BEHAVIORAL, NEUROLOGICAL, AND BIOCHEMICAL EFFECTS OF EARLY L-PHENYLALANINE PLUS DL-PARA-CHLOROPHENYLALANINE

A Thesis

Presented to

the Faculty of the Department of Psychology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

By

Ken M. Dobbins

December, 1974

ACKNOWLEDGMENTS

Thanks are expressed to the following people for their support in this study:

Dr. Joseph Schoolar, Director, Texas Research Institute of Mental Sciences for the predoctoral fellowship which made this study possible.

Dr. Ellis Nordyke, for performing the amino acid assays.

John McClure, for performing the assay for brain DNA.

Dr. Mary Roach and Dr. Harold Altschuler for their generous donations of laboratory facilities.

Dr. Marlyne Kilbey, Dr. William Braud, and Dr. Daniel Sheer for their ideas, help, and advice.

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ABSTRACT

Neonate rats were intubated daily from 0-29 days of age with l-phenylalanine plus dl-parachlorophenylalanine in order to produce an animal model of phenylketonuria (PKU). On the 29th day of intubation, the PKU group had elevated plasma phenylalanine and tyrosine levels and reduced body and brain weights as compared to an agar control group. At 85 days of age the PKU animals made significantly fewer bar presses than the agar controls, with the largest difference occurring when the subjects were shifted from a CRF to a DRL-5" schedule of reinforcement. The rats were decapitated at 330 days of age and the PKU animals had significantly less DNA and wet brain weight than the agar controls in the cerebellum only. Early drug treatment of the PKU animals produced behavioral, biochemical, and neurological characteristics that resemble those of human PKU.

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CHAPTER I

HISTORY OF THE PROBLEM

Phenylketonuria (PKU) is an inborn error in amino acid metabolism that was first described by Fölling, who detected excessive amounts of phenylpyruvic acid in the urine of institutionalized, severely retarded siblings (1934). The most important biochemical characteristic of PKU is an elevated level of phenylalanine in the blood and urine, which is caused by the virtual absence of the liver enzyme phenylalanine hydroxylase (Jervis, 1953; Lyman, 1963; Knox, 1966; Kaufman, 1971). In an effort to prevent the severe mental retardation that accompanies classical PKU, Woolf (1951) proposed a therapy of restricting the amino acid phenylalanine in the diet of infants diagnosed as phenylketonuric or hyperphenylalanemic. This low phenylalanine diet was first instituted by Bickel (1953) and has since then resulted in higher IQ's and better physical growth than that occurring with untreated PKU (Sutherland, 1966; Fuller, 1967; Berry, 1969). Although a low phenylalanine diet is the only available treatment for PKU, there are the disadvantages of a stressful long-term diet, the danger of over-restricting the essential amino acid phenylalanine, which can result in reduced growth, mental retardation, or death (Rouse, 1966) and the probability that treated PKUs are still vulnerable to the biochemical lesions of PKU (Cohen, 1969; Sanders, 1969; Perry, 1970). Thus, there

is still the need for a more effective therapy for PKU as well as a better understanding of the nature of this human disease.

Review of Literature

Although PKU is the best understood of all the amino-acid disorders, the exact cause of the biochemical damage of this disease is unknown. Eastham and Jancar (1968) have proposed that the lesions of all aminoacidopathies including PKU are caused by (1) the failure of the amino acid to enter the cell, or the failure of the cell to take up the amino acid, (2) an intracellular enzyme (or co-enzyme) deficiency that prevents normal amino acid metabolizm and (3) an abnormal 'leakage' of the amino acid or metabolite from the cell.

The specific hypotheses that attempt to explain the mental retardation of PKU can also be organized into three similar groupings. First, the retardation associated with PKU could be the result of a secondary inhibition of enzyme reactions by high levels of phenylalanine and the accompanying metabolic defects. Thus an inhibition of tryptophan hydroxylase by phenylalanine results in a drop in the level of tryptophan metabolites 5-hydroxytryptophan, 5-hydroxytryptamine, 5-hydroxyindole acetic acid, and kynureine (Eastham and Jancar, 1968; McKean, Boggs, and Peterson, 1968; Lutz, 1971). Researchers have also observed defects in glycolysis, reduced myelination, protein and proteo-lipid synthesis, and brain weight, and an overall depression of plasma amino acids besides phenylalanine (Eastham and Jancar, 1968; Linneweh and Ehrlich, 1962; Efron, Kang, Visakorpi, and Fellers, 1969; Martin and Schlote, 1969).

Secondly, PKUs may suffer from a deficiency of substances that are formed from phenylalanine, a few of which are melanin, tyrosine and its metabolites homogentisic acid, tyramine, phenol , and especially the neurotransmitters 3, 4-dihydroxy-phenylalanine (DOPA), norepinephrine, and epinephrine (Nadler and Hsia, 1961; Perry, Hansen, Tischler, Bunting, and Diamond, 1970; Lutz, 1971). Thirdly, clinical research has suggested that high levels of phenylalanine or its metabolites are directly toxic to the PKU child (Hsia, 1966; Knox, 1966; Perry, et al., 1970). Some of the urinary metabolites are phenylacetic acid, ortho-hydroxyphenylacetic acid, phenylacetyl-glutamine, N-acetylphenylalanine, phenylethylamine, indole, indoleacetic acid, indolelactic acid, and indican as well as phenylalanine itself (La Du and Zannoni, 1971).

Classical PKU with severe mental retardation is a rare disease that occurs in approximately 1:5000 - 1:18,000 (Bickel, 1971) of the births from the general population, but phenyl-ketonurics comprise 1.0 percent of institutionalized mental patients. Early epidemiological data seemed to indicate that PKU was primarily inherited by males despite contradictory evidence that PKU is genetically transmitted via the auto-somal recessive mode (Hsia and Dobson, 1970). However, later studies by Koch, Dobson, Hsia, and Woolf (1971) and others (Hawcroft and Hudson, 1973) have failed to find this prepon-derance of males in PKU but show instead that phenylalanine levels may rise more slowly in females after birth. Thus the widely used 'Phenistix' urine test or the Gutherie blood test

may give false-negative results if given only in the first forty-eight hours of life before adequate feeding with protein, particularly in females.

Of the several variant forms of classical PKU, the most common is hyperphenylalanemia. Mabry, Denniston, and Nelson (1969) believe that perhaps as much as .01 per cent of the general population has an undetected condition of hyperphenylalanemia which may or may not be reflected by sub-normal IQs. Fetuses exposed to high phenylalanine levels in utero through maternal PKU or hyperphenylalanemia may be born retarded even though they exhibit normal phenylalanine metabolism (Mabry, et al., 1963; Fisch and Anderson, 1971). Pregnant women who are PKUs or who have high blood levels of phenylalanine have been put on low phenylalanine diets and have given birth to small babies of normal intelligence (Arthur and Hume, 1970). In a short review of the research comparing classical PKU with its variant forms, Thalhammer and Scheiber (1972) concluded that hyperphenylalanemia appears to be genetically different from PKU, possibly through two different allels on the same phenylalanine hydroxylase locus.

One important issue remaining to be resolved concerns the optimal duration of dietary treatment. There is general agreement that PKU children who are treated early and whose phenylalanine blood levels are carefully monitored thrive far better than late or non-treated PKU children (Berry, 1969). Some research has shown that dietary treatment may be discontinued between the ages of five and eight without a drop in intelligence scores, presumably because the major portion

of cortical development has occurred by this time (Saunders, 1969). Other studies disagree with this termination date, and have noted that when the low phenylalanine diet is discontinued, PKUs exhibit undesirable behavioral consequences such as lethargy, irritability, or hyperactivity (Frankenburg and Goldstein, 1973).

In addition Grüttner, Maisch, and Bartelheimer (1971) have noted several instances in which dietary therapy has been initiated for the first time with children five or six years of age. In some cases these children have shown increases in IQ scores ranging from 7 to 34 points. Grüttner et al. (1971) have recommended that the theraputic diet be initiated even after the sixth year of life when the following symptoms are present:

1. Behavior disturbances such as apathy, lack of activity, hyperactivity and agressiveness, when they are so severe that they decrease the capacity of learning and sociability.

2. If severe seizures, uninfluenced by treatment, are present at the time of diagnosis, and considerably disturb the general condition of the patient.

3. If there is only moderate retardation of intellectual development or if the patient is just a borderline case for admission to a special school.

Grüttner et al. (1971) stress that when there is a change in behavior after the initiation of dietary therapy, "the change is always in the direction of normalization; the hyperactive children become more quiet and the apathetic children become more active."

Perry, Hansen, Tischler, and Sokol (1973) have suggested the temporary use of the low-phenylalanine diet with psychotic PKU adults. Perry et al. (1973) have observed cases in which

unrecognized PKU adults of normal intelligence were admitted to a mental institution for major psychotic illnesses and were given electroconvulsive shock and antipsychotic drugs. These patients were later diagnosed as PKUs by a routine urine screening test when it was determined that their fasting blood phenylalanine levels ranged from 14 to 17 milligrams per 100 milliliters of blood, the normal range being below 4 milligrams. These researchers concluded that lowering blood phenylalanine levels would have been a more appropriate treatment for these PKU patients during their acute psychotic illness. McKean (1972) appears to support this conclusion with data collected from other adult PKUs. McKean demonstrated that in vivo administration of phenylalanine to PKU adults severely disrupted cerebral serotonin and catecholamine metabolism, and that serotonin and dopamine levels could be improved by restricting phenylalanine intake with a possible improvement in norepinephrine synthesis as well. In conclusion, an early, permanent termination of the low phenylalanine diet in PKUs, and the possible utility of this diet during later life deserves further investigation.

Animal Models of PKU

A valid animal model of PKU would be of great value in attempting to assess the merits of various treatment methods or to examine the basic biochemical lesion or behavioral disorder. Karrer and Cahilly (1965) have reviewed some of the early studies that claimed to have made animals phenylketonuric and even prevented PKU. Karrer and Cahilly concluded that claims of having produced animal PKU were premature because no study had successfully met certain essential biochemical and behavioral criteria. The most frequent failures

in animal models of PKU occurred because blood serum phenylalanine levels were below 20 milligrams percent, or behavioral deficits that were reversible after the animals were taken off the high phenylalanine diet used to induce PKU. Perez (1965) supported Karrer and Cahilly in a study demonstrating that in rats when high levels of phenylalanine are administered by intraperitoneal injections from within hours after birth until 60 days of age and the animals tested 21 days after the last injection no learning deficit could be found.

Most previous attempts to produce animal PKU have increased the subjects' blood levels of phenylalanine either through diet or injections. One criticism of this approach has been that little phenylalanine hydroxylase inhibition occurs, and the levels of tyrosine are greatly increased rather than remaining sub-normal or normal as in human PKU (Perez, 1965; Perry et al., 1965; Karrer and Cahilly, 1965). With the exception of in utero damage to the fetus by high phenylalanine levels (Kerr et al., 1968; Chamove and Davenport, 1970; Chamove, Waisman, Harlow, 197C; Chamove and Harlow, 1973) with rhesus monkeys, only Schalock (1969) has produced with this model animal PKU that meets the behavioral criteria of irreversibility, and this study reported no biochemical data.

Koe and Waisman (1968) have attempted to produce another animal analogue of PKU by using dl-parachlorophenylalanine (PCPA) which inhibits the enzymes tryptophan hydroxylase and phenylalanine hydroxylase, decreases tyrosine and serotonin, and increases plasma and urinary phenylalanine. This model has been criticized for inadequate elevations of phenylalanine and its failure to produce

a long-term behavioral decrement (Watt and Martin, 1969). However, Hole (1972) claims to have produced an animal analogue of PKU using PCPA injections for the first seven weeks of life in rats and his data meet most of the criteria proposed by Karrer and Cahilly. Hole (1972) reported reduced serotonin levels during the injections, a permanent reduction in brain weight, and a reduced arousal level in the animals four weeks after the last injection.

Andersen and Guroff (1972) have discussed five basic errors that may occur in attempting to produce an animal model of PKU. The first of these errors is the chemical method of producing the model by the use of either phenylalanine or PCPA alone. Andersen and Guroff (1972) claim that by simultaneously administering phenylalanine plus PCPA, a treatment first proposed by Lipton, Gordon, Guroff, and Udenfriend (1967), that they were able to maintain high plasma phenylalanine levels, low or normal tyrosine levels, inhibit hepatic phenylalanine hydroxylase and thus produce most of the biochemical characteristics of PKU. The second error that they claim occurs frequently is attempting to induce hyperphenylalanemia past the period of rapid brain development. Andersen and Guroff treated their animals from birth until 21 days of age, a technique also urged by Kilbey and Harris (1971). A third common mistake has been to test the animals during the time of injections or diet when the animals are suffering from the nonspecific and reversible effects of the drug treatment. Anderson and Guroff ran their behavioral tests six months from the time of the last drug treatment when the animals were all fully mature. The fourth error in animal PKU studies has been to

make a single test of learning impairment which does not examine the complex array of behaviors which is changed in mental retardation. Andersen and Guroff therefore ran a series of tests to monitor motor activity, autonomic activity, active and passive avoidance abilities, appetitive learning, and aggression. Finally, there is usually no attempt made to correlate the changes in brain structure induced by the chemical treatment with the behavioral deficits. Andersen and Guroff examined the brains of their animals for possible neuropathology after the conclusion of testing by measuring brain weights and myelin content.

The animal model of PKU produced by Andersen and Guroff exhibited behavioral, morphological, and biochemical characteristics that strongly resemble the clinical characteristics of PKU. For instance, the clinical observation has been that human PKUs are hyperactive, and thus PKU animals should also exhibit hyperactive behaviors. The observed results from this experiment confirmed this prediction, with the PKUs having higher motor activity in an open field, superior active avoidance scores, and inferior passive avoidance scores compared to saline treated controls. In addition, untreated human PKUs are aggressive with severe temper outbursts and frequent seizures. Andersen and Guroff's data revealed that the PCPA plus phenylalanine treated rats were more often mouse killers than the saline controls, and also had lower seizure thresholds. Human PKUs also have reduced brain weights, usually associated with a myelin defect in young patients. The PKU treatment group had lighter brain weights than the saline controls although Andersen and Guroff were unable to observe any

myelin defect in these animals. Finally, human PKU patients not on the low-phenylalanine diet have increased phenylalanine to tyrosine ratios and reduced hepatic phenylalanine hydroxylase activity, conditions which were replicated with the animal PKUs but not in the saline-animals.

In a study designed to investigate maternal PKU, Foote and Tao (1968) administered PCPA plus phenylalanine to rats that were fourteen days pregnant until eight days after the birth of the pups. The young pups were then injected every third day with the same drug combination from six to thirty-one days after birth. The experiment produced variations in the brain lipids of developing rats, with the experimental group showing a retarded accumulation of oleic acid. The Foote and Tao study agrees with some clinical literature that has found abnormalities in the lipid content of human PKU brain, and extends the validity of PCPA plus phenylalanine treatment as a model of PKU. Unfortunately, Foote and Tao reported no behavioral data.

Longenecker, Reed, Lo, Chang, Nasby, White, and Ide (1970) performed an experiment in which they intubated newborn rats until one, three, or six days after birth with phenylalanine, or combinations of PCPA or amethopterin plus phenylalanine. They observed that treatment with phenylalanine plus an enzyme inhibitor was no more effective in inhibiting phenylalanine hydroxylase activity than treatment with phenylalanine alone. Longenecker et al. attributed these results to the toxicity

of PCPA or amethopterin, which resulted in mortality levels ranging from 50 to 100%, and the fact that phenylalanine hydroxylase activity is low in the one to six day old rat. Longenecker et al.'s results do not contradict Andersen and Guroff's observation (1972) that PCPA plus phenylalanine produces a more effective model of PKU than phenylalanine alone in older rats. Longenecker et al. do not report any behavioral observations for this study.

Butcher, Vorhees, and Berry (1970) administered a diet of 3% excess phenylalanine and 0.12% PCPA to rats from 20 until 48 days of age. The animals were then tested seven days later in an open field maze and Butcher et al. found no behavioral differences between the experimental and control groups. When the rats were tested in a water maze twenty days after termination of the diet, the PKU group made significantly more errors and spent more time in the maze than did the pair fed control group. Butcher et al. (1970) concluded that the experimental treatment had induced a condition analogous to late or untreated human PKU, complete with irreversible behavioral deficits. In a later study, Vorhees, Butcher, and Berry (1972) administered the same diet as above from 21 until 51 days of age and tested the rats from 2 to 10 days after diet termination. They found that the PKU rats were hypoactive in the open field test compared to pair-fed and ad libitum fed control groups. In addition, Vorhees et al. (1972) demonstrated that phenylalanine levels returned to normal 24 to 48 hours after diet termination, and that the

activity difference between the PKU and control groups was not due to weight differences at the time of testing. These results support the hypothesis that hypoactivity is consistently obtained in animals exposed to high phenylalanine levels during early development.

Schalock and Copenhaver (1973) also appear to support this hypothesis with data from a study utilizing PCPA plus phenylalanine treatments. Schalock and Copenhaver used a prepartum treated group, a postpartum group, and a control group of rats. The prepartum group received the drug in utero by injections to the mother from the 15th until 21st day of gestation. When the prepartum group was tested at 80 days of age, the PKU group showed decrements in initial DRL-12" learning, more fear, and less activity, exploratory behavior, and sociability. The postpartum PKU group, which was injected with the drug from 1 to 60 days of age was also significantly less active and exploratory than the postpartum control. The trend in the data for both of the experimental PKU groups was in the direction of hypoactivity, and the hypoactivity was not attributible to differences in body weight at the time of testing.

There is an apparent conflict between the results reported by Andersen and Guroff (1972) and the results reported by Butcher, Vorhees, and Berry (1970, 1972) and Schalock and Copenhaver (1973). The former researchers found that experimental PKU rats are hyperactive and the latter group found that the animals are hypoactive. One

major difference between the two studies is the time at which the animals were tested after the conclusion of drug treatment. Andersen and Guroff tested their animals six months after the last PCPA plus phenylalanine injection and the other group tested their animals from four to twenty days after the last drug treatment. Thus, the different behavioral characteristics reported by the two groups may be a function of the different recovery times.

Hypotheses

The following hypotheses were formulated on the basis of previous studies of animal PKU that utilized early PCPA plus phenylalanine administration:

1. The PKU rats should exhibit such acute biochemical characteristics as elevated serum phenylalanine levels between 10 and 20 mgs %, an increased phenylalanine to tyrosine ratio, and normal tyrosine levels.
2. The PKU rats should exhibit irreversible behavioral deficits after a sufficient time for recovery from acute drug effects.
3. The PKU rats should exhibit permanent changes in brain morphology after a sufficient time for recovery from acute drug effects.

CHAPTER II

METHOD

Subjects and Design

Fifteen litters of F344 albino rats born within one twenty-four hour period were pooled and evenly divided among the dams, which were randomly assigned to one of five experimental groups. These groups were intubated daily from birth until 29 days of age in the following manner:

1. High l-phenylalanine (HPHE) 200 mg/Kg body weight plus high dl-parachlorophenylalanine (HPCPA) 100 mg/Kg body weight -- four litters, 36 subjects.
2. HPHE plus low dl-parachlorophenylalanine (LPCPA) 50 mg/Kg -- three litters, 27 subjects.
3. Low l-phenylalanine (LPHE) 100 mg/Kg plus HPCPA -- three litters, 27 subjects.
4. Agar vehicle, 0.2% solution (AG) -- three litters, 23 subjects.
5. Handled control (HD) -- two litters, 23 subjects.

All neonate subjects were housed with the dams until 30 days after birth, at which time they were separated into individual cages. The subjects and dams were maintained in a 12 hour light/dark animal colony environment.

Apparatus

The blood plasma assays for free amino acids were done on a Hewlett-Packard gas chromatograph with a flame ionization

column, and the brain assays for DNA were done on a Beckman DB-G grating spectrophotometer. The subjects were trained in five sound-insulated operant conditioning chambers (Scientific Prototype, Model SPC-300) with plexiglass test cages and solid state circuitry. Reinforcers were 45 mg Noyes animal pellets and data collection was in cumulative response totals and cumulative records (Ralph Gerbrand Co.).

Procedure

Each pup in the five experimental groups was weighed daily and returned to its appropriate litter. Four of these groups were orally intubated in the volume of 10 ml/Kg body weight with a 0.2% agar solution. Three of the agar intubated groups were the HPHE plus HPCPA, HPHE plus LPCPA, and LPHE plus HPCPA drug-treated groups.

After thirty days of treatment, six female subjects from each group were chosen at random and sacrificed via decapitation four hours after the last intubation or handling. The blood plasma was collected in heparinized containers and assayed for free amino acids by gas chromatography (Pellizari, Brown, Talbot, Farmer, Fabre, 1971; Nordyke, 1972). The brains were then removed from the subjects and weighed on a Mettler H20-T balance. The remaining subjects in each group were separated into individual cages and weighed weekly until they were 77 days old, at which time the drug-treated and agar-treated animals weighed approximately the same.

When the subjects were 77 days old or when almost seven weeks had elapsed since the last drug or vehicle treatment, the young rats were put on a 23 hour food deprivation schedule until they were at 85% of their ad lib. food and water body weight. At 85 days of age, all subjects were given three days of 30 minute sessions in the operant conditioning chambers on a two-bar continuous reinforcement (CRF) schedule. The animals were then shifted to a one-bar Differential Reinforcement of Low Rate of Responding five second schedule (DRL-5") for two days with half of the subjects pressing the right bar and half pressing the left bar. All conditioning sessions were 30 minutes long and the subjects were evenly divided between left and right bars. The rats were trained on a one-bar DRL-10" schedule for four days alternating the reinforcing lever every two days.

Discrimination training was begun on the tenth day of conditioning and was identical to that used in a previous study (Kilbey, Harris and Aigner, 1972). The discrimination training schedule was a two-bar multiple DRL-15" - DRL-15" and the discriminative cue was the presence or absence of a rough plexiglass floor surface which was changed every two days along with the position of the reinforcement bar. The subjects were given eight days of discrimination training on the DRL-15" - DRL-15" schedule and tested with an extinction trial on the ninth day. This procedure was repeated for six extinction trials or for a total of 54 days of discrimination

training plus extinction trials.

After the conclusion of behavioral testing the subjects were put back on ad libitum food and water and maintained in their individual cages for approximately six months. When the rats were 330 days of age they were weighed, decapitated, and the brains removed and sectioned over ice into medulla, cerebellum, mid-brain, and cortex using a method suggested by Glowinski and Iverson (1966). The brain sections were then assayed with a Beckman DB-G Spectrophotometer for DNA using a modification of a method by Burton (1956) and Margolis (1969).

RESULTS

Neonate Intubation

During the 29 day intubation period the two groups receiving high levels of dl-parachlorophenylalanine (100 mg/Kg body weight), the HPHE plus HPCPA group and the LPHE plus HPCPA group experienced 95% and 92% mortality rates respectively and were dropped from the experiment. The remaining drug treated group, HPHE plus LPCPA experienced a 50% mortality rate but was retained for the experiment along with the AG and HD groups, which had 2% and 0% mortality rates. A sample of six female subjects from these three groups were decapitated and the remaining animals were arranged in the following fashion:

1. HPHE plus LPCPA group - composed of six males and five females.
2. AG control - composed of six males and five females.
3. HD control - composed of six males and six females.

The Duncan's Multiple Range Test (McGuigan, 1968) was used for all data analysis in this study. In the tabular data to follow, any two means that are underscored by the same line are not significantly different; any two means that are not underscored by the same line are significantly different. The level of significance is listed to the left of the group means.

When the animals were weighed and intubated or handled at 29 days of age, the AG group had a significantly higher ($p < .01$) mean body weight than the HPHE plus LPCPA group (see Table 1). Decapitation of the rats and removal of their brains revealed that the AG group also had a significantly higher ($p < .05$) mean wet brain weight than the HPHE plus LPCPA group (see Table 1).

Insert Table 1 about here

The blood that was collected from the whole body of the decapitated subjects was analyzed for plasma amino acids by gas chromatography. Table 2 gives the group mean amino acids and the significant between group differences. The significantly elevated levels of phenylalanine and tyrosine in the HPHE plus LPCPA group over the AG and HD groups are of particular relevance to this study.

Insert Table 2 about here

Bar Press Conditioning

Each animal's daily record of bar pressing was composed of four cumulative numbers.

1. Reinforcements (Rs) - the total number of food pellets earned by the subject, or during extinction trials, a hypothetical number of pellets that the subject could have earned.
2. Correct Bar Presses (CBPs) - the total number of responses on the bar controlling reinforcement, whether or not each response was reinforced.
3. Incorrect Bar Presses (IBPs) - the total number of responses on the bar not controlling reinforcement.
4. Total Bar Presses (TBPs) - the sum of CBPs and IBPs.

Figures 1-4 are the mean number of bar presses for each group during the first ten days of conditioning organized as Rs, CBPs, IBPs, and TBPs respectively. Figures 5 and 6 are the mean number of bar presses during six extinction trials, each trial being preceded by eight days of training on a two bar multiple DRL-15"-DRL-15" schedule.

The AG group made significantly ($p<.05$) more CBPs, IBPs, and TBPs than did the HPHE plus LPCPAs during the first ten days of training (See Table 3). The total number of Rs earned during this ten day period did not differ among groups, but on days two and three of conditioning, the AGs earned significantly more Rs than did the HPHE plus LPCPAs.

Insert Table 3 about here

The largest differences among groups in bar pressing behavior occurred on day four, which was the first day of DRL-5" training. The AG group made far more CBPs and TBPs on this day than did either the HD or HPHE plus LPCPA groups (See Figures 2 and 4).

Since the groups were not equal on day three of training, the data for each subject was transformed into a difference score by subtracting the bar presses on day three from those on day four. The difference scores represent a rate of change from one day to the next and demonstrate that the large group differences recorded during initial DRL-5" acquisition cannot be entirely the result of differences in CRF performance the previous day. Table 4 shows that the difference scores for the AG group are much larger than those of the HPHEN plus LPCPA or HD group ($p < .01$) for Rs, CBPs, and TBPs.

Insert Table 4 about here

The difference scores between day five (DRL-5") and day six (DRL-10") were in the opposite direction from those observed between day three and day four. The HPHE plus LPCPA group had a significantly larger ($p < .05$) mean difference score for TBPs than the AG group (See Table 4). Difference scores for CBPs and Rs were higher for the HPHE plus LPCPA group but were not statistically significant.

There were no significant differences among the groups in bar pressing behavior during extinction sessions, summed over all six trials (See Table 5). Visual inspection of

Graphs 5 and 6 and Table 5 reveal that the AG group tended to bar press more frequently than the HPHE plus LPCPA or HD groups which is in the direction predicted from initial conditioning data.

Insert Table 5 about here

Adult Morphology

There were no differences among the groups in body weight when the subjects were sacrificed at 330 days of age (See Table 6). Two of the HPHE plus LPCPA animals developed unilateral cataracts, although no other gross physical characteristics distinguished this group from the AG or HD groups.

The HPHE plus LPCPA group had a significantly lighter cerebellum than did the AG group ($p < .01$) although other group comparisons of wet brain weight of the medulla, mid-brain, cortex, and total were non-significant (See Table 6).

Similarly, the HPHE plus LPCPA group showed a significant reduction ($p < .01$) of total DNA in the cerebellum compared to the AG group, all other comparisons being non-significant (See Table 6).

—Insert Table 6 about here

CHAPTER IV

DISCUSSION

Hypothesis one

The PKU rats failed to exhibit serum phenylalanine levels elevated to between 10 and 20 mgs %, normal tyrosine levels, or an increased phenylalanine to tyrosine ratio. Therefore, the results of this study failed to support the first hypothesis. Since the serum phenylalanine and tyrosine levels were both significantly elevated, the results of this study suggest that the drug dosages of PCPA and phenylalanine were both too low. Extrapolation from the data of this study and other studies (Nordyke and Roach, 1973; Andersen and Guroff, 1972) suggests that PCPA dosage levels should be no lower than 100 mg/Kgs of body weight and phenylalanine dosage levels should be between 200 and 1000 mg/Kgs of body weight given daily in order to more closely reproduce the biochemical syndrome of PKU.

The PKU group in this study also exhibited significantly elevated levels of serum glutamine plus glutamic acid over the HD control group. The depression of these amino acids in PCPA plus phenylalanine treated rats has been reported by Nordyke and Roach (1974) who also report that amino acid compartmentation developed more slowly in the drug treated rats. Nordyke and Roach proposed that this retardation of amino acid compartmentation was related to the inhibition of neuronal growth and was reflected by the permanently reduced brain weights of their PCPA plus phenylalanine treated rats.

In the HD control group, serum alanine was significantly elevated over both the AG and PKU groups. One possible explanation for alanine elevation in the HD group of rats is that the neonates in these litters were overcrowded and were therefore mildly undernourished. Because of the unexpectedly high mortality rate of the PKU groups and an oversight in the experimental design, the HD groups had 12 neonates per litter as compared to 9 neonates per litter for the AG group and between 4 and 6 per litter for the PKU group. Therefore, the slight reduction in body weights that was recorded at 30 days of age in the HD group as well as the increased levels of alanine could have been due to the overcrowded litters. The evidence supporting this hypothesis comes from a study by Räihä and Schwartz (1973) in which newborn rats were divided into small and large litters. The small litter sucklings grew twice as fast as the large litter sucklings and presumably received larger amounts of protein. The rats in the smaller litters also had higher activity rates of the amino acid catabolizing enzymes alanine transaminase (L-alanine-2-oxoglutarate aminotransferase) and serine dehydratase. In this study, the activity of alanine transaminase was possibly lower in the HD group and possibly resulted in the higher serum levels of unmetabolized alanine. Additional evidence that the HD group was mildly undernourished is recorded in Table 1; the HD group had a significantly lower average body weight than the AG control groups at 80 days of age. The weight difference between the AG and HD control groups was no longer significant

at 330 days of age (See Table 6), but the HD group was still lighter than the other two treatment groups.

Hypothesis two

The PKU rats exhibited deficits in bar press conditioning 55 days after the last drug treatment and thus provided support for the second hypothesis of behavioral irreversibility. The direction of these behavioral deficits appears to be towards hypoactivity as previously reported by Butcher et al. (1970), Vorhees et al. (1972), Schalock and Copenhaver (1973) and not towards hyperactivity as reported by Andersen and Guroff (1972). The observation of hypoactivity in PCPA plus phenylalanine treated rats agrees with Hole's (1972a, 1972b) observations of hypoactivity in rats treated only with PCPA or phenylalanine during early development. Hole stated that the behavioral deficits observed in more complex tasks with PKU rats may be due to a basic defect in arousal or habituation. The nature of the irreversible behavior deficit seems to imply a long term change in brain amine levels and especially of serotonin in the PKU group of animals. The data from this study predicts that PCPA plus phenylalanine treatment during early development will, under certain conditions, result in a chronic elevation of serotonin after the termination of drug treatment. This chronic elevation of serotonin will in turn cause an arousal deficit towards hypoactivity in the PKU animals.

Schaefer, Buchanan, and Ray (1973) offer a resolution to the conflict between studies showing PKU rats to be hypoactive and studies showing them to be hyperactive. Schaefer et al. demonstrated that in newborn PCPA treated rats, the whole brain

levels of serotonin were related to whether the weaned pups were housed in an enriched versus an impoverished postweaning environment from 28 to 80 days of age. The PCPA treated rats raised in an enriched environment had chronically lower 5-HT levels and made more active avoidance responses than those PCPA animals raised in isolation who had higher 5-HT levels. The saline treated controls raised in an enriched environment had higher 5-HT levels and made fewer active avoidance responses than the isolation raised saline group who had lower 5-HT levels and made more active avoidance responses.

The Schaefer et al. study demonstrated three important points about animal models of PKU. First, they were able to find a long-term change in 5-HT levels almost 65 days after the last administration of PCPA, suggesting that serotonin is involved in the arousal deficits of animal PKU. Secondly, Schaefer et al. found an environment versus drug treatment interaction in the postweaning rat that could be responsible for the lack of agreement concerning the nature of long-term behavioral deficits. Andersen and Guroff (1972) kept their rats in their litter groups until six weeks of age and this enriched environment should have led to lower 5-HT levels in the PCPA plus phenylalanine group. Since conditions that reduce 5-HT levels also increase active avoidance (Schaefer et al., 1973), the PCPA plus phenylalanine group should have exhibited superior active avoidance and inferior passive avoidance which did occur (Andersen and Guroff, 1972). Of the PKU studies mentioned previously that report hypoactive PKU rats, only Hole (1972a) describes the preweaning environmental that was used. If these studies used the standard single animal

isolated cage environment for their postweaning rats, it would be predicted that these rats should be hypoactive PKUs. The third point that Schaefer et al. demonstrate is that to maximize the behavioral differences between PKU and control groups, the animals should be housed in an enriched environment.

In conclusion, there appears to be ample evidence of an irreversible behavioral deficit induced by early PCPA plus phenylalanine treatment. The lack of agreement between studies on the nature of this behavioral deficit may be at least partially due to different postweaning environments.

Hypothesis three

There was a permanent reduction in the cerebellar tissue weight and DNA content in the adult PKU rats as compared to the AG control group. This irreversible treatment effect in brain morphology is in the direction predicted and thus supports the third hypothesis. Other studies that have induced a PKU-like syndrome with PCPA plus phenylalanine also report reduced brain weights and/or DNA in rehabilitated adult rats (Andersen and Guroff, 1972; Nordyke and Roach, 1974a, 1974b). Adelmann, Mann, Calley, and Bass (1973) administered phenylalanine to neonatal rats, produced a reduction in the wet brain weight, and observed neuropathological lesions through the light microscope that were limited to the cerebellum. The cytoplasm of the Purkinje cells was vacuolated and heterochromatin severely decreased in granular cell nuclei in the cerebellum of phenylalanine treated neonatal rats. Adelmann et al. stress that high levels of phenylalanine had the greatest

effect on the rapidly developing cerebellar neurons and affected DNA, RNA, protein, myelin lipids, and total brain weight.

Although the mildly undernourished MD group had normal brain weight and DNA at maturity, several studies mention that the induction of a PKU model in animals is confounded by undernutrition in the experimental group. That is, unless a pair-fed control group is used, the dependent measures of biochemistry, behavior, and brain morphology could be a function of starvation rather than of a specific drug induced lesion. Even pair-fed controls gain weight more rapidly than experimental PKU animals, and thus pair feeding will not completely rule out the effects of undernutrition. However, Vorhees et al. (1972) were able to resolve this problem by a selective assignment of the heaviest rat pups to the PCPA plus phenylalanine group in addition to using pair-fed and ad libitum control groups. At the end of drug treatment there was no difference in the body weights between the PKU and pair-fed groups, but the PKU groups were consistently hypoactive in an open field test. Vorhees et al. were therefore able to demonstrate that behavioral deficits induced by PCPA plus phenylalanine are independent of the effects of malnutrition.

Table 1 - Neonate Intubation at 29 Days

Average body weights (gms.)

	PKU	HD	AG
($p < .01$)	38.13	42.48	48.75

Average wet brain weights (gms.)

	PKU	HD	AG
($p < .05$)	1.22858	1.28066	1.31174

Neonates at 80 Days

Average body weights (gms.)

	HD	PKU	AG
($p < .05$)	156.14	165.66	177.71

Table 2 - Plasma Amino Acids at 29 Days (mgs. %)

	AG	HD	PKU
phenylalanine ($p < .01$)	3.18	3.38	5.20
tyrosine ($p < .05$)	2.38	2.57	3.80

	HD	AG	PKU
glutamine plus glutamic acid ($p < .05$)	8.27	8.95	10.78

	PKU	AG	HD
alanine ($p < .05$)	5.60	5.87	8.78

All other comparisons non-significant

	PKU	AG	HD
valine	2.55	2.45	3.00
isoleucine	1.28	1.10	1.25
leucine, glycine, threonine	10.38	10.10	10.62
proline, serine	8.57	9.57	10.40
methionine	2.03	2.77	1.72

Table 2 - continued

	PKU	AG	HD
asparagine plus aspartic acid	2.22	2.43	2.22
lysine	7.75	7.47	8.92
total	60.16	56.27	61.13
parachlorophenyl-alanine	7.10	-----	-----

Table 3 - Cumulative Bar Presses

Average for first ten days

	PKU	HD	AG
CBPs ($p < .05$)	142.0	160.5	169.4
IBPs ($p < .05$)	57.1	61.9	77.1
TBPs * ($p < .05$)	182.9	203.0	223.0
Rs (n.s.)	60.5	63.1	65.0

Average for single days

Rs - Day 2 ($p < .05$)	55.3	78.2	82.2
Rs - Day 3 ($p < .05$)	102.6	112.8	131.7

*TBPs $\neq$ CBPs plus IBPs when averaged for the first ten days since there were no IBPs for the first three days of two-bar CRF training.

Table 4 - Bar Press Difference Scores

Day 4 minus Day 3

	PKU	HD	AG
CBPs ($p < .01$)	25.6	56.4	95.8
Rs ($p < .01$)	2.6	1.9	-37.6
TBPs ($p < .01$)	51.0	71.5	135.5

Day 6 minus Day 5

CBPs (n.s.)	85.5	76.0	56.3
Rs (n.s.)	73.3	56.8	52.2
TBPs ($p < .05$)	170.6	116.6	111.9

Table 5 - Average Bar Pressing for Six Extinction Trials

	HD	PKU	AG
CBPs (n.s.)	67.8	70.4	75.1
IBPs (n.s.)	59.8	55.24	60.64
TBPs (n.s.)	122.9	127.1	135.7
Rs (n.s.)	30.2	30.4	31.3

Table 6 - Adult Morphology at Time of Sacrifice

Average body weights (gms.)

	HD	AG	PKU
(n.s.)	259.9	273.1	278.4

Average wet brain weights (gms.)

	PKU	HD	AG
medulla (n.s.)	0.2685	0.2771	0.2707
cerebellum ($p < .01$)	0.2944	0.3076	0.3208
mid-brain (n.s.)	0.3325	0.3344	0.3415
cortex (n.s.)	1.1198	1.1419	1.1517
total (n.s.)	2.015	2.0611	2.0848

Average DNA per brain section (mgs.)

	PKU	HD	AG
medulla (n.s.)	0.2880	0.3025	0.3020
cerebellum ($p < .01$)	2.1470	2.2992	2.6000

Table 6 continued

	PKU	HD	AG
mid-brain (n.s.)	0.3900	0.4117	0.4460
cortex (n.s.)	1.5620	1.4567	1.3850
total (n.s.)	4.3870	4.4700	4.7330

Fig. 1

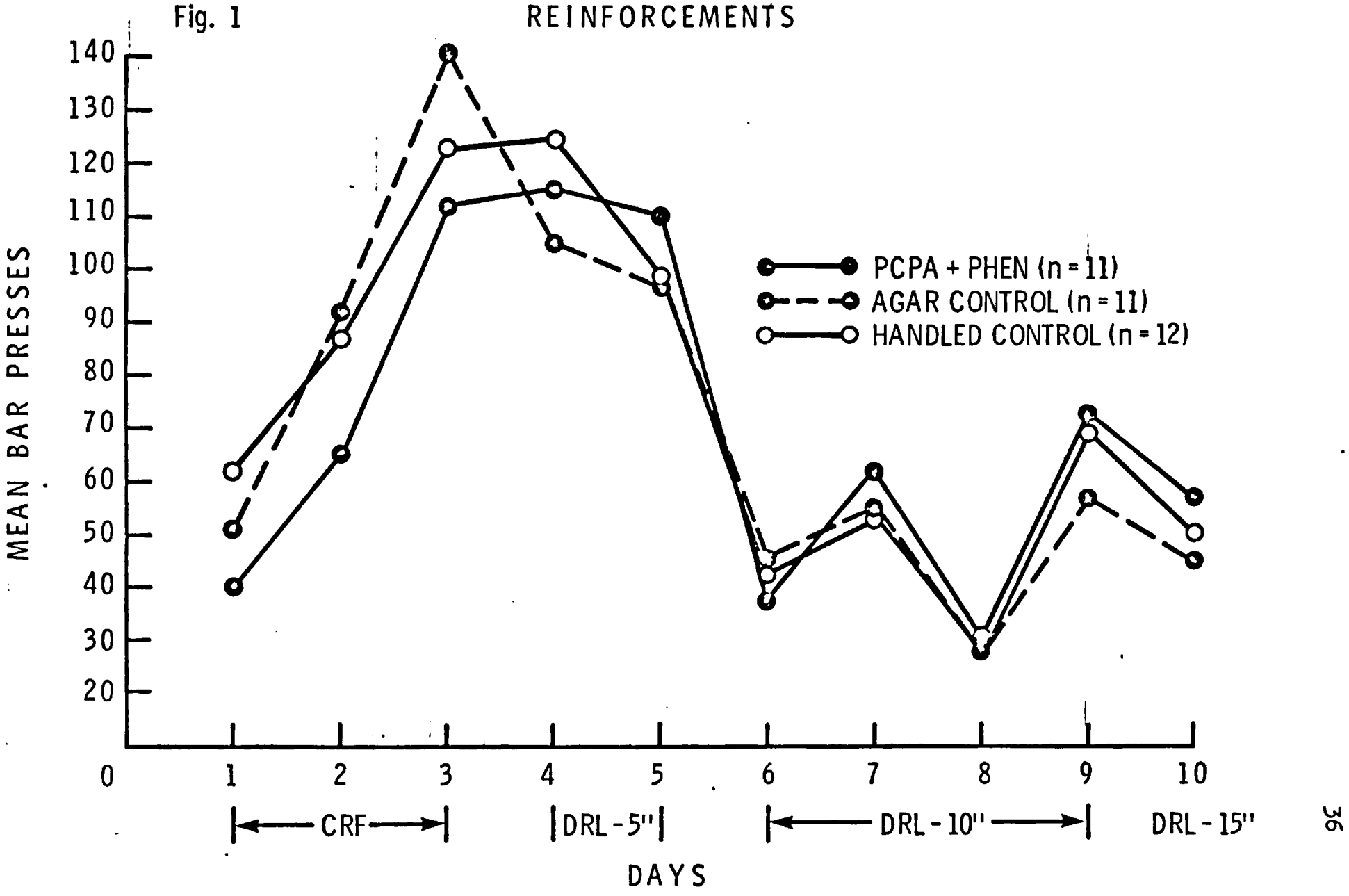
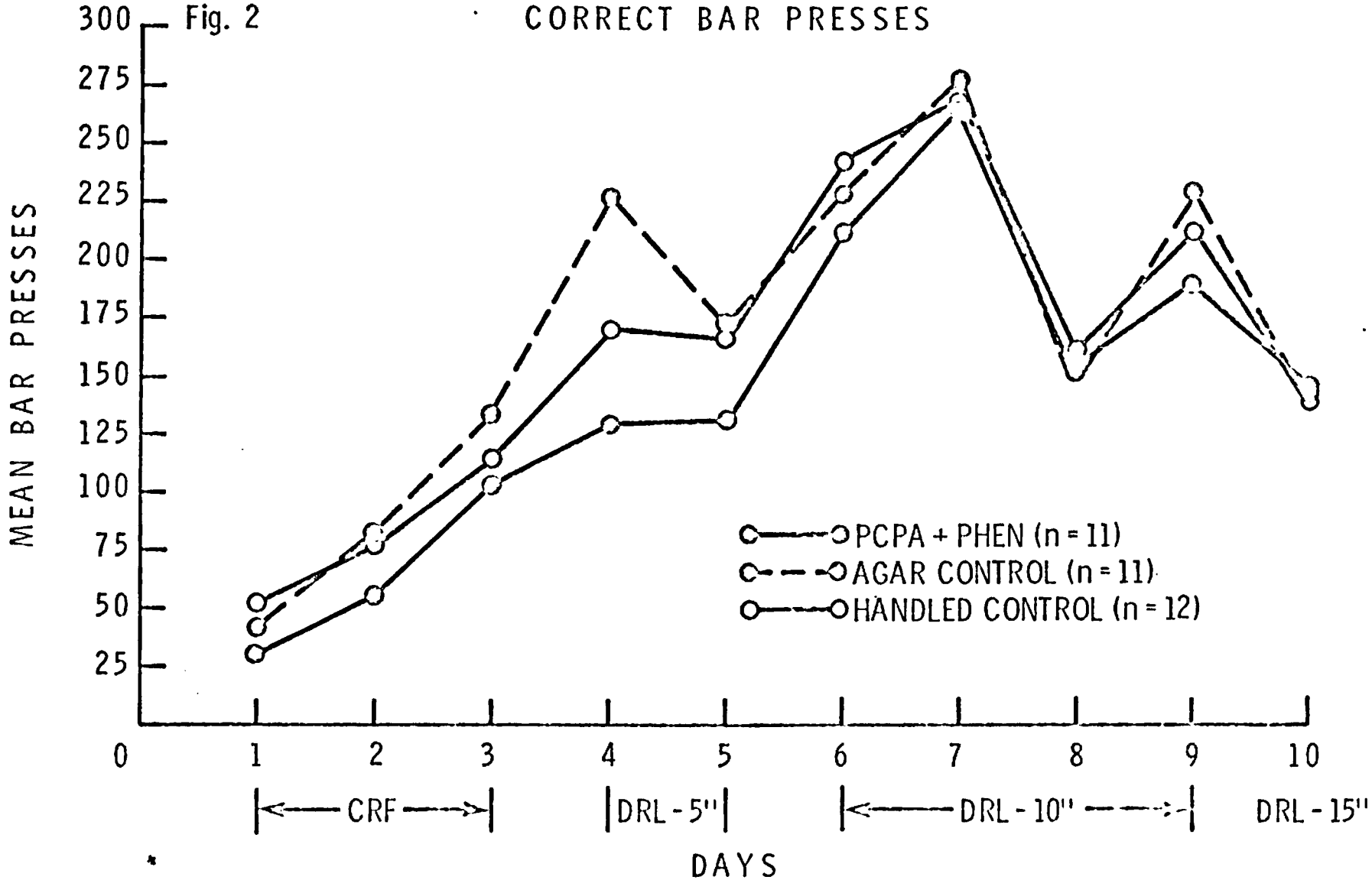

REINFORCEMENTS
MEAN BAR PRESSES
140
130
120
110
100
90
80
70
60
50
40
30
20
0
1
2
3
4
5
6
7
8
9
10
PCPA + PHEN (n = 11)
AGAR CONTROL (n = 11)
HANDLED CONTROL (n = 12)
CRF
DRL - 5"
DRL - 10"
DRL - 15"
DAYS

Fig. 2


CORRECT BAR PRESSES
MEAN BAR PRESSES
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275
250
225
200
175
150
125
100
75
50
25
0
1
2
3
4
5
6
7
8
9
10
PCPA + PHEN (n = 11)
AGAR CONTROL (n = 11)
HANDLED CONTROL (n = 12)
CRF
DRL - 5"
DRL - 10"
DRL - 15"
DAYS

Fig. 3

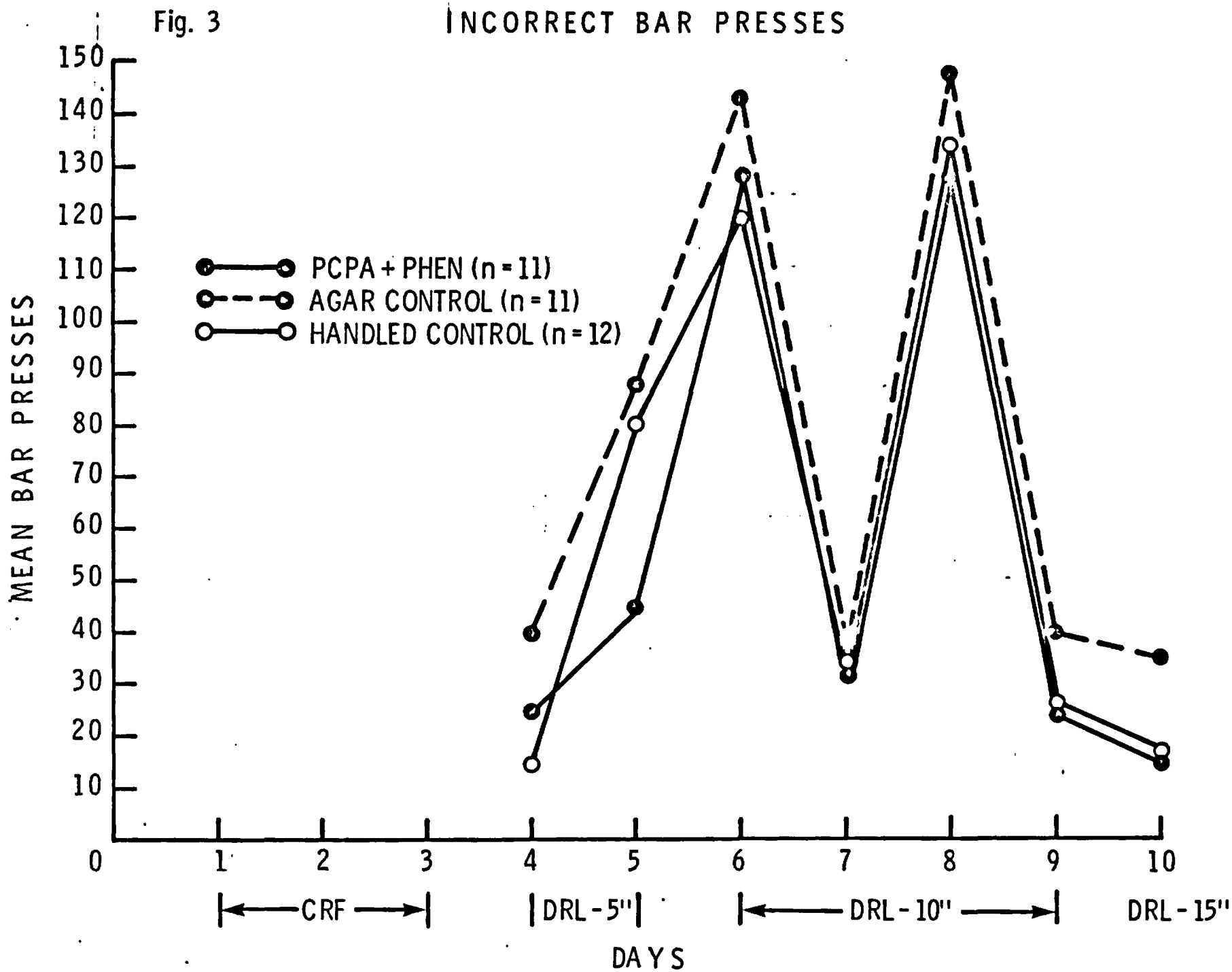

INCORRECT BAR PRESSES
MEAN BAR PRESSES
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
PCPA + PHEN (n = 11)
AGAR CONTROL (n = 11)
HANDLED CONTROL (n = 12)
1
2
3
4
5
6
7
8
9
10
CRF
DRL - 5"
DRL - 10"
DRL - 15"
DAYS

Fig. 4

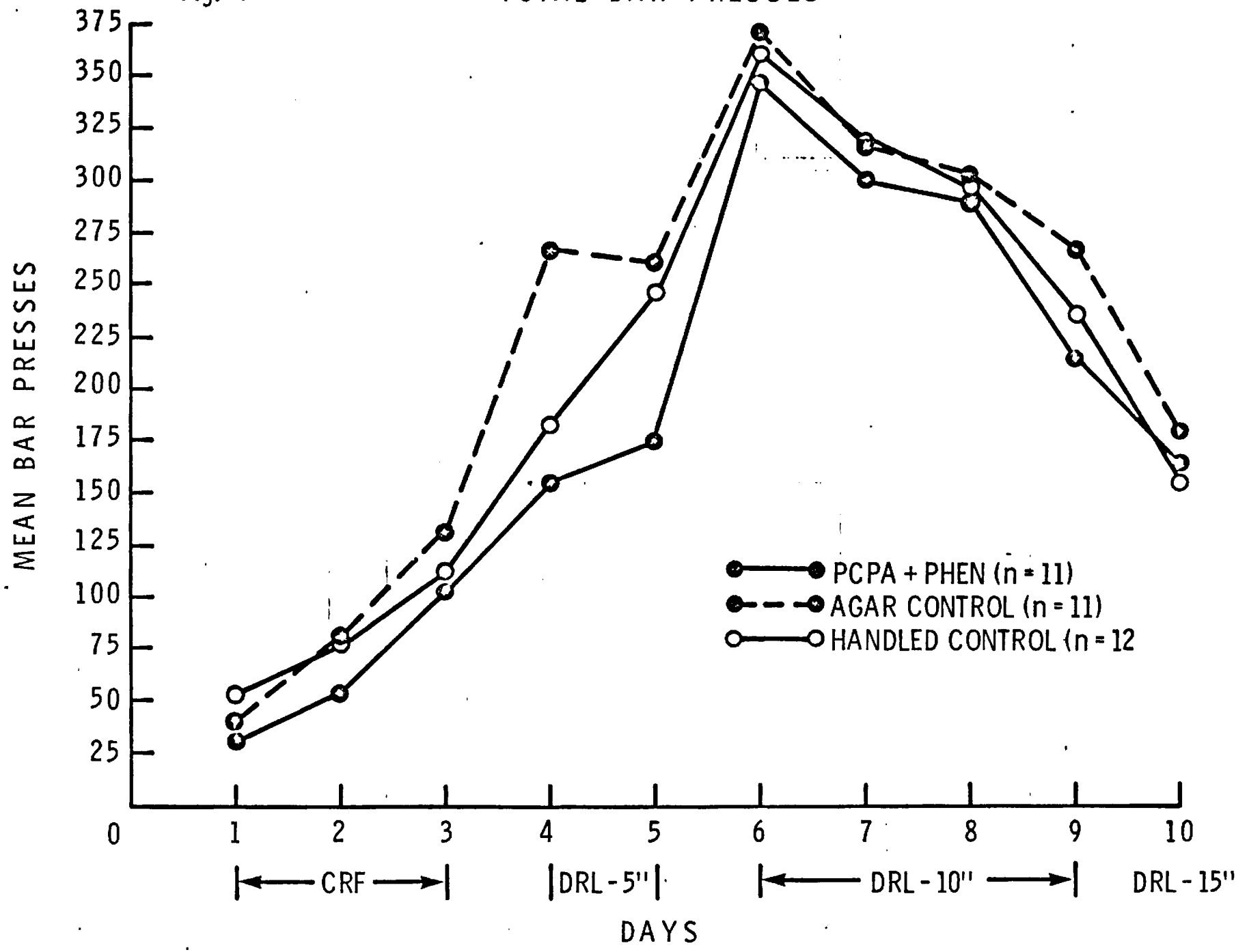

TOTAL BAR PRESSES
MEAN BAR PRESSES
375
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
1
2
3
4
5
6
7
8
9
10
PCPA + PHEN (n = 11)
AGAR CONTROL (n = 11)
HANDLED CONTROL (n = 12
CRF
DRL - 5"
DRL - 10"
DRL - 15"
DAYS

Fig. 5 EXTINCTION TRIALS

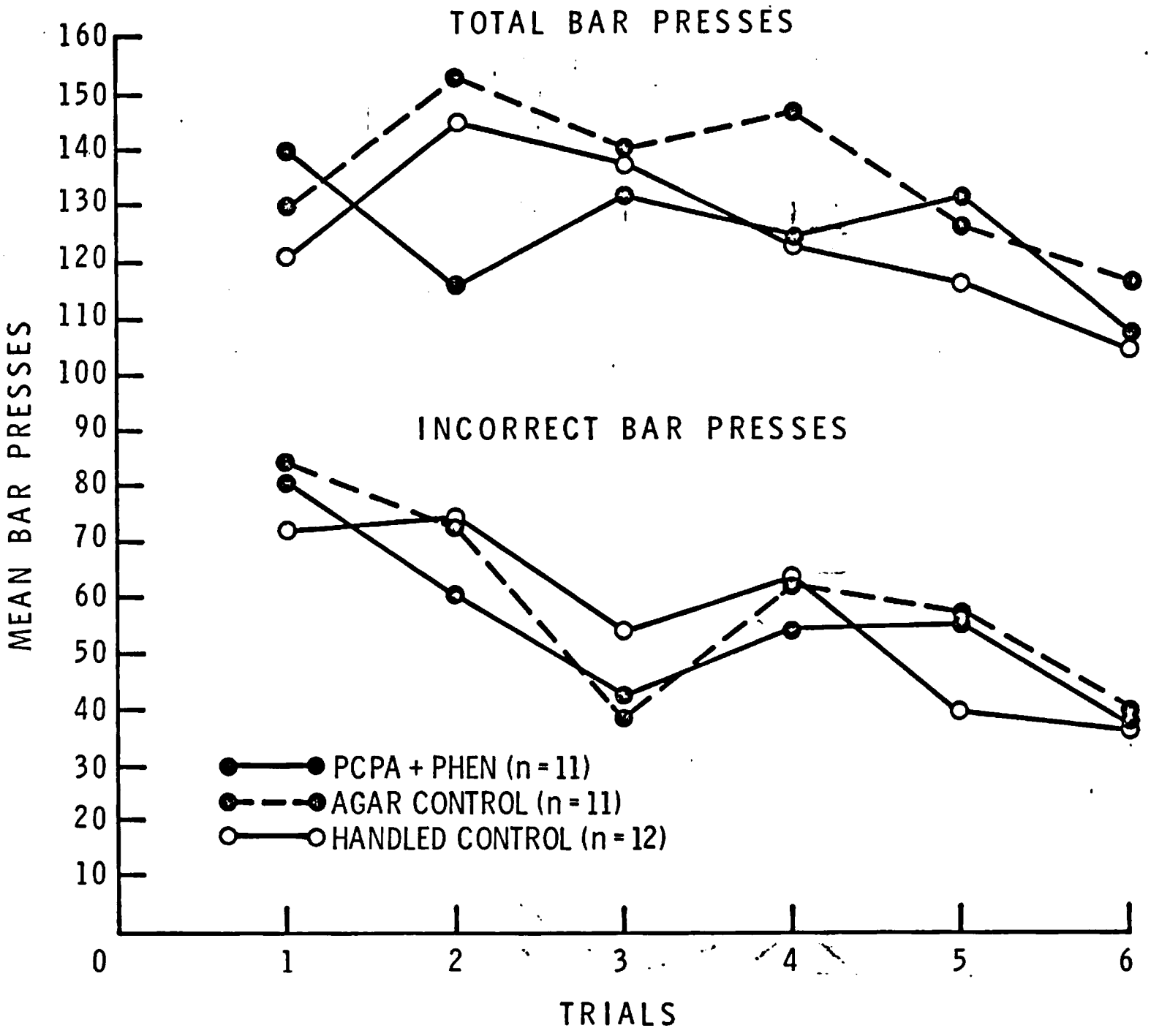
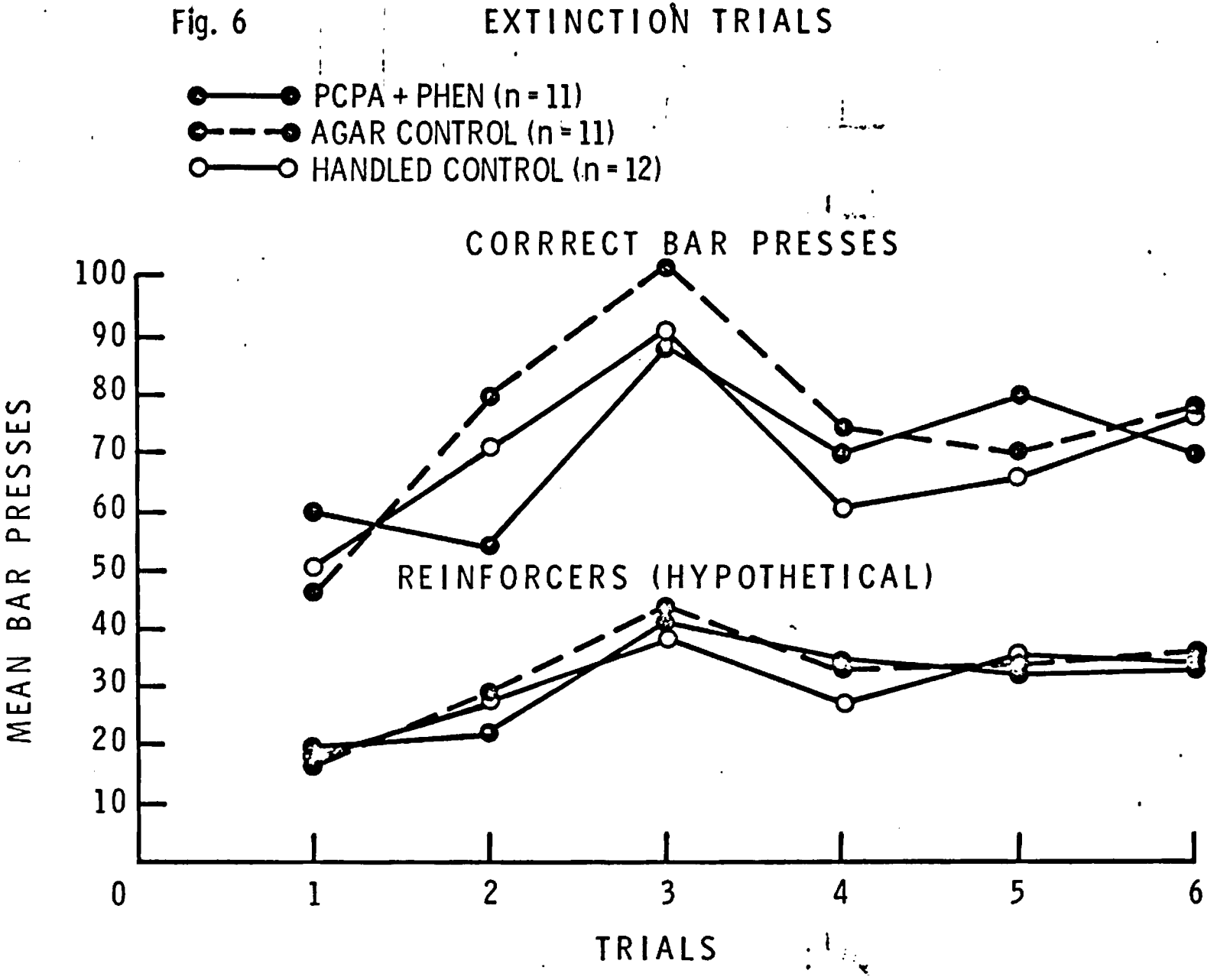


Fig. 6


EXTINCTION TRIALS
PCPA + PHEN (n = 11)
AGAR CONTROL (n = 11)
HANDLED CONTROL (n = 12)
CORRRECT BAR PRESSES
REINFORCERS (HYPOTHETICAL)
MEAN BAR PRESSES
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
TRIALS

BIBLIOGRAPHY

Adelman, L. S.; Mann, J. D.; Caley, D. W.; and Bass, N. H. Neuronal lesions in the cerebellum following the administration of excess phenylalanine to neonatal rats. Journal of Neuropathology and Experimental Neurology, 1973, 32 (3), 380-393.

Andersen, A. E. and Guroff, G. Enduring behavioral changes in rats with experimental phenylketonuria. Proceedings National Academy of Sciences, U.S.A., 1972, 69, no. 4, 863-867.

Arthur, L. J. H. and Hume, D. J. Intelligent, small for term babies born to oligophrenic phenylketonuric mother after low-phenylalanine diet during pregnancy. Pediatrics, 1970, 46, 235-239.

Berry, H. K. Phenylketonuria: diagnosis, treatment, and long-term management. Congenital Mental Retardation, University of Texas Press, Austin and London, 1969.

Bickel, H.; Gerrard, J. W.; and Hickmans, E. M. Influence of phenylalanine uptake on phenylketonuria. Lancet, 1953, 2, 812-813.

Burton, K. A study of the conditions and mechanism of the diphenylalanine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochemical Journal, 1956, 62, 315-323.

Butcher, R.; Vorhees, C.; and Berry, H. A learning impairment associated with phenylketonuria. Life Sciences, 1970, 9, 1261-1268.

Chamove, A. and Davenport, J. Differential reinforcement of latency (drl) in phenylketonuric monkeys. Developmental Psychobiology, 1970, 2 (4), 207-211.

Chamove, A. and Harlow, H. Avoidance learning in phenylketonuric monkeys. Journal of Comparative and Physiological Psychology, 1973, 84 (3), 605-612.

Chamove, A.; Waisman, H.; and Harlow, H. Abnormal social behavior in phenylketonuric monkeys. Journal of Abnormal Psychology, 1970, 76 (1), 62-68.

Cohen, B. E. A proposed methodology in the evaluation of dietary treatment in PKU. International Symposium on Phenylketonuria and Allied Disorders, ed. Bernard Cohen, Translator's Pool, Tel-Aviv, 1971, 140-165.

Eastham, R. D. and Jancar, J. Disorders of amino acids and related substances. Clinical Pathology in Mental Retardation, William and Witkins Co., Baltimore, 1968, 7-32.

Efron, M. L.; Kang, E. S.; Visakorpi, J.; Fellers, F. X. Effects of elevated plasma phenylalanine on other amino acids in phenylketonuric and normal subjects. Journal of Pediatrics, 1969, 74 (3), 399-405.

Fisch, R. O. and Anderson, J. A. Maternal phenylketonuria. In H. Bickel, F. Hudson, and L. Woolf, Phenylketonuria. Stuttgart: Georg Thieme, 1971.

Foote, J. and Tao, R. The effects of p-chlorophenylalanine and phenylalanine on brain ester-bound fatty acids of developing rats. Life Sciences, 1968, 7 (2), 1187-1192.

Frankenburg, W. K.; Goldstein, A. D.; and Olson, C. O. Behavioral consequences of increased phenylalanine intake by phenylketonuric children: a pilot study describing a methodology. American Journal of Mental Dificiency, 1973, 77 (5), 524-532.

Glowinski, J. and Iverson, L. L. Regional studies of catecholamine in the rat brain. Journal of Neurochemistry, 1966, 13, 655-669.

Grüttner, R.; Maisch, H.; and Bartelheimer, K. Evaluation of late initiation of therapy. In H. Bickel, F. Hudson, and L. Woolf, Phenylketonuria. Stuttgart: Georg Thieme, 1971.

Hawcroft, J. and Hudson, F. P. Sex ratio among phenylketonuric infants in the United Kingdom. The Lancet, 1973, 702-703.

Hole, K. Arousal defect in l-phenylalanine fed rats. Developmental Psychobiology, 1972, 5 (2), 149-156.

Hole, K. Behavior and brain growth in rats treated with p-chlorophenylalanine in the first weeks of life. Developmental Psychobiology, 1972, 5 (2), 157-173.

Hsia, D. V. Enzyme defects, Inborn Errors in Metabolism Part I, Yearbook, Medical Publishers, Inc., Chicago, 1966, 132-144.

Hsia, D. V. and Dobson, J. Altered sex ratio among phenylketonuric infants ascertained by screening the newborn. Lancet, 1970, 3, 905.

Jervis, G. A. Phenylpyruvic oligophrenia: deficiency of phenylalanine oxidizing system. Proceedings of the Society of Experimental Biological Medicine, 1953, 82, 512-515.

Karrer, R. and Cahilly, G. Experimental attempts to produce phenylketonuria in animals: a critical review. Psychological Bulletin, 1965, 64, 52-64.

Kaufman, S. The phenylalanine hydroxylating system from mammalian liver. Advances in Enzymology and Related Areas of Molecular Biology, ed. A. Meister, Interscience Publishers, New York, 1971, 245-316.

Kerr, G. R.; Chamove, A. S.; Harlow, H.; and Waisman, H. Fetal PKU: the effect of maternal hyperphenylalanemia during pregnancy in the rhesus monkey (macaca mulata). Pediatrics, 42 (1), 1968.

Kilbey, M. and Harris, R. T. Behavioral biochemical, and maturation effects of early d-para-chlorophenylalanine treatment. Psychopharmacologia, 1971, 19, 334-346.

Kilbey, M.; Harris, R. T.; and Aigner, T. G. Establishment of equivalent external- and internal-stimulus control of an operant behavior and its reversal. Proceedings of the Annual Convention of the American Psychological Association, 1971, 6 (2), 767-768.

Koch, R.; Dobson, J.; Hsia, D. Y.; and Woolf, L. I. Sex ratios among phenylketonuric infants in the United Kingdom. Journal of Pediatrics, 1971, 78, 157.

Koe, B. K. and Weissman, A. P-chloro-phenylalanine: a specific depletor of brain serotonin. Journal of Pharmacology and Experimental Therapies, 1966, 154, 499-516.

La Du, B. N. and Zannoni, V. G. Basic biochemistry in aromatic amino acid metabolism in phenylketonuria. In H. Bickel, F. Hudson, and L. Woolf, Phenylketonuria. Stuttgart: Goerg Thieme, 1971.

Linnewah, F.; Ehrlich, M. Zur Pathogenese des Schwachsinns bei Phenylalaninarmer. Klinische Wechselnschrammen, 1962, 40, 225-226.

Lipton, M. A.; Gordon, R.; Guroff, G.; and Udenfriend, S. P-chlorophenylalanine-induced chemical manifestations of phenylketonuria in rats. Science, 1967, 156, 248-250.

Longenecker, J. B.; Reed, P. B.; Lo, G. S.; Chang, D. Y; Nasby, M.W.; White, M. N.; and Ide, S. Temporary induction of phenylketonuria-like characteristics in infant rats. Nutrition Reports International, 1970, 1 (2), 105-112.

Lutz, P. Biochemical monitoring of dietary treatment. In H. Bickel, F. Hudson, and L. Woolf, Phenylketonuria. Stuttgart: Georg Thieme, 1971.

Lyman, T. Phenylketonuria. Springfield: Thomas, 1963.

Mabry, C. C.; Denniston, J. C.; Nelson, T. L. Maternal phenylketonuria: a cause of mental retardation on other amino acids in phenylketonuric and normal subjects. Journal of Pediatrics, 1969, 74, 399-405.

Margolis, F. L. DNA and DNA-polymerase activity in chicken brain regions during ontogeny. Journal of Neurochemistry, 1969, 16, 447-456.

Martin, J. J. and Schlote, W. Central nervous system lesions in disorders of amino-acid metabolism. Journal of the Neurological Sciences, 1972, 15, 49-76.

McGuigan, F. J. Experimental Psychology. Englewood Cliffs, New Jersey: Prentice-Hall, Inc., 1968.

McKean, C. M.; Boggs, D. E.; and Peterson, N. A. The influence of high phenylalanine and tyrosine on the concentrations of essential amino acids in the brain. Journal of Neurochemistry, 1968, 15, 235-241.

McKean, C. M. The effects of high phenylalanine concentrations on serotonin and catecholamine metabolism in the human brain. Brain Research, 1972, 47, 469-476.

Nadler, H.; Hsia, D. Y. Y. Epinephrine metabolism in phenylketonuria. Proceeding of the Society for Experimental Biological Medicine, 1961, 107, 721-723.

Nordyke, E. Para-chlorphenylalanine treated rats: a model system of phenylketonuria. Unpublished doctoral dissertation, University of Houston, 1972.

Nordyke, E. and Roach, M. K. Effect of hyperphenylalanemia on amino acid metabolism and compartmentation in neonate rat brains. Brain Research, 1974, 67, 479-488.

Nordyke, E. and Roach, M. K. Effect of hyperphenylalanemia on succinate dehydrogenase and glutamate dehydrogenase activities in brain regions of developing brains. Research Communications in Chemical Pathology and Pharmacology, 1974, 8 (2), 397-400.

Nordyke, E. and Roach, M. K. Effect of p-chlorophenylalanine and l-phenylalanine on deoxyribonucleic acid and protein content of developing rat cerebellum. Biochemical Pharmacology, 1974, 3, 2920-2922.

Pellizari, E. D.; Brown, J. D.; Talbot, P.; Farmer, R. W.; and Fabre, L. F. An evaluation of the gas chromatographic analysis of plasma amino acid. Journal of Chromatography, 1971, 55, 281-289.

Pellizari, E. D.; Rising, C.; Brown, J. D.; Farmer, R. W.; and Fabre, L. F. An improved plasma amino acid purification procedure for gas-liquid chromatography. Analytical Biochemistry, 1971, 44, 312-316.

Perez, V. J. Phenylketonuria or phenylpyruvic oligophrenia in the rat: behavioral and biochemical correlates. Journal of Mental Dificiency Research, 1965, 9, 170-181.

Perry, T. L.; Hansen, S.; Tischler, B.; Bunting, R.; and Diamond, S. Glutamine depletion in phenylketonuria. The New England Journal of Medicine, 1970, 282 (14), 761-766.

Perry, T. L.; Hansen, S.; Tischler, B.; Richards, F. M.; and Sokol, M. Unrecognized adult phenylketonuria. The New England Journal of Medicine, 1973, 289 (8), 395-398.

Räihä, N. C. R. and Schwartz, A. L. Development of urea biosynthesis and factors influencing the activity of the arginine synthetase system in perinatal mammalian liver. In F. A. Hommes and C. J. Van Den Berg, Inborn Errors of Metabolism. London: Academic Press, 1973.

Rouse, B. M. Phenylalanine deficiency syndrome. Journal of Pediatrics, 1966, 69, 246-249.

Saunders, C. Phenylketonuria 1969. Postgraduate Medicine, 1969, 159-162.

Schaefer, G. J.; Buchanan, D. C.; and Ray, O. S. The effects of early p-chlorophenylalanine administration and postweaning housing conditions on serotonin and behavior in rats. Life Sciences, 1973, 12 (1), 401-411.

Schalock, R. L. Induced phenylketonuria (PKU): lack of a critical period in development. Psychonomic Sciences, 1969, 17 (3), 191-192.

Schalock, R. L. and Copenhaver, J. H. Behavioral effects of experimental maternal hyperphenylalanemia. Developmental Psychobiology, 1973, 6 (6), 511-520.

Sutherland, B. S.; Umbarger, B.; and Berry, H. K. The treatment of phenylketonuria: a decade of results. American Journal of Disabled Children, 1966, 111, 505-523.

Thalhammer, O. and Scheiber, V. Genetic difference between hyperphenylalanemia and phenylketonuria. Neurcpädiatrie, 1972, 3, 358-361.

Vorhees, C. V.; Butcher, R.E.; and Berry, H. K. Reduced activity in rats with induced phenylketonuria. Developmental Psychobiology, 1972, 5 (2), 175-179.

Watt, D. D. and Martin, P. R. Phenylalanine anti-metabolite effects on development - I. Behavioral effects of dl-4-chlorophenylalanine in the young rat. Life Sciences, 1969, 8, 1211-1222.

Woolf, L. I. and Vulliamy, D. G. Phenylketonuria with a study of the effect upon it of glutamic acid. Archives of the Disabilities of Childhood, 1951, 26, 487.