PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 166, 360–363 (1981)

Influence of Protein–Calorie Malnutrition on the Circadian Rhythm of Pineal Melatonin in the Rat (41074)

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Abstract. Pineal melatonin was measured over a 24-hr period in 50-day-old Sprague–Dawley male rats that had been fed a low-protein diet for 30 days and in a group of age-matched controls that were maintained for the same period on a standard laboratory chow. The patterns of melatonin secretion were similar in both groups. The highest content and concentrations were recorded during the dark phase of the light:dark cycle, while the lowest were measured after the onset of light. At each of the time intervals studied, less of the antigonadotrophin was present within the pineal glands of the malnourished rats.

We have recently evaluated the endocrine profile of the protein–calorie malnourished rat (1). This form of undernutrition is quite prevalent in today's world (2) and seems to affect children more than any other age group (3). Because of this, we focused on selected time intervals between 20 and 50 days of age, a period in the life of a rat which would correspond to childhood and early adolescence in humans. The investigation demonstrated that serum and pituitary gonadotrophins, serum steroid levels, and hypothalamic LHRH were markedly reduced in the animals that were malnourished (1). The observed changes occurred rapidly and were, in general, sustained as long as the experimental conditions ensued.

Starvation and chronic underfeeding are two additional forms of undernutrition which lead to a reduction in gonadotrophin secretion (4, 5). Several laboratories have reported changes in pineal gland activity in rats that are chronically underfed (6–10). Knowing that the pineal gland secretes antigonadotrophic principles which are thought to exercise their physiologic actions directly on the hypothalamus and/or the pituitary gland (11), we questioned whether one of these hormones was influenced by protein–calorie malnutrition. The present report describes the rhythm of melatonin in 50-day-old male rats that had been maintained on a low-protein diet since weaning.

Materials and Methods. One hundred twenty-eight Sprague–Dawley male rats were purchased at 20 days of age from Simonsen Laboratories, Gilroy, California. They were separated into two groups and housed in temperature-controlled quarters having a light:dark cycle of 14:10. The lights were regulated automatically so that they turned on at 0500 hr and went off at 1900 hr. Half of the animals were provided with a standard laboratory diet containing 27% protein, 59% starch, 10% vegetable oil, and 4% salt mixture USP XIV. The remaining rats were fed a low 8% protein diet which also contained 78% starch, 10% vegetable oil, and 4% salt mixture USP XIV. The two diets were isocaloric (1) and were administered *ad libitum* for 30 consecutive days. The changes in the body weight and the amount of food consumed by each rat were followed on a daily basis. When the animals were 50 days of age, eight control and an equal number of malnourished rats were sacrificed by decapitation at each of the following time intervals: 0100, 0300, 0500, 0700, 1100, 1500, 1900, and 2300 hr. The pineal gland was removed from each animal and stored at −20°. During the dark phase of the light:dark cycle, the procedures were conducted using a red Kodak 1A safelight. The tissues were subsequently homogenized in 0.01 *M* phosphate–saline buffer, pH 7.0, and assayed for melatonin by radioimmunoassay (12). The protein

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0037-9727/81/030360-04$01.00/0

concentration of the homogenates was determined using the procedure of Lowry *et al.* (13). The results were combined according to experimental grouping and statistically analyzed using analysis of variance and the *t* test for comparison of differences between individual means.

Results. At the beginning of the study, the mean body weight of the animals was 40.4 ± 1.7 g. By Day 50, the rats fed the standard laboratory diet weighed 249.0 ± 7.1 g, while the body weight of the rats maintained on the low-protein diet was only 53.1 ± 3.1 g. The daily intake of food, kilocalories, and proteins by the malnourished rats was less than that observed by the controls.

The circadian rhythm of pineal melatonin is shown in Fig. 1. At each of the intervals examined, including those when the animal room was illuminated, significantly less melatonin was present in the pineal glands of the protein–calorie malnourished rats. From 0700 to 1900 hr, the percentage differences between the two groups ranged from 395 to 753%, while those computed for the remaining time varied from 177 to 390%. The general pattern of melatonin secretion was, however, similar in both the control and the malnourished animals. Maximal values were recorded during the dark phase of the light:dark cycle, albeit the zenith was attained in the control group 2 hr earlier. During the 4 hr between 1900 and 2300 hr, melatonin rose 9.1-fold in the pineal glands of the controls, while over the same time period, a 20.9-fold increase was measured in the rats maintained on the low-protein diet. At 0100 hr, significant differences ($P < 0.02$) were detected over values recorded at the previous sampling time for each of the two animal groups. Thereafter, no marked changes occurred until just prior to the onset of light, at which time melatonin levels fell precipitously.

Protein concentrations were found to be lower in homogenates of pineal glands from rats fed the low-protein diet. When the melatonin values were expressed in terms of milligrams of protein concentration of the tissue homogenates, a profile similar to that for total organ content of the indole amine was observed (Fig. 2). Lower values were again found in the experimental animals during the light phase of the light:dark cycle. Peak levels for both groups occurred after the animal room lights were extin-

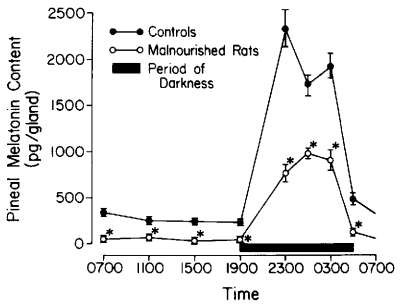

FIG. 1. Total pineal melatonin content over a 24-hr period in control and protein–calorie malnourished male rats. Each point represents the mean ± SE. Statistical comparisons were made between means from control and experimental animals at each time interval (*) $P < 0.001$.

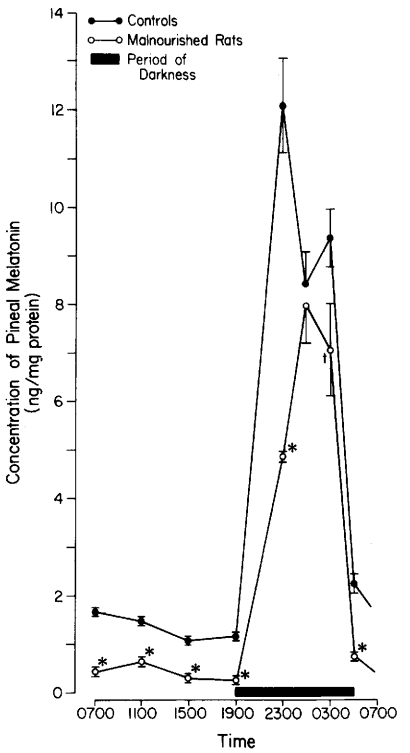

FIG. 2. Pineal melatonin concentration over a 24-hr period in control and protein–calorie malnourished male rats. Each point represents the mean ± SE. Statistical comparisons were made as described in Fig. 1. (†) $P < 0.01$, (*) $P < 0.001$.

guished and a marked decline in melatonin transpired near the end of the 10 hr of darkness. The magnitude of the differences recorded at any single time point between the control and malnourished rats was, however, less (Fig. 2 vs fig. 1).

Discussion The activity of the pineal gland appears to be sensitive to changes in the nutritional status of an animal. Under conditions of protein–calorie malnutrition, the content of protein within the pineal gland and the synthesis of melatonin were markedly depressed, even during the light phase of the light:dark cycle when pineal function is thought to be at its nadir (14). To our knowledge, the only other report in which daytime levels of a pineal compound were affected by experimentation is from Moore and Rapport (15). They found that the activity of the melatonin-forming enzyme, hydroxyindole-*O*-methyltransferase, was less in pineal glands collected 1 hr after the onset of light from rats whose preganglionic sympathetic nerve fibers had been transected as compared to that noted in the sham-operated controls. The rhythm in melatonin production was not affected by the malnourished conditions precipitated in our animals. However, there may have been some influence on the temporal pattern of melatonin secretion since maximal levels of the antigonadotrophin were observed 2 hr later in the experimental animals as compared to that measured in the controls. Shortened photoperiods (16) or maintaining animals in continuous darkness (12) has had little affect on the rhythms associated with pineal activity. Other experimental procedures, however, such as constant light (12) and bilateral superior cervical ganglionectomy have been effective in preventing the nocturnal rise in peripheral (12) or tissue content (17, 18) of the indole amine.

The relationships between the experimental observations made in the present study and the hypogonadotrophic state which exists in the protein–calorie malnourished rat (1) are not entirely clear. The pineal gland is thought to play a minor role in the regulation of the reproductive system of the rat (19). Minimal changes occur in the reproductive organs and in peripheral gonadotropic levels of this species after pinealectomy or blinding (19). Thus, the decrease in melatonin measured in the pineal glands of the undernourished animals may have had little or no influence on the activity of the hypothalmo-pituitary-gonadal axis. Moreover, the observations made may be entirely explained by the reduction in the amount of proteins present within the pineals from which the precursors for melatonin biosynthesis are generated. Alternatively, however, it is possible that protein–calorie malnutrition may have altered the metabolic activity of the pineal gland. Walker *et al.* (10) recorded an increase in oxygen consumption by pineal glands obtained from chronically underfed rats. If a similar acceleration in glandular activity transpired in our undernourished animals, then the turnover rate of melatonin has been increased to the extent that less of the indole was detected at any given point in time. Additionally, one would anticipate that larger quantities of melatonin would be secreted in response to an appropriate stimulus if the gland were truly hyperactive. This did occur in the malnourished animals after the onset of darkness. The percentage increase in indole content and concentration during the dark phase of the light:dark cycle was significantly greater in the pineal glands of these animals as compared to that recorded in the organs of the well-fed controls. If these points are considered in light of the reports which indicate that malnutrition increases the responsiveness of rats to the actions of the pineal antigonadotrophins (7, 20, 21), then it is possible that the levels of melatonin measured in the protein–calorie malnourished animals were sufficient to affect gonadotrophin secretion. Additional experimentaiton, however, will be needed to verify this premise and to further clarify the role of the pineal gland during undernutrition.

This project was supported by grants from the NIH (HD 10914) and NSF (PCM 77-05734). The authors wish to thank Bonnie Ferguson, Sheryl Green, and Donna Kenneally for their expert technical assistance in this study.

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Received May 8, 1980. P.S.E.B.M. 1981, Vol. 166.