were placed on nesting material in cages in the hibernating room. Normal squirrels so treated returned to 35°C within 3½ hours. None of the seven brain-damaged squirrels recovered normal temperature, nor could they be aroused at any time by handling or electric shock. They assumed no position other than the one in which they were first placed in the cold. Their brain temperature dropped to 8.0° to 8.5°C and remained there, without arousal, until they died 2 to 6 days later. The temperature curve of squirrel TAH 51 (Fig. 1) is typical of these seven squirrels; the other curves simply stop earlier or later. Two animals with lesions were able to raise their temperature and returned to normal within 4 hours. Histologic examination later showed that the lesions were too low and damaged only the optic chiasm. The seven squirrels (with lesions) that died in hypothermia and the two nonobese squirrels that died during hibernation had large lesions in both the preoptic and anterior hypothalamic areas, including the suprachiasmatic and arcuate nuclei, although the lesions extended more rostrally or caudally in different animals (Fig. 2). The furthest anterior extent of any lesion was at the level of the anterior commissure, and the furthest posterior extent was just anterior to the ventromedial nuclei. The lesions extended laterally to the lateral preoptic nuclei and the fornix, dorsally to just below the paraventricular nuclei, and ventrally to the optic chiasm and base of the brain.

Autopsy revealed that all seven hypothermic squirrels had large ulcers in the stomach or intestinal tract. None of the four squirrels that died in hibernation had any visible ulcers.

The brain-damaged squirrels rendered hypothermic, and the squirrels (with lesions) that entered hibernation normally, remained hypothermic until they died. However, the former died after 2 to 6 days, whereas the latter died after 11 to 12 days. This suggests that the latter group hibernated normally but died because they were unable to arouse. Results from other investigators support this view. Popovic (*3*) has shown that normal ground squirrels (*C. tridecemlineatus* and *C. citellus*) kept hypothermic at a body temperature of 10°C live approximately 110 hours. The brain-damaged hypothermic squirrels in this experiment lived 48 to 140 hours. In our laboratory, a period of 9 to 12 days is the longest that normal ground squirrels (*C. tridecemlineatus* and *C. lateralis*) remain in hibernation before arousing, at temperatures comparable to the 6°C used here. The hypothalamic-damaged squirrels that entered hibernation in this study died after 11 to 12 days.

The necessity for periodic arousals has not previously been proved. Hock (*4*) suggested that arousal takes place when some lethal substance has built up in the animal or when the amount of some nutrient has fallen critically. Thus, if the animal did not wake up, the concentration of the lethal substance would increase (or the amount of the nutrient decrease) to a point where he would be unable to arouse. My research corroborates Hock's view that arousal is necessary for survival and suggests (tentatively, since the number of animals is small) that the timing of the arousals is crucial; the normal animal wakes up just before the unknown, potentially lethal state becomes in fact lethal. The animal does not have much leeway in the time of arousal. If it does not wake up at approximately the outer limit of a normal dormant period, it dies quickly thereafter.

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27 December 1966

Phenylketonuria: Enduring Behavioral Deficits in Phenylketonuric Rats

Abstract. *The behavioral deficit in rats made "phenylketonuric" by ingestion of* L-*phenylalanine was evaluated 23 to 65 days following cessation of treatment. Animals treated from birth to 60 days showed significant deficit on reasoning and discrimination learning tests but not on discrimination reversal sets. Animals treated from 30 to 60 days showed significant deficit only on the reasoning test.*

When phenylalanine and related compounds are administered to rats, excess phenylketones are excreted in the urine, and phenylalanine of plasma is elevated. In addition there are frequent reports of deficits in behavior. Although the biochemical reactions are dependable, the occurrence and interpretation of the behavioral deficits are open to question (*1*), making doubtful the adequacy of this procedure as a model of phenylpyruvic oligophrenia.

One question concerns the time of testing of behavior. If the tests are given during or immediately after the administration of phenylalanine, any demonstrated deficit may be attributed to acute effects of this agent. For example, Polidora *et al.* (*2*) demonstrated that the deficit reported for rats treated from time of weaning disappeared after cessation of treatment with L-phenylalanine.

A second question involves the treatment period. Administration of the amino acid during fetal or neonatal life, a period of rapid neural development, is more apt to produce a permanent behavioral deficit, whereas treatment beginning at the time of weaning might not. Results of research with such subjects has been equivocal. Woolley and van der Hoeven (*3*) reported a deficit in mice treated from birth, but not in mice treated from weaning. However, testing occurred before an acceptable period of recovery from the toxic effects. Similarly, the deficit reported by Loo *et al.* (*4*) was measured when the subjects were still receiving phenylalanine.

Perez (*5*) reported no learning deficit in rats treated from birth, but his mortality figure of 70 percent could mask any effect of the amino acid through selective survival. Perry *et al.* (*6*) also reported no deficit, but the treatment periods did not extend beyond 8 days of age, a period too brief to produce permanent neurological or physiological deficit (*7*).

A third question involves the selection of assessment measures. Most tests used to evaluate effects of phenylalanine in rats have involved relatively simple discriminations that frequent-

Table 1. Errors through criterion on original discrimination and reversals (R).

Treatment	Animals (No.)	Errors (mean ± standard error of mean)				
		Control	R_1	R_2	R_3	R_4
L-Phenylalanine	22	16.70 ± 3.40	12.40 ± 2.80	10.10 ± 1.80	8.90 ± 2.00	7.40 ± 1.50
Saline and L-phenylalanine	17	13.40 ± 3.00	8.40 ± 1.39	6.35 ± 1.23	4.50 ± 0.90	4.80 ± 0.78
Saline	19	9.25 ± 1.90	5.70 ± 0.90	4.35 ± 0.97	3.35 ± 0.40	3.45 ± 0.19

ly fail to distinguish between normal and abnormal rats (*8*). We assessed various levels of intellective functioning, since the intelligence tests used to evaluate the human deficit in phenylpyruvic oligophrenia also sample various intellective levels. Our study demonstrates that L-phenylalanine treatment impairs performance on discrimination and reasoning tests, with the degree of impairment dependent upon the level of intellective functioning.

The subjects were 90 albino rats (Long-Evans strain) from the colony at American Lake Veterans Administration Hospital. The average litter size was nine pups; at birth the average weight was 6.23 g. Breeding procedure included placing two primiparous females with one male, and then transferring the females to maternity cages as soon as a marked weight gain was noted. Of the 90 subjects, 58 (31 female and 27 male) survived through the behavioral tests (*9*). The groups were initially balanced with respect to sex, litter mates, and body weight.

General procedures included intubation, recovery, and testing. During the intubation phase, and within 24 hours after birth, the subjects in each litter were divided into two groups and intubated (solutions were 40°C) with either L-phenylalanine or physiological saline. The L-phenylalanine solution was prepared daily as follows: 5 g of L-phenylalanine were added to 100 ml of sterile physiological saline, then heated to 90°C, and mixed by a Thermo-mix until the solution was clear (approximately 10 minutes). Twenty-two subjects assigned to the group receiving L-phenylalanine (P) were given 3 g of L-phenylalanine (in the described solution) per kilogram of body weight (*10*) daily from birth until 60 days of age by means of a polyethylene tube connected to a 20-ml syringe. The remaining subjects (36) received normal physiological saline (60 ml/kg) from less than 1 day to 30 days of age, by intubation. Half of these, the group receiving saline (S), continued to receive saline for an additional 30 days; the other half, the group receiving saline and L-phenylalanine (S-P), received L-phenylalanine (3 g/kg) in saline solution daily from 30 to 60 days of age (*11*). During this period, weekly blood and urine samples were collected from each subject, for determinations of serum phenylalanine (*12*) and phenylpyruvic acid (*13*), 105 to 150 minutes after intubation. Samples were also collected before each of the behavioral tests.

At the conclusion of a 23-day recovery period (*14, 15*), each subject was tested in a discrimination apparatus and on the Maier reasoning test (*16*). Half of the animals within each group were tested first on the discrimination problem, the other half on the reasoning test. All subjects were maintained on a 23-hour schedule of food deprivation during both the discrimination and reasoning tests, and all were fed at will for an hour immediately after testing.

The discrimination apparatus was a Y-maze 10 cm wide and 24 cm high throughout with a 38-cm short alley, 30-cm arms, and retractable guillotine doors located 10 cm into each arm. The alleys differed in color and floor texture. In order to ensure an easy discrimination, position, alley color, and floor texture were confounded cues; all or any could be used in a successful solution. An original discrimination, made against the subject's initial preference, and four reversals were conducted in the discrimination apparatus. Reinforcement for a correct response consisted of one 45-mg Noyes reward pellet. Twenty trials per day, noncorrective, were given during both the initial and reversal learning phases, with 17 correct responses in 20 successive ones constituting the learning criterion. The intertrial interval was 30 seconds.

Table 2. Reasoning scores obtained on the Maier reasoning apparatus.

Treatment	No.	Mean reasoning score	Standard error of mean
L-Phenylalanine	22	0.09	0.017
Saline and L-phenylalanine	17	.27	.028
Saline	19	.84	.009

The reasoning test which assesses higher mental processes and is sensitive to cortical injury (*16*) used three tables (77 cm high), connected by three elevated runways (132 cm long and 4 cm wide). Each table differed either in size, albedo, or floor texture from the other two. Animals were adapted to this apparatus by half-hour exploratory periods on 8 successive days with no food present. On each of the next 18 days, each animal was placed on one of the tables with six food pellets. The feeding table varied from day to day in a random fashion, with the restriction that the animal was fed on each table six times in 18 days. When the pellets were partially consumed, the rat was moved to one of the other tables and permitted to run once either to the table with food or the one without. After the 1st day, the erroneous response always consisted in running to the table on which the animal had been fed on the previous day. Reasoning scores were the ratios of the difference in frequencies of correct and incorrect responses to the total number of responses.

Subjects in the L-phenylalanine group were of lighter weight during days 1 to 30 [Student's t-test (t) = 5.15, degrees of freedom (df) = 59, $P < .001$], but by the time of testing there were no significant differences in mean weight among the three groups. Groups P and S-P did not differ in the mean amount of L-phenylalanine ingested (11.78 g as opposed to 12.06) because of slightly heavier weight of group S-P while receiving the L-phenylalanine.

During intubation, subjects receiving excess L-phenylalanine were biochemically phenylketonuric, that is, the mean concentration of phenylpyruvic acid (as phenylketone) in the urine was 87.15 mg per 100 ml for group P, and 83.3 mg for group S-P. The mean concentrations of serum phenylalanine (milligrams per 100 ml of serum) were also increased, group P to 24.7 and group S-P to 24.4. These levels dropped to normal within 24 hours after discontinuance of the excess amino acid and were normal at the time of behavioral testing (phenylpyruvic acid: groups P and S-P both with none; serum phenylalanine: group P with 3.5 mg, group S-P with 2.6). The means of the concentrations of phenylpyruvic acid and serum phenylalanine for group S were normal throughout (0 and 2.6 mg).

Group P made significantly more errors than group S on each of the discriminations (*17*) (see Table 1), while group S-P did not differ significantly from either of the other groups, although they consistently made more errors than group S and fewer than group P. All groups improved through the series of reversals, and all solved the fourth reversal with significantly fewer errors than on the first problem. However, there were no differences between the groups on this improvement trend.

Animals in group S did significantly better (Table 2) than either group P ($t = 12.50$, $df = 39$, $P < .001$) or S-P ($t = 8.90$, $df = 34$, $P < .001$). Group S-P did significantly better than group P ($t = 2.90$, $df = 37$, $P < .01$). Only one subject in group P and three in group S-P achieved scores as high as the lowest score in group S.

Since the groups did not differ in the amount of L-phenylalanine administered during the 60 days, the implication is that the age of treatment (or possibly duration of treatment) affected the later behavior. Since Polidora *et al.* (*2*) demonstrated that neither age nor duration of treatment influenced behavior of subjects treated from weaning, the former alternative is the most likely explanation of the difference in performance in the T-maze. In the reasoning test, significant deficit is found whether treatment is started at birth or at 30 days, with the degree of deficit inversely related to age at initiation of treatment. Although age and duration are confounded, the findings of Polidora *et al.* (*2*) are probably relevant for the reasoning test as well, implying that age at initiation of treatment (rather than duration) was the important variable. The reasoning test is a more sensitive instrument for assessing behavioral deficit than the discrimination test with the T-maze.

The improvement trend in the reversal problems, presumably a measure of reversal set, was not different for the various groups, although Rajalakschmi and Jeeves (*18*) suggest a correlation between intellective level and reversal performance. Response stereotypy and rigidity, militating against reversal sets, are not affected by treatment, whereas the ability to form new associations, and especially to combine disparate experiences, is sensitive to L-phenylalanine treatment. However, there may be another interpretation. These data differ from those typically obtained from rats, in that the mean performance on the first reversal was below that on original learning; they are similar to those obtained when successive discrimination problems with new cues (nonreversal shifts) are presented to rats (*19*). If the rats showed nonreversal shifts (*20*), then no reversal set could be formed, and differences in trends would not be expected.

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9. Causes of death and the frequency of each cause are: injection into lungs (12); perforated lungs (4); middle-ear disease (4); unknown (12). The mortality (all causes) for each group was (percent): L-phenylalanine: 39; saline: 32; saline and L-phenylalanine: 35.
10. We thank Sandoz Pharmaceuticals and Ames Company, Inc., for the L-phenylalanine, Phenistix, and Pheniplates.
11. Electrolyte measurements were made to determine the effects of the excess (3 g/kg) ingested L-phenylalanine upon electrolyte balance. Average values (meq/liter) for group P were: Na, 144.8; K, 7.5; Cl, 117; CO_2, 10.4. Average values (meq/liter) for group S were: Na, 143; K, 8.6; Cl 115.9; CO_2, 12.4.
12. Pheniplate PKU assay kit, Ames Company (Division of Miles Laboratories), Elkhart, Indiana.
13. Phenistix reagent strips, Ames Company (Division of Miles Laboratories), Elkhart, Indiana.
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13 December 1966

Fighting and Death from Stress in a Cockroach

Abstract. *Fighting behavior, leading to the establishment of stable dominant-subordinate relationships between pairs of males, is described for the cockroach* Nauphoeta cinerea. *Deaths, which do not appear to be due to external damage, occur in subordinate animals as a result of fighting. The situation is likened to death from stress as found in mammals.*

Intraspecific fighting between males has been described for a number of insects including field crickets (*1*), cicada-killer wasps (*2*), ants where fighting occurs between colonies of the same species (*3*), and for a wood roach where the male defends a mating chamber against rivals (*4*). Although no published records have been found, fighting can be observed commonly between adult males of the cockroach *Nauphoeta cinerea*, where it appears to be associated with a loose territorial system. Fighting, which first appears on the 2nd and 3rd days after the imaginal molt, involves a complex sequence of events that eventually establishes a stable dominant-subordinate relationship between members of a fighting pair.

In an encounter between two more-or-less evenly matched males, a fairly consistent sequence of events can be observed. Both animals, with heads lowered, extend upwards the last three or four abdominal segments, simultaneously lifting the body high off the ground (Fig. 1). This posture may be assumed on sight, when one aggressive male crosses the path of another, or after brief but rapid antennal flagellation (fencing) between the two animals. This posture could be described as aggressive. It always preceeds fighting but may cause a less aggressive male to flee. Following this display, two aggressive animals charge towards each other with their heads lowered and butt on contact. If one cockroach successfully engages its pronotum under that of its opponent, it may toss the rival in the air so that it falls on its back. Less frequently, males may grapple with their legs locked together and bite at each other as they roll over and over. A critical stage is usually reached within a few minutes, and one animal emerges superior.

The behavior of the loser is quite characteristic. After prolonged chasing by the dominant, it suddenly lies still