Deviations in food and water consumption and urinary electrolytes with frequency of measurement in rats*

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Metabolic readings taken 1, 2, and 6 times per day from ad lib rats maintained under constant illumination revealed phasic increases in food and water consumption with each change in the number of daily measurements by the E. A temporary dilution in urinary electrolyte concentrations was noted during the first day following the switch from 2 to 6 readings per day. It is recommended that the number of reading periods be fixed from the outset of investigations concerned with metabolism. If this is not possible, then appropriate control groups should be employed to partial out the effect of any changes in the frequency of E presence from those due to the experimental manipulation.

Kissileff & Epstein (1969) have proposed that prandial drinking is a learned response to a dry mouth and facilitates the ingestion of food. Fitzsimons & Le Magnen (1969) have distinguished food-associated drinking from prandial drinking in part by demonstrating that rats learn to anticipate the increased future metabolic water requirement imposed by replacement of a carbohydrate diet with a protein diet. Thus, food-associated drinking more adequately provides for metabolic water need than prandial drinking. In each case, an association is apparently formed between internal stimuli, e.g., dry mouth, gastric osmoreceptors, gastric distention, and drinking behavior. Also of importance are contingent associations formed between consummatory behavior and external environmental setting conditions. For example, Bolles (1961) has proposed that the 24-h feeding cycle in rats kept under constant light can be triggered by the presentation of fresh food and water on a set daily schedule. And Tapp et al (1970) have reported that rats given ad lib food and water increase their activity with the onset of various stimuli, such as light, tone, and odor.

The present investigation focuses attention upon possible associations between frequency of E presence and the consumption of food and water. Informal observations taken over the course of earlier work had suggested that whenever the E removed the foodcups and drinking cylinders to measure and refill them, the animals

investigated and often ate and drank when the containers were restored. Given this potential source of experimental error in studies of metabolism, an investigation of the effect was considered necessary. The present experiment is thus concerned with the influence of frequency of metabolic measurements upon food and water consumption. It is hypothesized that just as activity increases with the onset of environmental stimulation (Tapp et al, 1970), so may food and water intake vary as a function of the number of E manipulations of the food and water containers.

METHOD

Eighteen experimentally naive male albino Holtzman rats, approximately 120 days of age, were adapted to unit metabolism cages (Acme Metal Products) under constant illumination in a sound-isolated room at a temperature of 23°-24°C for a 7-day preobservational period. This adaptation period was necessary in view of evidence indicating that constant light may inhibit fluid consumption during the initial 3 days of maintenance (Gutman et al, 1969). The designated reading periods were the only times the E entered the room. The animals were maintained on ad lib tap water and powdered Wayne Mouse Breeder Blox (content: 0.42% Na, 0.77% K). When there was only one metabolic reading period per day, it was conducted between 0900 and 1000 h. The second reading period per day, when instituted, came between 2100 and 2130 h, and when six reading periods were employed, they were initiated at 0100, 0500, 0900, 1300, 1700, and 2100 h.

The body weight of each animal was determined once a day between 0900 and 1000 h. Water and food consumption and urine volume excretion were measured at each

reading period. Urine was collected under mineral oil, and a sample was retained from each animal for each reading period. Urinary sodium and potassium concentrations were determined by flame photometry (Instrumentation Laboratories, Model 143) and urine specific gravity by refractometer (American Optical Company, Model 10400 TS meter). The metabolism cage funnels were washed daily, rinsed in distilled water, and thoroughly dried in an adjoining room following the morning reading period.

RESULTS

Mean water consumption, urine volume excretion, and food intake corrected for body weight changes for each of the 7 days following the week of adaptation are offered in Fig. 1. During these 7 days of observation, one metabolic reading was taken at 0900-1000 h. The mean $\pm$ standard error (SE) water intake across this 7-day period was 32.3 ± 2.1 ml/day, and urine volume output was 16.5 ± 1.8 ml/day. Mean food intake was 15.9 ± 0.9 g/day. The mean $\pm$ SE sodium concentration across the initial 7 days was 221.9 ± 19.7 mEq/L. The comparable potassium values were 195.2 ± 17.8 mEq/L.

The next day following the change from one to two metabolic readings per day (first vertical intermittent line) indicated an increase in water intake to 49.2 ± 1.8 ml/day, which was significantly higher than the seventh and last day of one reading per day (t for correlated measures = 7.81, $df = 17$, $p < .01$; all t tests were for correlated measures). The urine volume excretion for Day 8 also increased to 23.8 ± 1.1 ml/day and was different from that of Day 7 ($t = 5.20$, $df = 17$, $p < .01$). Food ingestion increased from a Day 7 mean of 15.8 ± 0.7 to 23.7 ± 0.9 g/day on Day 8 ($t = 9.87$, $df = 17$, $p < .01$). The mean urinary sodium and potassium concentrations of Day 8 were 228.0 ± 18.9 and 229.8 ± 16.5 mEq/L, respectively, and were not different from the Day 7 values.

Days 9-15 represent a 7-day period of maintenance with two readings per day and are also presented in Fig. 1. The mean water intake during this period was 29.4 ± 2.2 ml/day and was not different from the Days 1-7 period ($t = 1.35$, $df = 17$, $p > .10$). Mean urine volume excretion was 16.1 ± 1.6 ml/day and was also not different from the Days 1-7 period ($t = 0.27$, $df = 17$, $p > .10$). The mean food intake during Days 9-15 was 15.7 ± 0.8 g/day and was not different from Days 1-7 ($t = 0.24$, $df = 17$, $p > .10$). The mean urinary sodium and potassium levels during the second week were 199.9 ± 16.4 and 216.8 ± 14.5 mEq/L and did

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Fig. 1. (Upper part) Mean $\pm$ SE water and urine volumes (milliliters/body weight $\times$ 100) for the three measurement conditions: once (24 h), twice (12 h), and six (4 h) times per day. (Lower part) Mean $\pm$ SE food consumption (grams/body weight $\times$ 100) noted for the three measurement conditions over days.

not differ from the levels of the first week.

With the change from two to six metabolic readings per 24 h on Day 16 (second vertical intermittent line), there was an increased mean water intake to 37.7 ± 1.9 ml/day, and urine volume output also increased to 21.4 ± 1.7 ml/day, as did food intake, which increased to 20.2 ± 1.1 g/day ($t = 3.41, 2.80, \text{ and } 4.89$, respectively, $df = 17$, $p < .05$). The mean urinary sodium concentration of Day 16 was 174.1 ± 9.8 mEq/L, significantly lower than the Day 15 mean of 198.4 ± 11.2 mEq/L ($t = 2.32$, $df = 17$, $p < .05$). Urinary potassium level for Day 16 was 177.7 ± 10.4 and was lower than the Day 15 mean of 208.2 ± 14.5 mEq/L ($t = 2.37$, $df = 17$, $p < .05$). The 3 days of six readings

subsequent to Day 16 indicated recovery back to baseline values not different from the established norms.

DISCUSSION

The results of this experiment indicate substantial increases in food and water intake and urine volume excretion concomitant with increases in the number of metabolic reading periods per day. These changes were phasic, for within 2 days after the alteration, baseline values were reattained. Although no changes in urinary sodium and potassium concentrations were noted with the switch from one to two readings per day, there were significant decreases in concentration with the change from two to six daily measures.

The influence of time of day upon metabolic characteristics of the rat has

been well documented (Bare, 1959; Verplanck & Hayes, 1953). For instance, Young & Richey (1952) have found that morning food deprivation has much less effect than evening deprivation due to the rat's persistence to eat the major portion of its food during the 12 dark hours of the diurnal cycle. The results of this investigation further indicate that the frequency of measurements taken per day can influence these levels. The methodological cautions stemming from these findings suggest that the number of measurements be fixed from the outset of the experiment or that an adaptation period of at least 2 days be employed after altering the number of reading periods. If the above is not possible, then the influence of a change in measurement schedule can be partialled out from the experimental manipulation by the employment of appropriate control groups.

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