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BRIEF COMMUNICATION

Automatic Measurement of Drinking in Rats: Effects of Hypophysectomy[1]

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KURIBARA, H., T. HAYASHI, M. R. ALAM, S. TADOKORO AND T. MIURA. *Automatic measurement of drinking in rats: Effects of hypophysectomy*. PHARMAC. BIOCHEM. BEHAV. 9(5)697–702, 1978.—A new apparatus for the continuous measurement of drinking in the rats was assembled. The principle of the device is as follows: a cartridge which makes water drops (0.05 ml) is inserted between a water tank and a drinking spout. When a rat drinks, water falls into the cartridge drop by drop and the number of drops is electrically counted. The total count of drops per day, as well as counts at definite intervals, can be automatically printed out. To test apparatus reliability and applicability, drinking behavior in hypophysectomized rats was investigated in the light and dark phases, alternating every 12 hr. Activity and feeding in these phases were also observed. In the sham-operated rats, the total daily water intake was 30–40 ml, which corresponded to 10–15% of the body weight, and 85–95% of the total daily drinking counts were recorded in the dark phase. In the hypophysectomized rats, a large amount of water was drunk immediately after the operation. However, the high rate of drinking rapidly returned to near the normal level within a few days. Drinking in the dark phase decreased to about 75% of the total daily, but synchronization with the light-dark cycle was still maintained. The daily patterns of activity and eating ran nearly parallel with the drinking behavior. These results indicate that our drinkometer could have extensive applications within many fields of research.

Automatic drinkometer Drinking rhythm Activity rhythm Feeding Hypophysectomy Rats

MANY ANIMAL behaviors and physiological functions synchronize with the day-night cycle, thus showing a circadian pattern [1]. Many rodent species, including laboratory rats, are nocturnally active, with most of the locomotor activity, eating and drinking occuring in the dark. On the other hand, a large proportion of these quiescence or sleeping occurs in the light [8]. Since it has been technically difficult to observe all these behaviors continuously and quantitatively, there are few comprehensive reports except those on locomotor activity alone [7,8].

In the present work, we succeeded with our newly assembled drinkometer, in recording the drinking pattern of the rat continuously and quantitatively. Furthermore, to provide a sample of change in the drinking pattern, we investigated hypophysectomized rats.

METHOD

Animals

Thirty-eight adult male rats of the Wistar strain were used. The strain has been maintained for about 25 years by brother-sister mating in the breeding colony of Gunma University, Medical School. At the start of the experiment, these animals were 3 months old, weighing about 250 g. Each rat was housed in an individual wire mesh cage of 38 (D)×25 (W)×17 (H) cm and allowed to eat solid diet MF (Oriental Yeast Co., Tokyo) ad lib. Also, water was freely available through a drinking spout (SE, TV-15, O'hara & Co. Ltd., Tokyo), set on a side wall of the cage.

Room Condition

In the light-dark cycle, the two phases alternated at 12 hr intervals. The light phase began at 6:00 a.m. and ended at 6:00 p.m. Illumination was provided by fluorescent lamps, and the intensity of illuminance on the experimental cages was about 100 Lux. Room temperature was maintained at 22 ± 2°C throughout the experiment. Humidity was not controlled.

[1]The authors thank Prof. John L. Falk for comments on this paper.

TABLE 1
EXPERIMENTAL CONDITIONS AND GROUPING OF RATS

Operation	Time of the measurement after the operation	N and marking	Items of observation
Sham-operation	immediately	3 (C1–C3)	Drinking
	10 days	5 (C4–C8)	Drinking
	90 days	5 (C9–C13)	Drinking
	90 days	5 (M1–M5)	Motor activity
Hypophysectomy	immediately	5 (H1–H5)	Drinking
	10 days	5 (H6–H10)	Drinking
	90 days	5 (H11–H15)	Drinking
	90 days	5 (HXM1–HXM5)	Motor activity

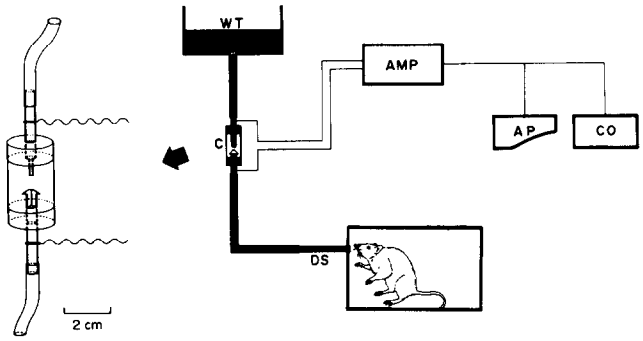

FIG. 1. Schematic structure of the automatic drinkometer. WT: Water tank. C: Cartridge. DS: Drinking spout. AMP: Amplifier. AP: Automatic printer. CO: Electromagnetic counter.

Surgery

The thirty-eight rats were divided into 8 groups of 3–5 animals each. Under ether anesthesia, they were hypophysectomized or sham-operated by the transtracheal method. The experimental conditions for the groups of animals are shown in Table 1.

Water Drinking Measurement

The amount of water drinking and its 24-hr pattern were measured with an automatic drinkometer which was assembled by us in collaboration with a medical instrument maker (O'hara & Co. Ltd., Tokyo). The principle of the device and the method of measurement are as follows: a water tank is connected with a drinking spout through a cartridge, which makes 0.05 ml water drops accurately. When a rat drinks water, water falls drop by drop. The short circuit generated by each drop was amplified to activate an electromagnetic counter. Thus the apparatus permits the exact determination of the amount and rate of water drinking. The cartridge is made of acrylfiber and stainless steel tubes. The schematic diagram of the automatic drinkometer is shown in Fig. 1. By the use of one unit of these devices, recording for 10 animals can be performed separately and simultaneously. In the present experiment, drinking counts of each 1-hr period were automatically printed out. The data for the first 3 days were eliminated owing to imperfect adaptation to the new experimental environment. The total period of observation lasted for 15–30 days.

Locomotor Activity Measurement

Locomotor activity was measured with an ANIMEX DS (AB Farad, Sweden), and the activity count was printed out at 1-hr intervals by an automatic printer (Muromachi Kikai Co. Ltd., Tokyo). During the observation period, the rats were housed in a Plexiglas cage with sawdust floor in place of the wire mesh cage. The sensitivity settings used were 20 and 30 for low and high sensitivities, respectively. Data for the first 3 days in the experimental boxes were excluded, and observations were continued for 15–20 days.

RESULTS

Figure 2 shows 24-hr drinking patterns of three groups of the sham-operated rats (C1–C3, C4–C8, C9–C13) over a period of 10 days. The drinking of these rats was measured immediately, 10 and 90 days after the operation, respectively. In this figure, the vertical bars indicate the drinking counts (1 count=0.05 ml) at 1-hr intervals, and the abscissa is the time scale. The shaded parts in the lower channels denote the dark phase. For rats C9–C13, average body weight had increased to about 350 g, at the start of measurement.

The total daily water intake in the sham-operated rats was 30–40 ml, which corresponded to 10–15% of the body weight, and remained nearly constant every day. The 24-hr drinking patterns of the rats were well synchronized with the light-dark alternation, with a large proportion (85–95%) of the total daily volume of water consumed in the dark phase.

Figure 3 shows, in the same way as in Fig. 2, the drinking patterns of three groups of hypophysectomized rats (H1–H5, H6–H10, H11–H15) over a period of 10 days. The drinking behaviors of these rats were measured immediately, 10 and 90 days after the operation, respectively. After hypophysectomy, the body weight of the rats gradually decreased, and rats H6–H15 weighed about 200 g.

Immediately after hypophysectomy, the rats drank a large amount of water (c. a. 70 ml/day), and synchronization with the light-dark alternation disappeared for 2 days. Although the synchronization with the light-dark cycle was recovered within a few days, an increase in drinking in the light phase remained for long periods. This behavioral change persisted as late as 3 months after the operation as seen in the drinking patterns of rats H11–H15. The total amount of water drinking of hypophysectomized rats accounted for about 15% of

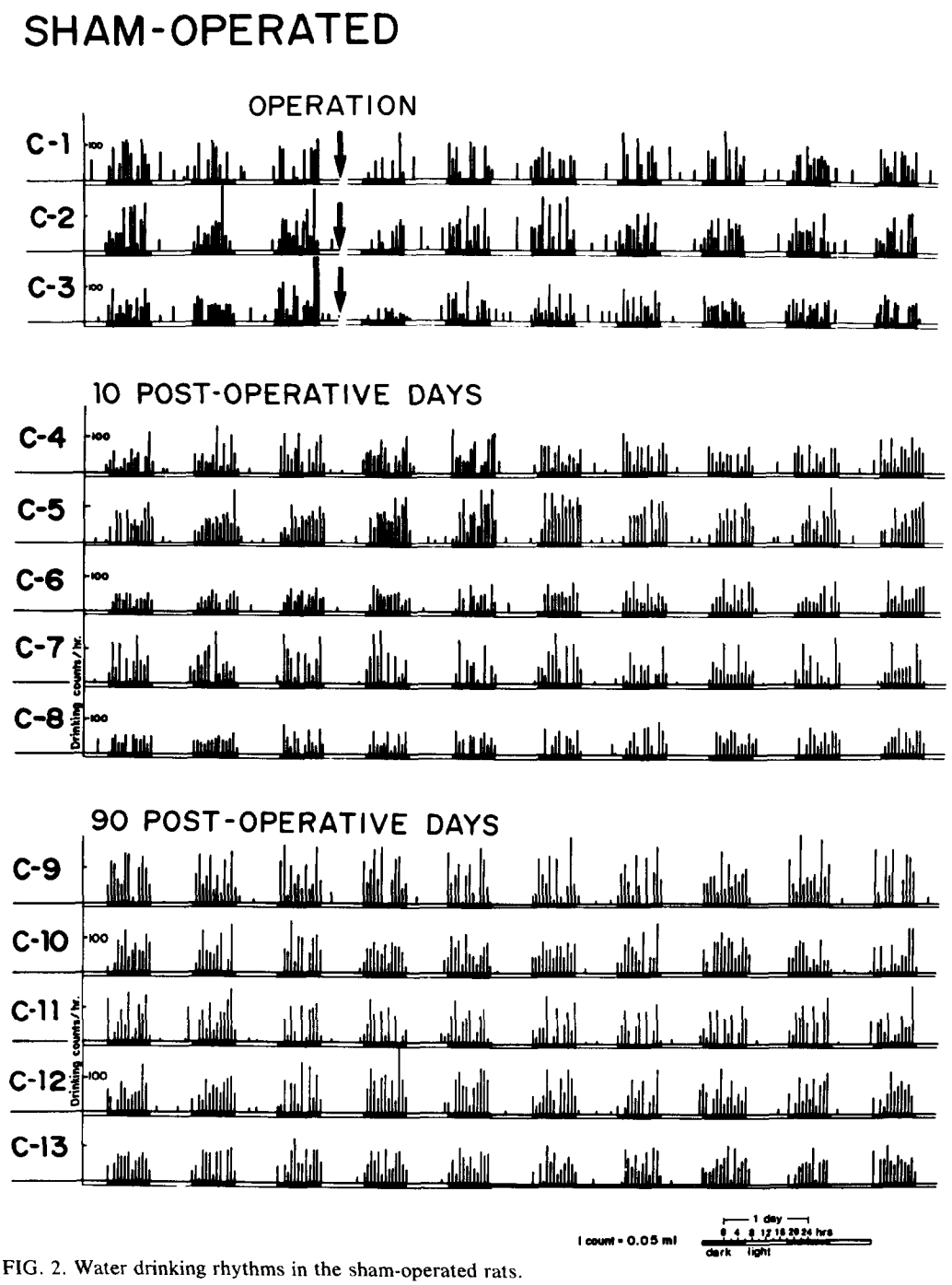

FIG. 2. Water drinking rhythms in the sham-operated rats.

the body weight, which tended to be higher than those for the sham-operated groups, though not significantly. The mean percent drinking counts in the dark phase for the two groups of hypophysectomized rats (H6–H10, H11–H15) were both about 75% of the daily totals, which were significantly lower than those for the sham-operated groups ($p<0.01$, Student's t-test).

Figure 4 shows the count of locomotor activity for each of the sham-operated rats (M1–M5) and hypophysectomized rats (HXM1–HXM5) at 1-hr intervals for 10 consecutive days, from 90 post-operative days. Because the qualitative patterns of the activity measured at sensitivities of 20 and 30 were analogous, only the data at sensitivity 30 are presented in Fig. 4. The vertical bars indicate the activity counts at 1-hr intervals, and the abscissa is the time scale. The lower shaded lines denote the dark phases.

The 24-hr patterns of locomotor activity of the sham-operated rats changed with the light-dark cycle, with about 75% of the total daily locomotor activity counts recorded in the dark phase. Individual differences were marked, though for any one case, the pattern remained nearly constant from day-to-day.

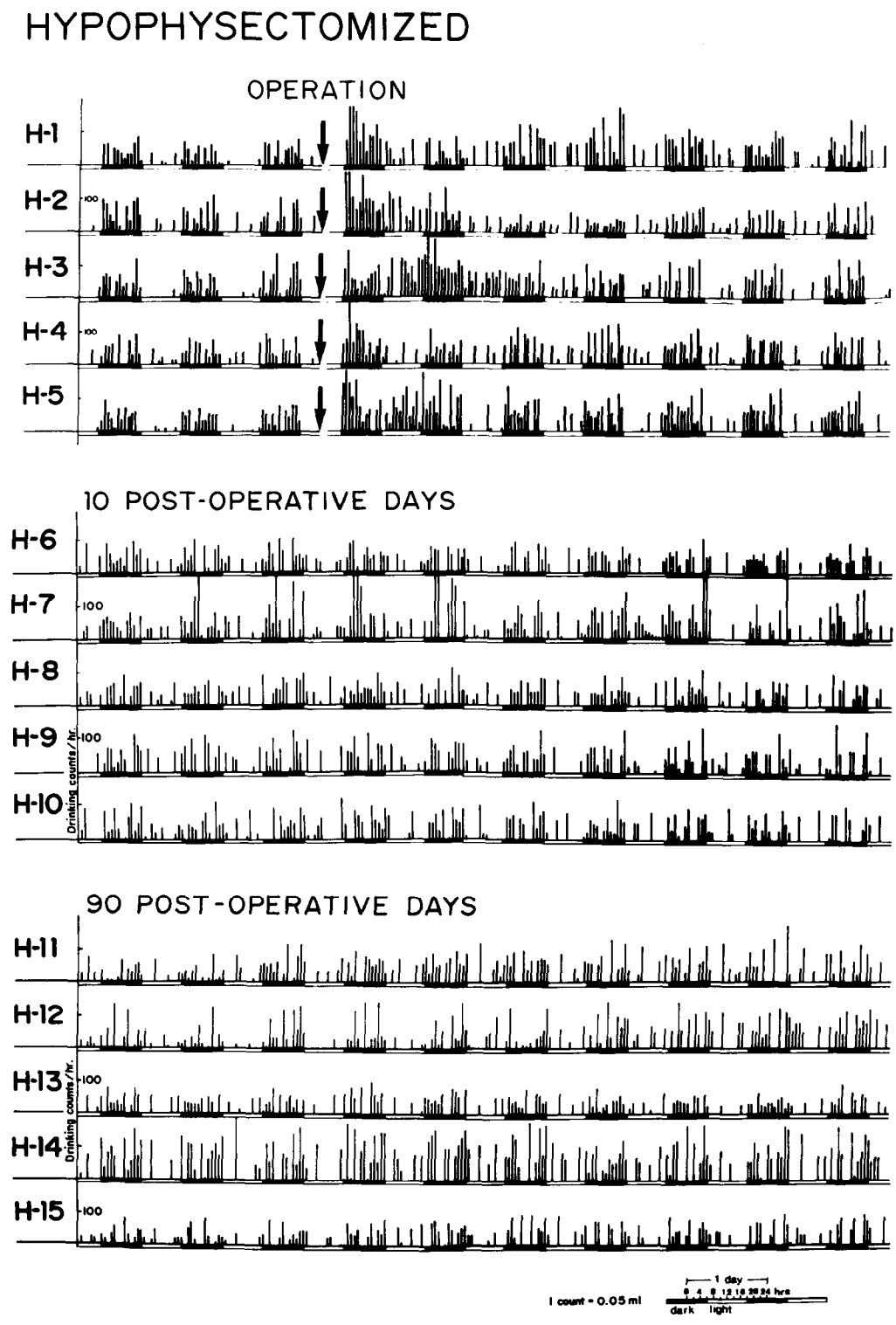

FIG. 3. Water drinking rhythms in hypophysectomized rats.

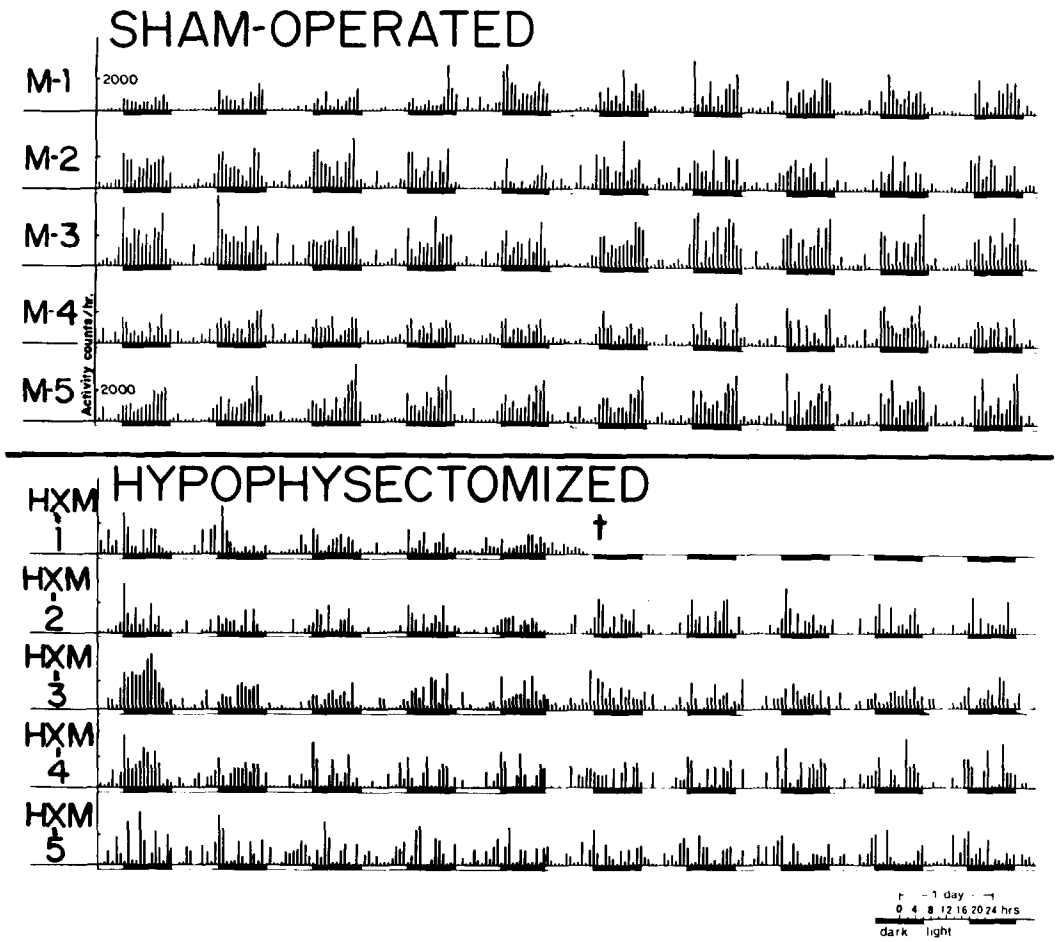

FIG. 4. Locomotor activity rhythms in the sham-operated and hypophysectomized rats.

One hypophysectomized rats (HXM1) died during the observation period, and its data were excluded from the calculations. The activity pattern of each hypophysectomized rat also changed synchronously with the light-dark cycle. However, the activity in the light phase increased and conversely decreased in the dark phase. Average percent activity of the total in the dark phase decreased to about 65% which was significantly lower than that for the sham-operated group ($p<0.05$). According to gross observation, the eating ran nearly parallel with the drinking activity.

DISCUSSION

The present experiment corroborated that drinking and eating behaviors, and locomotor activity of rats synchronize with the light-dark alternation, thus showing regular 24-hr rhythms. With regard to behaviors in the dark phase compared to the daily total, drinking yielded about 90%, while locomotor activity showed about 75%. Thus the rate for the former was greater than the latter, and furthermore the drinking revealed smaller individual differences. Therefore, it may be more convenient in studying change in behavioral rhythm in the rat to take drinking as the indicator.

Drinking behavior runs nearly parallel with eating, as reported by many investigators [3,5]. Therefore, one may be able to estimate the eating pattern by measuring the drinking behavior alone. On the other hand, differences were observed in the drinking and the locomotor activity patterns between the sham-operated and hypophysectomized rats. As pointed out by Stephan and Zucker [9], the latter was more liable to display drinking in the light phase than the former. The effect of hypophysectomy evidently persisted as late as 3 months after the operation. This means that the disorder in this behavior caused by hypophysectomy is probably irreversible. As for the mechanism of induction of the behavioral disorders following hypophysectomy, various factors can be considered, among which the blocking of pituitary-thyroidal or -adrenocortical pathways, or lack of ADH secretion from the posterior pituitary gland may be important. Especially, the drinking burst observed the first and second days after hypophysectomy was probably due to the lack of ADH secretion. These are problems to be solved in future manipulations involving endocrinological determinants.

Although hypophysectomized rats displayed distorted drinking and locomotor activity patterns compared to the sham-operated animals, their 24-hr behavioral rhythms still conform to the light-dark alternation cycle. This result indicates that the synchronization is not greatly affected by the hypophysectomy. According to Halberg *et al.* [4] and Ferguson *et al.* [2], however, the rectal temperature of

hypophysectomized mice showed a free-running rhythm. Further elucidation of the relation between these physical rhythms and the behavioral rhythms must await future study.

The automatic drinkometer, which was assembled by us and used in the present experiment, does not aim to measure the total amount of water consumed as did the conventional ones [9,10], but can measure temporal changes in drinking behavior exactly. The arrangement allows an accurate moment-to-moment measure of licking behavior and the actual amounts consumed since spillage from the spout is virtually impossible. This is so because fluid flow from the reservoir to the spout is a demand function of the licking behavior. Actually, we [6] have reported that the licking counts ran parallel with the drinking counts. The apparatus is of particular use when the surface tension or viscosity of the liquid is different from that of water (e.g., ethanol solution) where leakage can be a problem.

REFERENCES

1. Bünning, E. *The Physiological Clock.* New York: Springer-Verlag, 1967, Revised Second Edition.
2. Ferguson, D. J., M. B. Wisscher, F. Halberg and L. M. Levy. Effects of hypophysectomy on daily temperature variation in C_3H mice. *Am. J. Physiol.* **190:** 235–238, 1957.
3. Fitzsimons, J. T. and J. LeMagnen. Eating as a regulatory control of drinking in the rat. *J. comp. physiol. Psychol.* **67:** 273–283, 1969.
4. Halberg, F., J. H. Galicich, F. Unger and L. A. French. Circadian rhythmic pituitary adrenocorticotropic activity, rectal temperature and pinnel mitosis of starving, dehydrated C mice. *Proc. Soc. exp. Biol. Med.* **118:** 414–419, 1965.
5. Kissileff, H. R. Food associated drinking in the rat. *J. comp. physiol. Psychol.* **67:** 284–300, 1969.
6. Kuribara, H. and S. Tadokoro. Effects of psychotropic drugs on interpellet distributions of lever pressings and water drinking under a fixed interval 1.5 min schedule of food reinforcement in rats. *Jap. J. Pharmac.* **27**(Supple.): 57, 1977.
7. Richter, C. P. *Biological Clocks in Medicine and Psychiatry.* Springfield: Chas. C. Thomas, 1965.
8. Richter, C. P. Sleep and activity: their relation to the 24-hour clock. In: *Sleep and Altered States of Consciousness. Proc. Ass. Res. nerv. ment. Dis.* **45:** 8–27, 1967.
9. Stephan, F. K. and I. Zucker. Rat drinking rhythms: central visual pathways and endocrine factors mediating responsiveness to environmental illumination. *Physiol. Behav.* **8:** 315–326, 1972.
10. Zucker, I. Light-dark rhythms in rat eating and drinking behavior. *Physiol. Behav.* **6:** 115–126, 1971.