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Phenobarbital Effects on Weight Gain and Circadian Cycling of Food Intake and Body Temperature[1] (41007)

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Abstract. Rats fed a diet supplemented with phenobarbital at a concentration of 0.25% gained less weight than rats fed the unsupplemented diet. The reduced weight gain in the phenobarbital-treated rats accompanied the induction of marked hepatomegaly. Circadian cycling of food consumption in control rats followed a biphasic pattern, with the first feeding episode occurring during the middle of the 12-hr dark phase and the second occurring as the dark phase ended. In rats on phenobarbital, eating activity was confined to the first feeding episode, with the level of intake during this interval increasing to compensate for the absence of significant subsequent feeding, so that the daily levels of food consumption were similar in both groups. Measurements of circadian cycling of deep body temperature showed that ingestion of 0.25% dietary phenobarbital approximately doubled the amplitude of the temperature cycle and advanced the time at which peak temperature was attained by approximately 2 hr. It is suggested that the lower weight gain in rats chronically exposed to 0.25% dietary phenobarbital results primarily from alterations in hepatic metabolism, but phenobarbital-mediated changes in the circadian cycling of food intake and deep-body temperature may also contribute to the growth reduction.

Studies in this laboratory have shown that 0.05% dietary phenobarbital enhances hepatic tumorigenesis in rats previously fed the hepatocarcinogen, 2-acetylaminofluorene, for a brief (2- to 3-week) interval (1–4). In recent experiments we compared tumorigenic enhancing effects of dietary phenobarbital at various dose levels. During the course of these studies we observed that 0.25% dietary phenobarbital reduced the growth rate of the rats and produced noticeable somnolence between approximately 1100 and 1500 hr daily, although lower dietary phenobarbital levels did not produce somnolence or a growth reduction. Our initial interpretation of these observations was that the behavioral changes induced in the rat by the high dietary phenobarbital probably caused a reduction in food intake, with a consequent slowing of growth. This interpretation required empirical examination, however, since it was necessary to determine whether our tumorigenesis studies, which involved the use of different dietary phenobarbital concentrations, would be complicated by phenobarbital-mediated changes in the level of food intake. The present study, therefore, analyzed the effects of 0.25% dietary phenobarbital on growth rates and food intake levels in rats. In addition, in view of the cyclic behavioral change noted above, phenobarbital effects on the circadian fluctuations in deep-body temperature and feeding activity were measured by means of an automated feeding and monitoring system. The results suggest that phenomena other than reduced food intake underlie the growth suppression produced by 0.25% dietary phenobarbital.

Materials and methods. Male Sprague–Dawley rats at 22 days of age were obtained from Charles River Laboratories, Wilmington, Massachusetts, and were maintained for 25 days (five rats per cage) on a pelleted 30% casein, semi-synthetic diet (Teklad Test Diets, Madison, Wisc.), which has served as the control diet in our tumorigenesis studies (4). Food and water

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were provided *ad libitum*. Temperature was maintained at 23°, and lights were on for 12 hr each day.

The foregoing procedure was common to the experiments outlined below. These experiments were initiated on the 26th day (Day 0 of each experiment). Subsequent details specific to each experiment are as follows.

In Experiment 1 (see Fig. 1—Results) 35 rats were placed on the 30% casein diet supplemented with phenobarbital to a concentration of 0.25%; 40 rats remained on the control diet. Five rats from each group were killed at the intervals shown (five control rats on Day 0), and whole body weights and liver weights were measured. Lights were on from 0600 to 1800 hr each day.

In Experiment 2 sixteen rats were placed in controlled-environment rooms where each animal's deep body temperature and food consumption could be continuously monitored electronically. Each rat was caged individually, with the control and experimental rats interspersed in an alternating arrangement. A detailed description of the automated facility will be given elsewhere (Meinert *et al.*, in preparation). Briefly, body temperature was measured via intraperitoneally implanted radiotelemeters, and food consumption was detected as changes in the weights of the food hoppers, each of which was freely suspended from an electronic strain gage. Raw data were automatically collected on magnetic tape for subsequent computer processing.

Following implantation of the telemeters, the animals were maintained for 11 days under a regimen involving alternating 12-hr intervals of illumination and darkness. The food used during this interval was the control diet. On Day 15 eight rats were shifted to the 0.25% phenobarbital diet. Data were collected from Days 23 to 37.

Results. Figure 1 compares the body weights and liver weights in rats fed the phenobarbital or control diets over an 11-day interval. The data show that both a suppression in total body weight gain and an increase in liver size were produced by 0.25% dietary phenobarbital; these responses became apparent by the third day

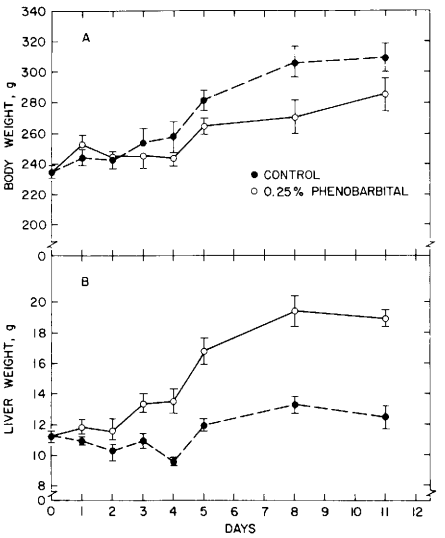

FIG. 1. Changes in body weights (A) and liver weights (B) in rats fed a control diet (dashed line, solid circles), or a diet supplemented with phenobarbital to a level of 0.25% (solid line, open circles), over an interval of 11 days. Each data point represents the mean and one standard error (vertical bar) for a group of five rats.

after the dietary change. After Day 5 the difference between the phenobarbital and control groups became relatively constant with respect to both parameters. Considered together, therefore, the results in Fig. 1 suggest that the effects of 0.25% dietary phenobarbital on total body growth and liver size emerge after a short delay, and subsequently remain constant for the duration of the exposure to phenobarbital. The present data on weight gain are in agreement with the results of our tumorigenesis experiment (manuscript in preparation) in which the body weights of rats on 0.25% dietary phenobarbital averaged 25% less than those of rats on the control diet, over an interval of 575 days.

In addition to the stimulation of hepatomegaly and the reduction of weight gain, consumption of the 0.25% phenobarbital diet substantially altered the pattern of *ad libitum* food intake during the rats' normal feeding interval. Figure 2 compares the food intake patterns over a 15-day interval in the control and phenobarbital-fed groups; each pattern represents the average

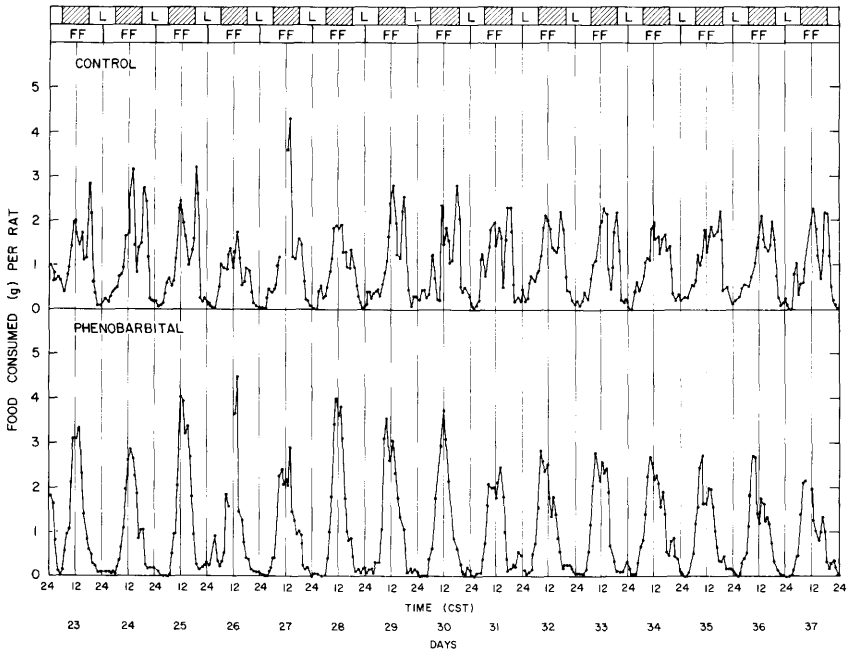

FIG. 2. Automated measurement of food intake over an interval of 15 days in rats habituated to a control or 0.25% phenobarbital diet. The Group data were averaged from eight control rats and eight rats fed phenobarbital. Lights were out during the intervals designated by the cross hatched bars; L denotes the interval of illumination; FF denotes *ad libitum* feeding. The days along the abscissa denote the elapsed time that the rats were in the automated facility.

of the intake patterns of all the rats in the group. The scattered short gaps in the patterns represent brief interruptions in monitoring. Figure 3 depicts the average daily food intake pattern in the control and phenobarbital groups calculated from the data in Fig. 2. The data show that in rats fed the control diet *ad libitum* two episodes of food consumption occurred during the dark phase. The first and most prolonged feeding interval (approximately 3 hr) occurred during the middle third of the dark phase, with a second brief burst of intense feeding occurring as the dark phase was ending. Rats receiving the 0.25% phenobarbital diet showed only a single feeding interval that began slightly earlier than in the control rats and occupied approximately the first two-thirds of the dark phase. In addition, the level of food consumption by the phenobarbital-fed rats during this interval was increased to an extent that compensated for the failure of these rats to indulge in the second episode of feeding shown by the control rats; hence the average total daily food intake of the phenobarbital-fed rats was similar to that of the controls (Fig. 3).

As indicated earlier, the rats housed in the automated facility were monitored with respect to deep body (intraperitoneal) temperature as well as food intake. Figure 4 shows the circadian cycling of temperature over an interval of 14 days in four representative rats from the control and phenobarbital groups, respectively. The data indicate clearly that habituation to 0.25% dietary phenobarbital was accompanied by an increase in the amplitude of the circadian temperature oscillation and an advancement in the time at which maximum temperature was attained.

In order to obtain a quantitative assessment of the magnitude of these phenobarbital effects, the 14-day temperature data for all eight rats in each group were combined and subjected to statistical analysis

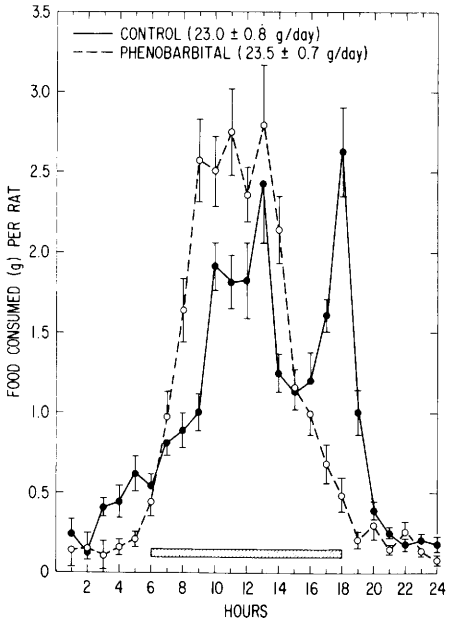

FIG. 3. Average daily food intake in control rats and those receiving 0.25% dietary phenobarbital. Each data point on the appropriate curve represents the mean and one standard error from eight control rats or eight phenobarbital-fed rats averaged over an interval of 15 days. The data were calculated from the automated measurements depicted in Fig. 2. The cross-hatched bar along the abscissa denotes the dark phase of the light–dark cycle. The numbers in parentheses indicate the average total daily food intake per rat over the 15-day interval.

by means of the cosinor method (5–7). As diagrammed in Fig. 5, the results of this analysis indicate that 0.25% dietary phenobarbital doubled the amplitude of the circadian oscillation (from 0.68 ± 0.145 to 1.17 ± 0.135°) and advanced the time at which peak temperature was attained by approximately 2 hr (from 1316 ± 0052 to 1108 ± 0029 hr). (The error shown for each value defines the 99% confidence limit.)

Discussion. The current study demonstrates that 0.25% dietary phenobarbital substantially reduces body weight gain without influencing the quantity of food consumed per day. In view of the marked hepatomegaly produced by phenobarbital treatment (Fig. 1B) and the myriad known effects of phenobarbital on liver biochemistry (8), the possibility arises that chronic exposure to 0.25% dietary phenobarbital alters hepatic metabolism in a manner that favors catabolic processes, with a consequent reduction in the efficiency of food utilization for growth. This interpretation is supported by evidence that dietary phenobarbital produces an increase in the excretion of urinary nitrogen (9).

Although the overall daily food consumption was similar in control and phenobarbital-treated animals, the present data (Figs. 2 and 3) show that the circadian pattern of food intake differed substantially in the two groups. Thus, whereas both groups consumed the bulk of their daily ration during the dark phase, the rats on phenobarbital ate more rapidly and finished earlier than did the control rats. It is noteworthy that the feeding behavior of the control rats during the dark phase involves two distinct feeding episodes. Additional evidence for this type of feeding pattern was obtained recently by Rietveld *et al.*, (10), who used an automated system for monitoring the frequency of approaches to the food as an indication of eating activity. This indicator, which correlated well with food intake on an individual rat basis, closely resembled the biphasic pattern obtained through the direct automated measurements of food intake obtained in the present study.

The possibility that phenobarbital-induced changes in the daily food intake pattern might also contribute to a reduction in weight gain is suggested by studies in which food intake was restricted to a single meal given at different phases of the circadian cycle (11–14). These studies showed that body weights were lower in subjects given a single meal only at the beginning of their normal activity phases (light phase for humans, dark phase for rodents). Such findings are compatible with the present observation that weight gain was reduced in rats conditioned, by the presence of 0.25% phenobarbital in the diet, to restrict their overall daily eating activity to a shorter, earlier segment of the normal feeding interval.

Prior studies have examined barbiturate

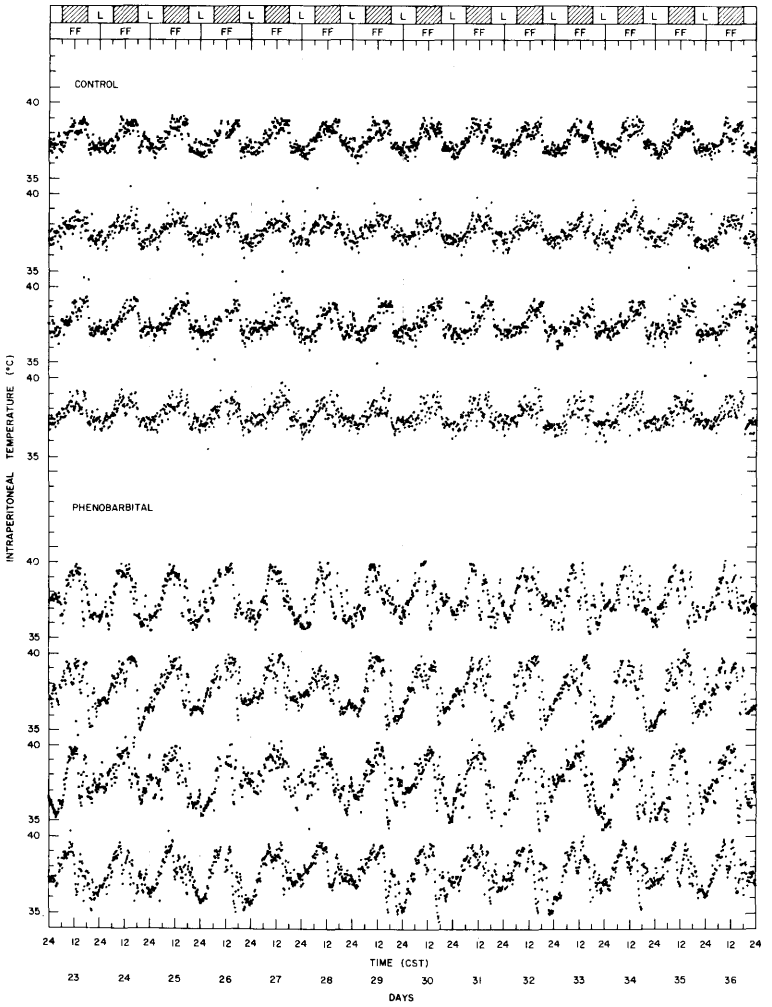

FIG. 4. Interperitoneal temperature measured every 15 min over an interval of 14 days in the rats described in Figs. 2 and 3. Data are shown for four representative animals from each group.

effects on the circadian oscillations of deep body temperature in rats (15, 16). A single injection of pentobarbital produced a delay in the phase of these temperature oscillations, the magnitude of the delay depending on the time of day at which the injection was administered. On the other hand, dietary phenobarbital available *ad libitum* to rats in continuous darkness tended to randomize the temperature patterns, indicating a disordering of circadian regulation (16). Using oscillations of food intake and deep-body temperature as indicators, however, the present results suggest that dietary phenobarbital available *ad libitum* under the appropriate conditions can amplify rather than reduce the ordered expression of circadian control processes. In comparing the current and prior (16) dietary phenobarbital studies it is essential to note the differences in the lighting protocols used. Retention of a light–dark cycle in the present study maintained the existence of a strong periodic feeding stimulus and a con-

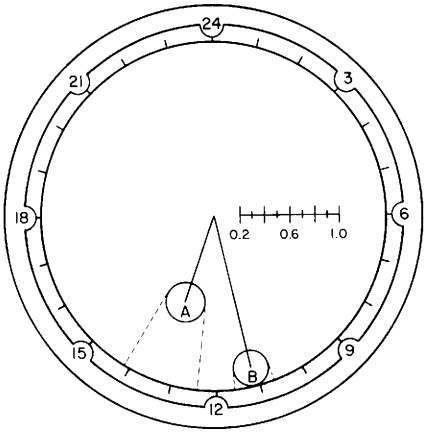

FIG. 5. Statistical analyses (cosinors) of the circadian oscillations of deep body temperature as represented in Fig. 4. Group A represents the data from the eight control rats and Group B represents the data from the eight rats receiving 0.25% dietary phenobarbital. The lines radiating from the center of the cosinor diagram point to the time of day in hours (clock face) at which the temperature reaches its maximum (acrophase). The length of these lines defines the amplitude (°C) of the oscillation and can be read from the scale given in the right half of the cosinor (0.2–1.0). The 99% confidence limits for the measurements of the temperature oscillation amplitude and for the temporal location of the acrophase are indicated by the enclosed areas around the tips of the lines. The portion of each line that lies within this area defines the error in amplitude, and the segments of the clock perimeter that are subtended by the dashed lines define the error limit for the position of the acrophase. The shaded area on the perimeter of the cosinor indicates the dark phase of the light–dark cycle.

sequent periodic intake of phenobarbital. This episodic pattern of exposure to phenobarbital may evoke responses more akin to those produced by barbiturate injection (i.e., a shift in the phasing rather than a disruption of circadian control processes (15).

The doubling in the amplitude of the circadian temperature fluctuations in the rats fed phenobarbital (Figs. 4 and 5) may also be responsible in part for their reduced body weight gain. Thus, the phenobarbital-treated rats increased their body temperatures daily at twice the rate required of the controls, and it is possible that this accelerated temperature response is less energy efficient than the more gradual circadian temperature elevations in the control rats. Should this be the case, the resultant caloric deficit in the phenobarbital-treated rats, in the absence of a compensatory increase in food intake, might be expected to contribute to the phenobarbital-induced reduction in body weight gain observed in the present study.

At present there exists little information bearing on possible mechanisms by which phenobarbital influences the circadian cycling of food intake and deep body temperature. Phenobarbital and other barbiturates have been shown to alter the output of certain exocrine and endocrine glands, as well as the metabolism of endocrine hormones (17–19). In addition, phenobarbital enhances the circadian rhythmicity of dopamine in specific brain areas (20). These observations suggest that the effects of phenobarbital reported in this study may derive in part from phenobarbital-mediated alterations in endocrine balance and in the function of relevant regulatory elements within the central nervous system.

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