crease in liver L-α-glycerophosphate concentration and in incorporation of intravenously injected palmitic acid-1-$C^{14}$ into liver triglycerides. It is suggested that these metabolic alterations may play an important role in the pathogenesis of fatty liver and of hypertriglyceridemia produced by ethanol. The changes could be observed only during the first few hours following ingestion of ethanol.

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## A Behavioral Deficit Associated with Phenylketonuria in Rats.* (28500)

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It has been established in this laboratory that certain conditions of dietary intake of excessive phenylalanine by rats(1) or monkeys(2) result in urinary excretion of phenylketones and elevated blood phenylalanine levels. Since it has been shown that phenylketonuric monkeys have severe and presumably permanent decrements in learning abilities and other complex behaviors(2), it was of interest to determine if rats with similar biochemical evidence of phenylketonuria also have deficits in higher-order behavior. This preliminary report describes a behavioral testing procedure which has been reliable in rapidly detecting and quantifying a decrement in the maze performance of phenylketonuric rats.

The selection of this procedure was dictated in part by several previous experiments which may be described briefly. The initial goal of this research has been to devise a learning task simple enough for rapid mastery by the rat, but difficult enough for re-

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liable detection of relatively small differences in learning ability. However since it was also considered necessary to choose a learning situation which utilized motivating conditions compatible with the exact dietary control necessary, any task employing a food deprivation-food reward motivating system was ruled out. We therefore turned first to a water deprivation-water reward motivating system in spite of the fact that water deprivation also decreases food intake and, thereby, the amount of phenylalanine.

Our initial series of 6 experiments employed a water-rewarded learning of successive reversals of a position response (left or right) in a single-unit Y-maze. The results were equivocal. Careful examination of the data and observation of the animals being tested, however, led to the conclusion that, compared to the controls, the phenylketonuric rats were hypermotivated when water-deprived, possibly due to an interaction with their self-imposed state of food deprivation. This possibility was not experimentally tested.

Finally, we chose to employ a naturally occurring (rather than an experimentally induced) tendency, the rat's aversion to water and swimming, to motivate it to learn to swim a maze route in order to gain exit from the water.

*Method.* The task employed was thus the learning of a swimming water maze where escape from water was the only motivating condition. A 6-unit, water-filled T-maze described by Biel(3), and first used in this context by Schanberg, *et al.*(4), was used.

A total of 44 Long-Evans strain rats were used. At 21 days of age litter mates were assigned, equally divided as to sex, to either the phenylalanine (P) or the control (C) group. Each animal was housed individually with *ad lib.* water in a 3-hour cycle light-dark room(1). The C-group was fed a commercial rat diet from weaning throughout the experiment. The P-group received the same diet supplemented by 5% L-phenylalanine for the first 10 post-weaning days, followed by 7% L-phenylalanine for the remainder of the experiment. Under these conditions this diet reliably induces phenylketonuria and elevated blood phenylalanine in rats(1).

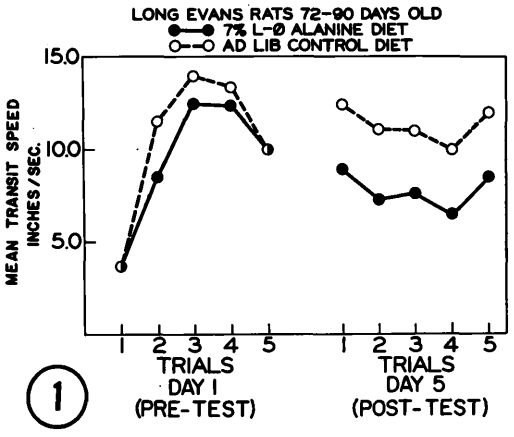
Three replications of the same basic experiment were performed. The first 2 replications were done on consecutive weeks and the third followed 2 weeks later. On the first day of testing the rats in the 3 replications were 72, 87 and 90 days of age.

Testing continued for 5 consecutive days. On the first day (pre-test) 5 trials in a 50-inch straight channel were given to acquaint the animals with swimming and to test the comparability of swimming speeds between the 2 groups. On the second, third and fourth days, 7 trials per day were given through the maze. The fifth day (post-test) consisted of 5 trials through a straight channel comparable in length to the maze (100 inches) to determine group swimming speed and fatigue comparability. In all cases a trial consisted of placing the rat in the water ($22° \pm 2°$C) at the starting point, and retrieving it as it crawled up the exit ramp at the finish point. Successive trials for a given rat were spaced at least 10 minutes apart, and, after each, the rat was dried with a towel.

Elapsed time between start and finish was recorded. Transit speed was then computed by dividing the elapsed time into the minimal linear extent of the maze or straight channel. Errors (whole-body entries into a blind alley) were also recorded on maze days.

*Results.* Fig. 1 shows mean transit speeds in the straight channel for the pre-test and post-test, days 1 and 5 respectively. Since there was no significant difference between replications, all P- and C-group data were pooled. It can be seen that the group transit speeds were comparable on the pre-test but that the P-group completed the swim more slowly on the post-test. However, it was noted that on the post-test day many of the P-group rats turned, backtracked and circled in the straight channel, while those in the C-group did not. Since this observation suggested that the group transit speed differences resulted from the greater distance traveled by the P-group, a test of this possibility was made using a graphic plot of actual distance traveled for each rat on most trials of

the post-test. True swimming speeds, computed on the basis of the actual distance traveled rather than the minimal linear extent of the channel, were found to be practically identical for the two groups.



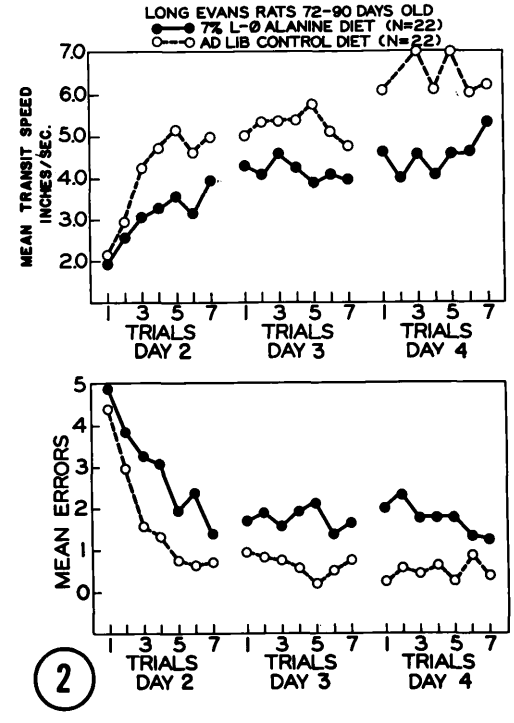




FIG. 1. Mean transit speeds (inches/second) through a straight channel on pre-test and post-test days 1 and 5 for all rats receiving L-phenylalanine (P-group) and control (C-group) diets in the 3 replications.

FIG. 2. Mean transit speeds (inches/second) through water maze on test days 2, 3 and 4 for all rats receiving L-phenylalanine (P-group) and control (C-group) diets in the 3 replications.

An analysis of variance of transit speeds for the three maze test days, 2, 3 and 4, revealed: (a) no significant difference between replications, (b) a significant difference between P- and C-groups ($p<.01$), (c) a significant effect of trials ($p<.01$), days ($p<.001$) and trials by days interaction ($p<.001$), and (d) no significant sex difference. Subsequent Duncan multiple range tests(5) on these maze data indicated that, on the whole, trials 5, 6 and 7 did not differ significantly and that each of the 3 maze days differed from each other. An analysis of variance of errors gave essentially identical results except that the group, day and trial effects were significant at $p<.001$.

Since there was no statistically significant difference between replications, the transit speeds and errors of all P- and C-groups for the 3 maze days were pooled, and their means are presented graphically in Fig. 2. These data show that both groups learned the maze but that the P-groups performed more slowly and made more errors than the C-groups. The slower transit speeds of the P-groups resulted mainly from their higher error rate, their slowness to gain exit from blind alleys, and their tendency to turn in the wrong direction once having exited. All indications were that group swimming speeds *per se* were comparable.

An infrequent case of mild alopecia, and a 38% lower mean body weight compared to the controls was observed in the P-groups. There was no apparent muscular weakness in any of the rats.

*Discussion.* These data demonstrate first, that rats fed a L-phenylalanine diet from weaning display a deficit in maze performance by 72 to 90 days of age, and second, that the described behavioral testing procedure reliably detects this deficit. A suggestion of an additional behavioral deficit of the P-group rats was noted during the post-test day of these experiments. As reported above, these animals turned, backtracked and circled in the straight channel considerably more than the controls. The possibility exists that this inappropriate behavior, which was

not seen on the pre-test day in the straight channel, was indicative of a diminished ability to change a previously effective response pattern. That is, after learning to make turns through the maze, the P-group may not have been able to adapt so well as the controls to the post-test straight channel condition where swimming straight ahead was effective. Experiments designed to test the reversal learning ability of phenylketonuric rats are in progress.

Although behavioral differences have been reported by others(4,6,7,8,9), questions regarding the adequacy of the phenylalanine concentration, dietary control, or behavioral testing procedure employed remain unanswered in many of these researches. To answer these questions pair-fed experiments designed to clarify the effects of dose, duration on the diet, and weight loss *per se* are in progress using the behavioral testing method as described, except that on the 3 maze days, only 5 trials per day are given. The effects of nonspecific toxic agents, D-, DL-, and L-phenylalanine and the possibility of a "critical period" of susceptibility to excess phenylalanine will also be studied.

The present experiments also bear on the question of when the dietary regimen should begin in order to produce behavioral deficits in the rat. It is now clear that it is not necessary to feed the excess phenylalanine diet to the mother in order to provide it to the fetus *in utero,* and to the pup during the suckling stage, as was previously suggested(10). It may now be concluded that the weanling rat is a satisfactory experimental animal for use in the study of behavioral, as well as biochemical, parameters of phenylketonuria.

*Summary.* A behavioral testing procedure utilizing a swimming water maze has been reliable in rapidly detecting and quantifying a performance decrement in rats made phenylketonuric by feeding excessive quantities of L-phenylalanine from weaning.

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## Evidence for Genetic Factors in the Resistance of the Rat to Dietary Cirrhosis.* (28501)

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When experimental animals are fed diets deficient in one or more nutrients, often only a limited number of animals develop signs of specific malnutrition. In the case of rats fed a cirrhosis-producing diet (deficient in protein and choline) the same basic diet produced a fairly predictable response: 70 to 80% of male, Sprague-Dawley rats developed

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