Numerous studies have indicated that sublethal whole body irradiation causes a decrease in food intake, body weight, and growth in young animals, and a weight loss in adult animals (15). This is due chiefly to a decrease in food intake during the first week following exposure. Thus any effect of whole or partial body irradiation on kidney cell proliferation will be complicated by effects due to decreased food intake. The role of food intake suggested by previous radiation studies (5,6) is confirmed in this report.

Summary. The effect of variation in food intake and of unilateral nephrectomy on cellular proliferation in the kidney has been measured in weanling rats by means of autoradiography following flash labeling with tritiated thymidine. Labeling was used as an index of the proportion of cells engaged in DNA synthesis and thus in active proliferation. The labeling index decreased markedly from 0.65% to 0.06% after 24 hours of fasting. Refeeding after a 20-hour fast resulted in increased labeling after a 16-hour interval with return to normal levels at 24 hours. The stimulus of unilateral nephrectomy, which causes an increase in labeling index to 1.9% in animals fed *ad libitum*, failed to increase the index above 0.1% in fasting animals or in animals given 6 gm of food/day following a 4-day fast. The results suggest that renal work load may be the major factor stimulating cellular proliferation in the kidney, since loss of renal mass caused by nephrectomy failed to cause cellular proliferation in animals deprived of high levels of new metabolic products by fasting.

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Facilitation of Avoidance Conditioning by Barbiturate* with Stimulant Properties† (33069)

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Phetharbital (*N*-phenylbarbital, Pyrictal), an anticonvulsant developed for the prophylactic treatment of febrile convulsions (1), is less sedative than phenobarbital and appears to enhance mental alertness in some patients with epilepsy. The present investigation was

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undertaken to determine in more detail the effect of phetharbital on the behavior of laboratory animals, and to examine its potential value as a therapy for disorders of learning and memory. Modifications of locomotor activity induced by phetharbital were correlated with the concentrations of drug in the brain, and possible effects on learning and memory were assessed by the alterations observed in passive avoidance conditioning. The actions of phetharbital were compared with those of methylphenidate, a central nervous stimulant of proven value in the management of some behavior and learning disabilities of children (2,3).

Methods. Male albino mice, 21–39 gm weight and 6–10 weeks old, were used. Locomotor activity was measured by means of 6 cylindrical chambers fitted with lights and photoelectric cells, and experiments were conducted in a semidark room. Animals were tested individually, and interruptions of the light beams in each chamber were recorded automatically and cumulatively on digital computers (4). Phetharbital in 10% acacia was given intraperitoneally in anticonvulsant doses (1) of 40, 60, and 75 mg/kg to three groups of mice; minimum of 5 animals was tested at each dose level. Eight control animals received the acacia vehicle and their activity was determined simultaneously.

Brain levels of phetharbital were estimated in mice sacrificed at intervals after administration of a dose that had been found to alter activity significantly. The methods of extraction and measurement by ultraviolet spectrophotometer (5) were similar to those employed by Butler (6) for barbituric acid derivatives.

The effects of phetharbital on memory and passive avoidance of a learned experience were examined in mice by means of a cylindrical chamber with an 11-inch diameter grid floor arranged to deliver a series of weak electroshock stimuli (50 V, 0.2-sec duration, 2/sec frequency) for a 5-sec period. The animal was placed on a 3-inch square platform, 1.5″ high, located in the middle of the grid floor, and the time taken to step down with at least 3 feet on the grid was called the learning trial-latency period (7,8). The dimensions of the platform had been selected by trial and error so that the latency period on the first day was less than 20 sec in the majority of animals; the occasional mouse remaining on the platform for more than 30 sec was rejected from the learning trial. The mouse was exposed immediately to the shock experience and then replaced in its home cage. The test and shock experience were repeated on 2–4 subsequent days at intervals of 24 hours, and the latency periods in these memory retention trials were compared in drug treated and control animals. A 2-min cutoff time was employed for all memory retention-latency period determinations, and 7–10 animals were included in each trial group. In order to elucidate the mechanism of changes in avoidance conditioning induced by phetharbital, the effects were examined in relation to brain concentrations of the drug and modifications of locomotor activity; they were compared with the effects of methylphenidate administered in a dose known to stimulate activity (9).

Results. The effects of phetharbital on the locomotor activity of mice are shown in Fig. 1. Control animals were hyperactive initially and quiet after the first 0.5 hour. The mean activity count per 0.5-hour period for the total number of animals in the three groups receiving 40, 60, or 75 mg/kg doses of the drug was significantly lower than controls in the first 0.5 hour ($p<.01$), and the activity was significantly increased during the periods 1–1.5, 1.5–2 and 2.5–3 hours, after administration of phetharbital ($p<.05$). The initial suppression and subsequent stimulation of activity by phetharbital were dose related, the responses being greater after 75 mg/kg than at the lower dose levels employed. In the group of animals receiving 75 mg/kg of phetharbital, the mean activity counts, with standard errors, for the first and final 0.5-hour periods of observation were 189 $\pm$ 28 and 213 $\pm$ 57, respectively, and the differences from control values recorded during the same periods (378 $\pm$ 36 and 39 $\pm$ 10) were significant ($p<.02$; $p<.05$).

Brain levels of phetharbital in mice that received 75 mg/kg, a dose that induced significant changes in locomotor activity, are

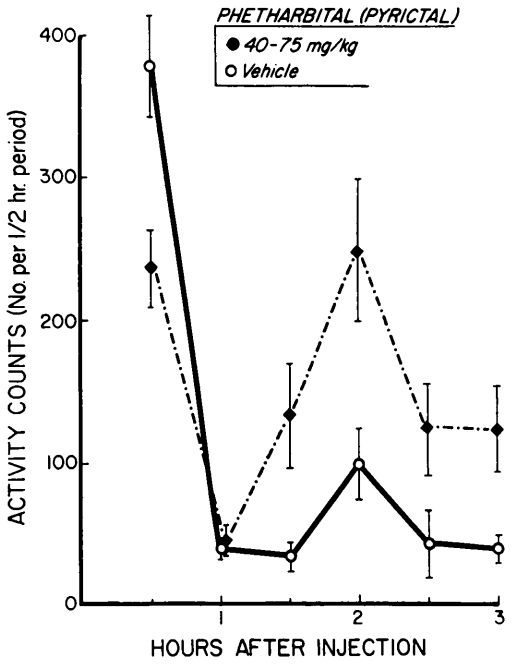

FIG. 1. Effect of phetharbital on locomotor activity of mice. Each point represents mean activity (with SE) of a group of 8 control animals that received the vehicle and a combined total of 17 animals treated with the drug in doses of 40, 60, or 75 mg/kg. See text for separate listing of activity counts significantly different from controls in the 5 animals that received the 75 mg/kg dose.

shown in Fig. 2. The maximum concentration was reached at 2 hours after drug administration, the time of maximal stimulation of activity. Brain levels at 0.5 hour, the time when activity was suppressed, were significantly lower than at 2 and 3 hours (t = 4.95, 6.25; $p<.05$). The differences between concentrations at 1 and 2 hours and 2 and 3 hours were not significant (t = 0.74, 2.09; $p>.05$).

The ability of mice to learn and retain a passive avoidance response to an electroshock stimulus was demonstrated in preliminary studies of 2 groups of control animals (Fig. 3). The mean latency preshock periods in learning trials on the first test day were 4.5 and 3.5 sec, with standard errors of 0.65 and 0.45, respectively. The mean latency periods were essentially unchanged when the mice were tested on the following day at either 0.5 or 2 hours after injection of 10% acacia. Significant increments in the mean latency periods occurred in both groups of animals on the third and fourth test days. Two shock experiences 24 hours apart were necessary for the acquisition of the passive avoidance response in control mice.

The effects of phetharbital on learning and retention of the passive avoidance response are compared with those of methylphenidate in Fig. 4. In mice tested at 0.5 hour after injection of phetharbital, when brain concentrations were relatively low and locomotor activity was suppressed, the mean latency period was not significantly modified on the second day but was shorter than the control on the fifth consecutive day of testing (27.0 ± 17.4 compared to 85.0 ± 18.1 seconds; t = 2.5, $p<0.05$). In mice tested at 2 hours, the time of maximal brain concentration when stimulation of locomotor activity is expected, the mean latency period was significantly prolonged on the second day (t = 2.96; $p<0.025$) but was not different from the controls on subsequent days. In mice treated with methylphenidate, locomotor activity was increased whereas the mean latency period in tests for memory retention of a shock experience was shorter but not significantly different from controls.

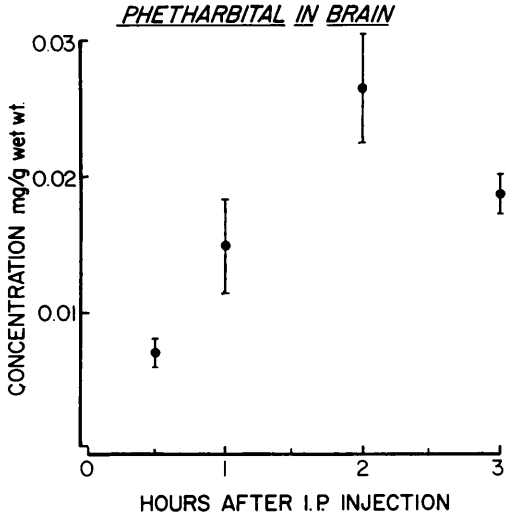

FIG. 2. Concentrations of phetharbital in mouse brain after a dose of 75 mg/kg by intraperitoneal injection. Each point represents the mean (with SE) of determinations in 5 or 6 animals.

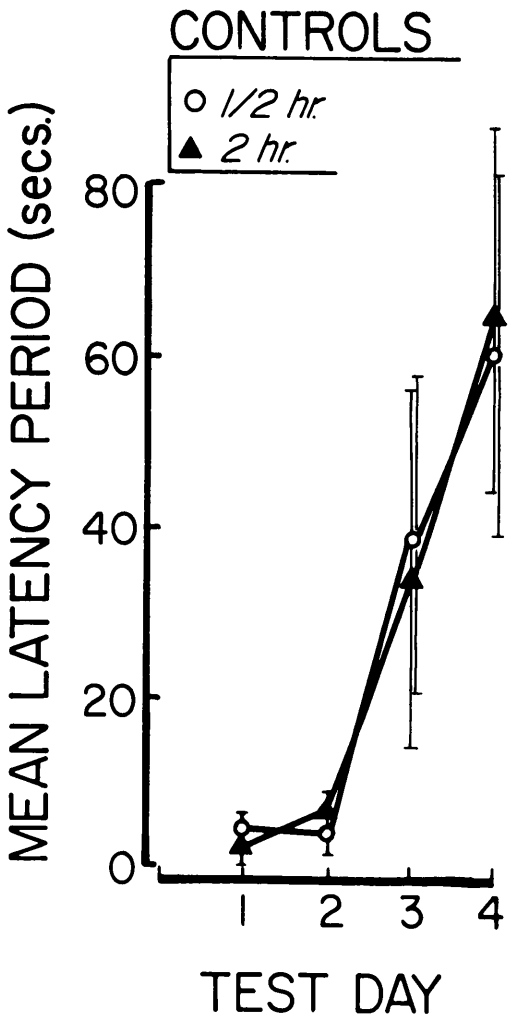

FIG. 3. Mean latency periods (with SE) in learning and memory retention trials of passive avoidance in 2 groups of control animals that received 10% acacia in water, intraperitoneally, (i) at 0.5 hour, and (ii) at 2 hours before repeat testing on the second, third, and fourth days.

Discussion. Phetharbital administered to mice in anticonvulsant doses has been found to modify locomotor activity in a biphasic manner, the responses correlating with the time of testing and differences in the concentration of drug in the brain. Suppression of motor activity was observed at 0.5 hour after administration of phetharbital when the concentration in the brain was relatively low, whereas stimulation occurred at 2 hours when maximal concentrations had been reached. The effects of phetharbital on motor activity were similar to those observed in response to phenobarbital (4) but stimulation was obtained at lower and nontoxic dose levels. Previous experiments in our laboratory have shown that phenobarbital in doses of 100 and 150 mg/kg caused hyperactivity in mice, but stimulation was complicated by ataxia of gait. A biphasic effect with initial suppression of activity was recorded only in response to anesthetic dose levels, and the subsequent stimulation of activity occurring between 2 and 6 hours after drug administration might possibly have been attributed to a withdrawal effect. In the present study, however, the behavioral responses have been correlated with brain concentrations of phetharbital, and hyperactivity was a direct effect of the drug.

The method employed for the investigation of effects of phetharbital on learned behavior was suitable for small animals, and the choice of mice permitted observations in sufficient numbers for statistical analyses of results. The ability of mice to learn and retain a shock avoidance response (7,8) has been confirmed in the present study, and modifications of the avoidance conditioning induced by phetharbital have been examined in relation to changes in locomotor activity.

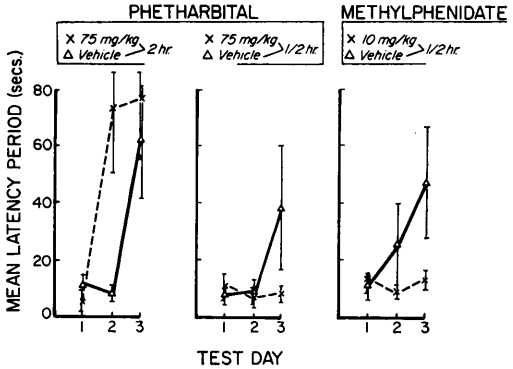

FIG. 4. Mean latency periods (with SE) in learning and memory retention trials in 3 control groups of mice that received 10% acacia in water, and in 3 test groups treated as follows: (i) phetharbital at 2 hours, (ii) phetharbital at 0.5 hour, and (iii) methylphenidate at 0.5 hour, before each repeat test on the second and third days of the experiment.

With a dose and brain concentration of phetharbital that stimulate activity, a shortened latency period might have been expected in a test of passive avoidance conditioning, and levels of the drug that suppress activity might lengthen the latency period and delay the movement of the animal from the platform to the grid. In our experiments, however, the opposite effects obtained; phetharbital in stimulant concentrations caused an increase in the latency period and appeared to enhance the acquisition of a passive avoidance response whereas concentrations that lessened activity impaired conditioning and the avoidance of a shock experience.

That phetharbital may facilitate learning is suggested by these results in mice, but the mechanism and significance of the alterations observed in avoidance conditioning are not completely defined. The failure of methylphenidate, a locomotor stimulant in animals, to accelerate the learning process confirms previous findings in laboratory experiments (10), although beneficial effects have been observed in some children receiving methylphenidate for the treatment of hyperactive behavior and perceptual disorders (2,3). The stimulant properties of phetharbital apparently are different from those of methylphenidate and the effects on learning are obtained with doses that are anticonvulsant and nontoxic. An increased alertness or reduced anxiety may be postulated, and the exact mechanism warrants further investigation in animals of different species and in patients with disorders of learning and memory.

Summary. The effects of phetharbital on behavior and learning have been studied in mice and correlated with concentrations of the drug in the brain. A dose of 75 mg/kg caused reduction of locomotor activity at 0.5 hour and stimulation at 2 or 3 hours after injection. Maximum concentrations in the brain were attained at 2 hours. The acquisition of a passive avoidance response was facilitated by phetharbital in high concentrations and impaired by low concentrations in the brain. Methylphenidate in stimulant doses failed to enhance passive avoidance conditioning in mice. Phetharbital caused prolongation of passive avoidance-latency periods and an apparent acceleration of the learning process despite an associated stimulant effect on motor activity.

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