Circadian Rhythms of Plasma 17-Hydroxycorticosteroids in the Infant Monkey (34471)

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A daily rhythm in plasma concentrations of cortisol or 17-hydroxycorticoids (17-OHCS) is a well-known characteristic of the adult rhesus monkey, *Macaca mulatta* (1). The steroid levels are highest after a night of sleep and decline during the waking, daytime activities. This same rhythm occurs in the human, in whom it seems to be entrained by sleep–waking cycles, and not by light–dark cycles *per se,* since the cycle has been observed to be reversed in humans working at night and sleeping by day (2). Furthermore, the cycle can be altered considerably in length to correspond to altered sleep–activity cycles of human volunteers (3). Finally, data in the human infant (4), who exhibits a polyphasic sleep pattern throughout the day, indicate no appearance of a circadian plasma 17-OHCS cycle, and the 17-OHCS cycle only gradually emerges to the full adult pattern by 3–13 years of age, lagging considerably behind the emergence of a "fully developed" adult sleep–waking cycle which occurs between 5–8 months of age.

The age at which the plasma 17-OHCS cycle is initiated has not been previously reported for the monkey. Such data would be of interest in helping to understand possible mechanisms of the rhythm, in correlating the development of the rhythm with neural or behavioral maturation, and in providing data useful for the design of adrenocortical studies utilizing the infant monkey.

Materials and Methods. The infant monkeys used in the present studies were separated from their mothers the day of birth and were caged individually in a monkey nursery under 24-hr light conditions. They were hand fed a milk formula every 2 hr, as previously described (5).

In Experiment I, two blood samples of about 1 ml each were taken with heparinized syringes from either the saphenous or femoral vein of each of 10 infant monkeys, 2–5 days of age. The first sample was taken at 9:00 PM and the second 12 hr later at 9:00 AM. Plasma was separated within 30 min and stored at $-15°$ until assayed. Plasma concentrations of 17-OHCS were determined by an ultramicro Porter-Silber method which has been found to measure mainly cortisol in the adult monkey (6).

Experiment II was a more extensive experiment designed after the results of Experiment I. Blood was drawn from 15 monkeys after 5 consecutive days beginning at 2–3 days of age. One sample was taken from each monkey daily at either 8:00 AM, 12:00 PM, 4:00 PM, 8:00 PM, or 12:00 midnight according to a Latin-square order. Three 5×5 Latin squares were randomly chosen to determine these orders and each monkey was assigned to one of the 15 rows of these three squares. This design spaced the blood sampling sufficiently to avoid the likelihood that the stress of one sampling would affect steroid levels at the next sampling, and also spaced the blood loss of the monkeys. All procedures of storage and assay of plasma, and all other conditions of treatment of the infant monkeys were as described for the first experiment.

Systematic observations on sleeping and activity were made on 10 of the monkeys of Experiment II. Every 30 min, an experimenter observed whether the monkey at that time was (a) asleep, (b) awake (eyes open) but inactive, or (c) awake and active (*i.e.,* eyes open plus limb movement). These observations were pooled over all monkeys and days to obtain curves giving the frequency with which monkeys exhibited each of these activities for each 2-hr block throughout the day. The data obtained on these 10 monkeys were similar to that obtained for a more extensive series of infant monkeys not included in the present steroid measurements.

Results and Discussion. In Experiment I, concentrations of 36.1 ± 4.6 and 28.9 ± 4.8 μg/100 ml 17-OHCS (means ± SE) were observed at 9:00 AM and 9:00 PM respectively. Thus, the percentage of relative change (100 times the AM minus PM difference divided by the average of the AM and PM values, as described by Franks (4) was —22.2. The difference was in the same direction as would be predicted from data on circadian changes in the adult monkey and was statistically significant ($t = 1.98$, $df = 9$, $p < .05$ for a one-tailed test). These values were comparable to those of 32.7 ± .97 (*SE*) and 21.4 μg/100 ml reported by Migeon *et al.* (1) for six adult rhesus monkeys at 9:00 AM and 9:00 PM respectively, for which the percentage of relative change was —41.8.

In Experiment II, the infant monkeys exhibited their highest steroid levels at 8:00 AM, namely 35.0 ± 3.6 μg/100 ml; these values tended to decline throughout the day to 24.0 ± 3.0 μg/100 ml at 12:00 midnight (Fig. 1). Analysis of variance indicated that this change across hours was significant ($F = 3.07$, $df = 4$ and 33, $p < .05$). There was also an unexpected significant change in steroid values over the first 5 days of the experiment ($F = 3.69$, $df = 4$ and 33, $p < .025$). The daily mean steroid values were 36.3, 26.7, 27.4, 23.2 and 26.7 μg/100 ml. There was thus a tendency for the steroid values to be highest on the first day of the study (at Day 2–3 of life), and not subsequently different. The high initial value may have represented some postparturition trauma.

It was interesting that these infant steroid levels were similar to those reported earlier by Migeon *et al.* (1) for the adult monkey, as indicated in Fig. 1, although not so high as those of the adult at 8:00 AM nor at 12:00 midnight. The percentage of relative change of the infant monkeys from 8:00 AM to 8:00 PM was —41%, and for the adult monkeys described by Migeon *et al.* (1) was 59%.

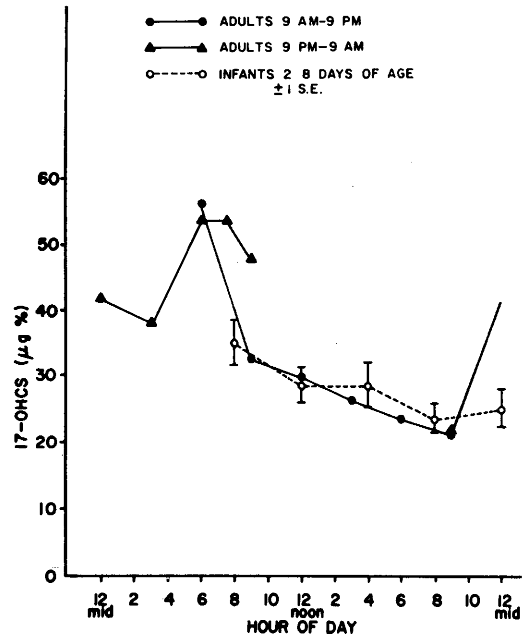

FIG. 1. Plasma 17-OHCS changes in infant monkeys throughout the day, as compared with data on the adult monkey as reported by Migeon *et al.* (1955).

Thus, these data suggest that the infant rhesus exhibits a less pronounced circadian 17-OHCS secretion than does the adult rhesus.

These steroid data fit very well with the measures of sleep and activity taken on the infant rhesus (Fig. 2). Although the infant monkey exhibited a polyphasic sleep pattern, and could be found sleeping 60–70% of the time throughout the day, there was, nevertheless, some emergence of the adult-type circadian sleep–wake pattern. In particular, the infant was most likely to be asleep at 3:00 AM and 5:00 AM (71% at both hours), and was least likely to be awake and active at those hours (8% and 7% respectively). From 7AM to 11:00 PM, the infant was found awake and active 20–25% of the time and asleep about 65% of the time. The steroid data thus appeared to be correlated with the awake and active state, or with its converse, namely, the asleep plus the awake but inactive states.

It would appear, therefore, that the neural mechanisms contributing to circadian changes in adrenocortical function were already active

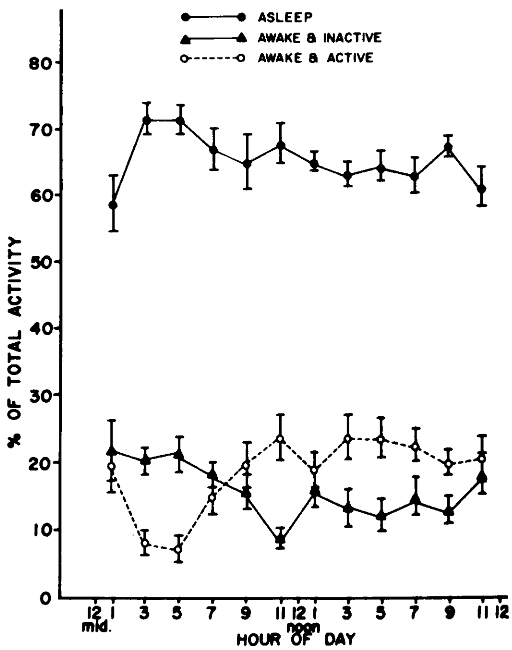

FIG. 2. Percentages of total activity in each 2-hr block throughout the day in which the infant monkeys were either asleep (●—●), awake and inactive (▲—▲) or awake and active (O- - -O) ± 1 SE is plotted around each point.

in the 2- to 8-day-old rhesus monkey, and that insofar as the monkeys exhibited an attenuated 24-hr sleep–activity cycle, they also exhibited an attenuated circadian 17-OHCS pattern. This finding appears dissimilar to those in the human infant (4), but is consonant with previous results in which we have found the infant rhesus to have an adrenocortical system reactive both to ACTH (7) and to restraint stress (8). As has been noted elsewhere (9), the 17-OHCS response of the infant or young rhesus to ACTH is significantly higher than that of the adult monkey. Thus, the CNS–hypophyseal–adrenocortical system of the monkey appears to be functional soon after birth in all major aspects so far examined.

Summary. Plasma 17-hydroxycorticosteroids (17-OHCS) exhibited a significant circadian rhythm in rhesus monkeys (*M. mulatta*) in the first week of life. In two experiments, plasma 17-OHCS levels averaged 35 μg/100 ml at 8–9:00 AM, and declined to levels of 29 μg/100 ml at 9:00 PM (Expt. I) and 24 μg/100 ml at 12:00 midnight (Expt. II). These changes were about half the magnitude reported by others in the adult monkey. Correlated with this steroid rhythm, the infant monkey, although having a polyphasic 24-hr sleep pattern, also exhibited a tendency to sleep more at 1:00 and 3:00 AM than at other times of the day. This suggested a relationship between sleep–activity cycles and steroid cycles in the infant monkey similar to that in the adult monkey.

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