests that LH may play a regulatory role in that phenomenon[4,12–14]. Unfortunately, reports of diurnal variation in plasma LH in male rats are conflicting and do not clarify the discrepant testosterone results. Dunn, Ariumur and Scheving[9] have found a circadian periodicity in serum LH values with peak levels occurring at 20.00 h, whereas Ter Haar and MacKinnon[15] report a circadian serum LH rhythm with peak values at 09.00 h and minimum levels at 21.00 h. Shin and Kraicer[16], Yamato, Diebel and Bogdanove[17], and Lawton and Smith[18] were unable to demonstrate a significant circadian periodicity in serum LH.

We are unable to explain the discrepancies between the testosterone rhythm found in this study and that reported by Kinson and Liu[8] nor also in the LH patterns described in the literature. Many factors are known to influence circadian rhythms including stress, lighting, feeding and temperature[19]. It is possible that methodologic differences in the studies account for the differences in the circadian gonadal rhythms observed. Another possible explanation is that there may be a seasonal variation in the timing of gonadal rhythms in the male rat. Haus and Halberg[20] have shown a seasonal shift in the acrophase of the circadian corticosterone rhythm in the mouse and recently Reinberg[21] has presented evidence suggesting that there is a seasonal variation in the acrophase of the human testosterone circadian rhythm.

We conclude from these studies that in our laboratory, at the time and under the conditions specified, there is a circadian variation in serum testosterone in the adult male rat with a late morning acrophase. Because of the variation in the timing of rat gonadal rhythms now reported, we feel it is unwise to assume the presence or timing of any gonadal rhythm in the adult male rat without confirming it under local conditions.



Mean serum testosterone concentration in male rats killed at different times of day. The vertical lines represent the SEM. N for each point is 6.

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