gas admixed to buccal contents varies from 24 to 75 percent (*5*). Calculations based upon a simple mixing system give comparable results.

However, the percentage of admixture of the gas leaving the nostrils is presumably less important than is the percentage of admixture to the gas being shifted into the lungs. Gas concentration during a single ventilatory efflux indicates that the coherent stream of pulmonary gas must bypass pockets of air in the buccal cavity. The gradual reduction of efflux concentration after the rapid attainment of a concentration peak could only result from the mixing between portions of such pockets and the slowing air stream. Furthermore, occasional high-velocity exhalations result in maximum concentrations of pulmonary gas in the nostril efflux of much lower concentrations. The gas introduced into the lung (diluting its argon or nitrogen concentration) must then have been in the buccal cavity at the time of the maximum efflux. The introduced gas must consequently have had a significantly lower concentration than that leaving the nostrils during the previous cycle; that is, it could not have been mixed with lung efflux during the period of high outflow.

Our results support the concepts that (i) the buccal oscillations serve to flush out the buccal chamber between ventilatory cycles and are definitely respiratory, (ii) the pulmonary gas crosses the buccal cavity in a coherent stream, and (iii) the gas entering the lungs during ventilation is positioned in the posterior portion of the buccal chamber ventral to the glottis and hence subject to minimum admixture during outflow from the lungs.

Recent reports by Bishop and Foxon (*6*) and McMahon (*7*) suggest that the filling of the buccal cavity with air by a hyoid movement and the exhalation of the pulmonary contents through this buccal chamber occur in lung fish and may consequently be phylogenetically old. Frogs have apparently retained this basic pattern but have increased its efficiency by reducing mixing.

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Early Experiences Accelerate Maturation of the 24-Hour Adrenocortical Rhythm

Abstract. *Rats reared under 12-hour alternating periods of light and dark were killed at times corresponding to the times at which the maximum or minimum plasma corticosterone concentrations occur in mature animals. The characteristic 24-hour adrenocortical rhythm was first observed in rats 21 to 25 days old. In rats handled or stimulated with electric shock, the rhythm developed as early as 16 days.*

Although circadian rhythms in mature organisms have been observed in physiological systems virtually wherever they have been sought, frequently they cannot be detected in newborn animals. We first sought to determine when, in the life of the laboratory rat, the light-synchronized 24-hour rhythm in plasma corticosterone concentrations could first be detected. Allen and Kendall (*1*) have reported that the adrenocortical rhythm becomes evident at 30 to 32 days of age. Our data indicate that it develops about 10 days earlier. It was also determined that maturation of the adrenocortical rhythm could be influenced by stimulation of the infant rat by handling or electric shock, stimuli which alter subsequent behavioral and physiological processes and resistance to disease (*2*).

Pregnant Charles River (CD) rats were individually housed in transparent plastic cages containing nesting materials, food, and water. These cages were located in modified office-type cabinets

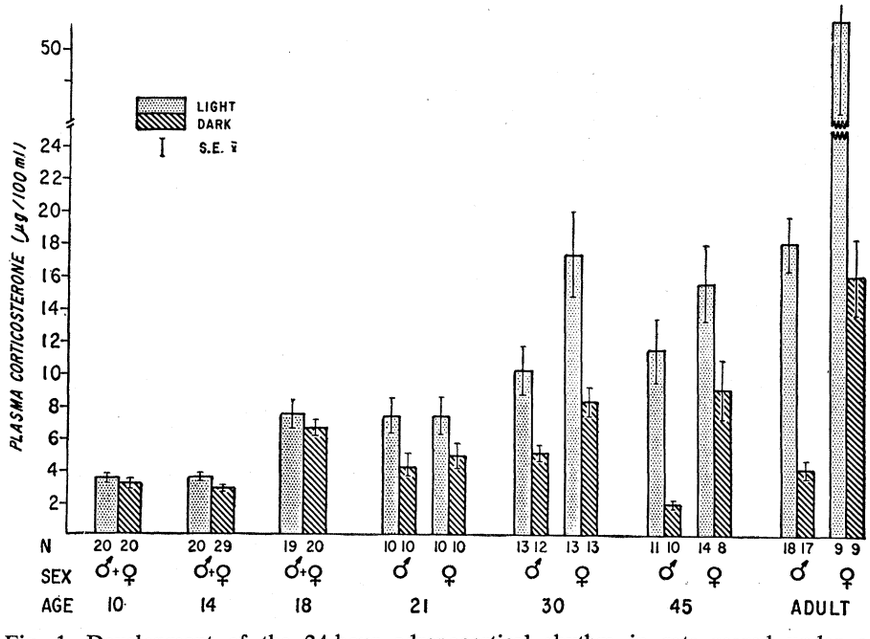

Fig. 1. Development of the 24-hour adrenocortical rhythm in rats reared under a 12-hour light-dark cycle. "Light" values were obtained 2 hours before the onset of the period of darkness, and "dark" values were obtained 2 hours before the onset of the light period. The values for adult animals were obtained from a previous study (*5*).

(*3*). Five such cabinets housed up to 50 litters and permitted as many as five different light-dark schedules within a single room.

At birth the litters were split and culled to a maximum of ten animals. Thereafter, the cages were not cleaned, and, except for replenishment of food and water, the animals were not disturbed. The rats sampled after 21 days were weaned at that time; the mother was removed from the cage, and the pups were separated by sex but remained in the nesting cages inside the cabinets. In mature rats maintained under a 12-hour light-dark regimen (LD 12 : 12), corticosterone concentrations are maximum approximately 2 hours before the onset of darkness and minimum approximately 2 hours before the onset of light (*4, 5*). Animals were decapitated, and individual samples of trunk blood were frozen for subsequent analysis for corticosterone by a modification (*6*) of the method of Glick *et al.* (*7*). Samples were obtained at 10, 14, 18, 21, 30, and 45 days of age at times of maximum and minimum adrenocortical concentrations in mature animals.

The corticosterone values obtained during the period of light (2 hours before darkness) were significantly greater than those observed during the period of darkness, as early as 21 days of age (Fig. 1). The rhythm was clearly present at 30 days, by which time the difference between males and females had also become evident. The development of the 24-hour rhythm in adrenocortical activity apparently begins at approximately 21 days, and the difference between maximum and minimum values progresses very gradually to that seen in adults.

The handled animals or those given electric shock (0.1 ma increasing with age to 1.0 ma) were treated exactly as above, except that they were housed in an open colony room. Control animals remained undisturbed, and the experimental groups received stimulation for 3 minutes daily up to, but not including, the day of sacrifice. Samples were obtained on days 16, 18, 20, 22, and 25. Analyses revealed an interaction between treatment, time of sampling, sex, and age ($F = 1.98$, d.f. = 8/524, $P < .05$). The unmanipulated animals did not show a difference between the corticosterone values sampled 2 hours before darkness and 2 hours before light, until 25 days of age (Fig. 2). In contrast, previously handled rats showed a difference at 20 days

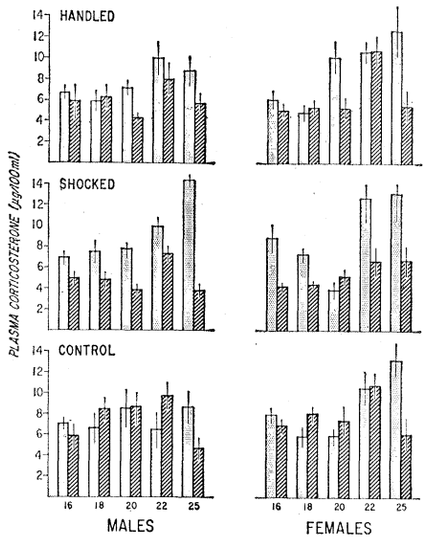

Fig. 2. Development of the 24-hour adrenocortical rhythm in handled, shocked, and unmanipulated (control) rats reared under a 12-hour light-dark cycle (light bars, maximum values; hatched bars, minimum values; vertical lines, standard error of the mean). The mean values are based on groups of 8 to 17 animals.

of age. There were no differences at 22 days, but the rhythm was again evident at 25 days. Because the lack of a difference at 22 days appears to be due to the relatively high corticosterone concentrations observed during the period of darkness and because relatively innocuous stimulation superimposed upon the trough in the adrenocortical cycle is particularly effective in eliciting an elevation in corticosterone levels (*5, 8*), this finding may have resulted from some extraneous environmental stimulation. With the single exception of the group of females sampled on day 20, animals that had been given electric shock showed a rhythm in corticosterone concentrations as early as 16 days of age. It is possible, then, that with appropriate stimulation development of the adrenocortical rhythm might mature before 16 days of age.

The repetitive handling and shock stimulation occurred at a constant time each day and could have acted as an additional cue for a 24-hour period. In order to eliminate this possibility an additional group of animals was stimulated by electric shock at a different time each day, and samples were taken beginning at 14 days of age. There was no difference between the corticosterone values 2 hours before the onset of light and 2 hours before darkness at 14 days, but the significant differences observed beginning at 16 days of age confirmed the above findings.

The observation of such a daily rhythm at 21 days of age is in agreement with the statement to this effect by Fiske and Leeman (*9*), although no such data have been published. In the second population of experientially naive animals the 24-hour rhythm was not evident until 25 days. This difference may reflect the greater degree of environmental control afforded by the use of the closed cabinets relative to the housing of animals in an open colony room. This may also be partially responsible for the discrepancy between these findings and those of Allen and Kendall (*1*). Another possibility is that the times of the daily samples in the study by Allen and Kendall were not optimum.

Maturation of the 24-hour adrenocortical rhythm at 21 days of age is still relatively late in comparison with development of the organism's capacity to respond to "stressful" stimuli, with an elevation in concentration of corticosterone in plasma (*10*). While the functional significance of an accelerated maturation of the 24-hour adrenocortical rhythm remains to be determined, these results do indicate that experiences occurring early in life can influence the development of neuroendocrine control mechanisms.

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