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TWO SELECTED NUTRITIONAL DEFICIENCIES AND ASSOCIATED BEHAVIOURS IN RATS

by

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ABSTRACT

The effects of three diets, iron-copper deficient, protein-calorie inadequate, and adequate, upon the behaviours and activity levels of 33 female Wistar rats, was studied by means of observation during a 24-day period of dietary manipulation and subsequent 24-day period of dietary recovery. Behaviour was recorded at eight points in time during each dietary period in a familiar environment (home cage) and at two points in time at the end of each dietary period in a novel situation, with lists of 28 or 20 respectively, mutually exclusive and exhaustive behaviours adapted from a study in the literature. For analysis the behaviours were grouped into four categories (vertical, horizontal, grooming, and maintenance). The changes from behaviour to behaviour, the number of rotations made in an activity wheel, latency and the number of squares crossed in an open field provided the measures of activity level. Each activity measure and each behaviour category was analyzed separately by split-plot two-way analysis of variance. Results showed the animals of both deficient groups engaged in more vertical behaviour and more behaviour changes in the home cage than rats fed an adequate diet, which may have given the impression of 'restlessness' in the malnourished animal. However no behaviours which

could have suggested fatigue of the iron-copper deficient animals was indicated. In fact they remained more active than the control rats until placed in the open field. In this novel situation the protein-calorie malnourished rats engaged in more vertical behaviour than rats of the other two groups, the iron-copper deficient in more horizontal behaviour and the adequately nourished in more grooming behaviour. During nutritional recovery the group differences were evident on only four days in the home cage in the horizontal, grooming, and maintenance categories, and may be indicative of the recovery process. Similarly, group differences in the open field occurred only in the rate of square crossing which also may be an indication of recovery. The unexpected behaviour of the anemic animals, that is, increased activity during the initial period of nutritional deprivation, may partly explain the difficulty researchers have had in finding consistent behavioural correlates of anemia. The results demonstrate that the observation technique and the behaviour categories used are sensitive to behavioural changes. If a theoretical framework such as Selye's stress model is used as a basis in future research, more of the detailed results may be better explained.

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CHAPTER I

Hereditary and environmental factors interact in a complex interdependent manner. They function simultaneously and continuously to determine the anatomical, chemical, and physiological characteristics of the body and the consequent development of physical, intellectual, social, and emotional behaviours of the organism. In the past the social and cultural facets of the environment received most attention as being the determiners of behaviour. However, today the focus has broadened to include consideration of the effects of nutritional and chemical components of the environment upon behaviour.

The importance of food to the physical and psychological well-being of the organism has been investigated under two broad classifications: severe protein-calorie malnutrition and specific nutrient deficiencies. Research of the physical concomitants of both the general protein-calorie and specific deficiencies suggests deleterious effects for the developing organism (Winick, 1970; Underwood, 1971). However, research of the psychological effects has produced few conclusive results. Intellectual deficits (Winick, 1970) and abnormal social and emotional development (Frankova, 1973; Frankova and Barnes, 1968; Levitsky and Barnes, 1972) appear to be related to severe protein-calorie malnutrition.

Studies of specific nutrient deficiencies, which today include trace elements such as iron, copper, mercury, and lead, also suggest behavioural correlates, but the behaviours can only be specified when the deficiency is severe.

In respect to investigations of both protein-calorie and specific nutrient malnutrition, whether animal or human, the behavioural correlates reported have generally been in terms of behavioural constructs such as intelligence or emotional reactivity, or in vaguely descriptive terms such as hyperactivity or lassitude. The use of such terms and constructs has been a limiting factor in the attempt to study the behavioural correlates of malnutrition. Such terms and constructs, though intuitively inferred from sets of concrete and discrete behaviour units, have generally not been derived from analysis at this finer level. Perhaps the subtle behaviour changes associated with malnutrition could be more effectively studied in terms of discrete behaviours. It was the purpose of this study to explore in detail the behaviours shown by rats during a progressing protein-calorie malnutrition, a developing iron-copper deficiency, and later through a period of nutritional recovery.

Following is a review of the physiological and psychological effects of protein-calorie malnutrition in animals, a discussion of the effects of iron-copper deficiency, and the statement of the problem.

Protein-Calorie Malnutrition Research

Physiological Effects in Animals

Undernutrition at any stage in life has physiological consequences. However, the earlier the undernutrition, the more far-reaching the observed effects. In the early stages of life, when growth is at a peak, inadequate protein intake affects tissue, cellular growth patterns, myelination, brain chemical composition, and some metabolic pathways in animals.

Malnutrition from birth to weaning results in a permanent reduction in brain weight in both rats and pigs (Dobbing, 1964; Dobbing & Widdowson, 1965; Widdowson, 1966; Widdowson & McCance, 1960; Widdowson, Dickerson & McCance, 1960). Both neurons and glia in the spinal cord and medulla permanently degenerate in rats, pigs, and dogs raised on protein deficient diets (Platt, 1962; Platt, Heard & Stewart, 1964). Certain enzymes in rat brains show delayed appearance and reduction in ultimate quantity with early malnutrition (Zeman & Stanbrough, 1969).

Winick (1970b) reported that the phase of cellular growth in which a system is engaged at the time of the nutritional insult has different effects upon cellular development. If the nutritional insult occurs during a time of rapid cell division (hyperplasia) and continues during the period of limited cell division and cell growth (hyperplasia and hypertrophy), the number and size of the organ cells is

affected permanently, regardless of adequate feeding at a later date. Winick (1970a) found that by the sixteenth day of the fetal life of the malnourished rat, reduced cell division in all areas and in all cell types was evident. Those animals deprived during pre- and post-natal development were severely retarded in growth and their brains contained only 40% of the expected number of cells.

A study of the myelination process in the rat (Bensted, Dobbing, Morgan, Reid, & Payling, 1957) indicated that most myelination occurred from 10 to 21 days after birth. Malnutrition during this period permanently reduced the amount of myelin in the brain and the rate at which it was laid down.

Currently other processes which may be disturbed by early malnutrition are being investigated. Some temporary changes have been reported in brain serotonin and norepinephrine levels of the newborn after only eight days of poor nutrition (Sereni, Principi, Perletti, & Sereni, 1966), but normal levels were later established even if the malnutrition persisted. The ratio of ribonucleic acid to deoxyribonucleic acid (RNA/DNA) in the brain was found to increase with malnutrition beginning at birth, though with persisting nutritional deficiency, the levels returned to normal (Winick, Fish & Rosso, 1968). Generally, the physiological studies of malnourished animals indicate that the earlier the deprivation the more far-reaching and permanent the effects.

Physiological changes have also been associated with an enriched environment. Minimal handling during infancy permanently alters endocrine mechanisms (Denenberg, 1964; Levine, 1957, 1962; Levine & Lewis, 1959). Rosenzweig (1971a) reported greater cerebral cortex weight and depth, and greater total activities of acetylcholinesterase, cholinesterase and hexokinase in rats reared in an enriched environment. The responses of protein-calorie malnourished rats raised in an enriched environment are found to be no different than responses of adequately nourished animals but to be different from protein-calorie malnourished animals raised in an impoverished environment (Zimmerman & Zimmerman, 1972). An enriched environment could therefore mask the effects of reduction in cell number. This issue has been extensively discussed by Denenberg and Zarrow (1971), Rosenzweig (1971b), and Zimmerman and Zimmerman (1972).

Psychological Effects of Protein-Calorie Malnutrition in Animals

Postweaning. Many of the crucial physiological developments in the rat are completed by 21 days of age. After this age, the effects on the central nervous system diminish and are more temporary (Winick, 1970). Studies of malnutrition beginning after 21 days of age also suggest a variety of effects upon learning. Anderson and Smith (1932), who underfed male albino rats, found the underfed rats superior to adequately fed rats in maze learning with food reinforcement. In contrast, Ruch (1932) found that rats with a diet limited to maintain body weight learned

to escape from a water maze more efficiently than those fed ad libitum. Bernhardt (1936a),using a water maze with an escape incentive, found protein deficient rats did not perform as well as adequately nourished animals. Later, Bevan and Freeman (1952) reported that though an amino acid deficiency did not affect the learning of a tunnel maze with either food or water reinforcement during the relearning period, the deficient rats engaged in more retracing of the maze which they described as "nervous" behaviour.

Other investigations indicate that learning efficiency may be associated with type of reinforcement or with strain differences. Griffiths and Senter (1954), reared rats on either a balanced or a protein-free diet, and used either a balanced or protein-free diet as reinforcement in a multiple Y-maze. They found that those raised on the protein-free diet erred least when reinforced with a balanced diet but also erred less than the control group even when the protein deficient diet was used as reinforcement. Hughes and Zubek (1965), using hooded rats selectively bred as "bright" and "dull" maze learners, tested them on a Hebb-Williams maze with a food reinforcement. They reported that supplements of glutamic acid to the regular diet reduced the errors and the time scores of the dull strain but had no effect on the bright strain.

Differences in behavioural patterns have also been reported. Bolles (1963) found that during deprivation, patterns of behaviour in the home cage were redistributed. Animals on

the deprived diet spent less time sleeping and more time in alert rest. Levitsky and Barnes (1972) manipulated protein levels from 21 days of age to seven weeks and tested the animals on the open field after 17 weeks of recovery. The malnourished animals showed more locomotor activity, less exploratory behaviour, and more fighting behaviour than the well-nourished animals.

Of the eight studies in which the diet was manipulated after weaning, five showed effects on learning. Of these, three supported the hypothesis of reduced learning capacity with malnutrition (Bevan & Freeman, 1952; Bernhardt, 1936; Ruch, 1932) and two showed learning efficiency to be affected by the type of reinforcement (Griffiths & Senter, 1954) and genetic potential (Hughes and Zubek, 1956). Observation of behavioural patterns also indicated a variety of changes associated with malnutrition induced after 21 days of age (Bolles, 1963; Levitsky & Barnes, 1972).

Preweaning. Inspection of the literature indicates that induction of nutritional deprivation before weaning (generally prior to 21 days of age) involves one or a combination of the following methods: (a) deprivation of the pregnant dam, (b) deprivation of the lactating dam, (c) manipulation of litter size during lactation, or (d) deprivation of previous generations. In this section the research reviewed is restricted to studies in which behavioural consequents in the offspring of the deprived pregnant and lactating dam was investigated.

Frankova and Barnes (1968) studied the effects of a low protein-calorie diet during the lactation of Holtzman dams on the quality and intensity of the progeny's exploratory behaviour. During the deprivation period, systematic observations indicated that the deprived animals exhibited a higher level of exploratory behaviour than the controls. However, after diet repletion they observed a decrease in rearing behaviour and locomotor activity. Frankova (1973) also studied the effects of low calorie and protein intake during lactation and post-weaning on activity and social interaction. The deprived rats developed social behaviours at a slower rate than the well-nourished. Prior to weaning they took a longer time to approach a second animal, they engaged in less social grooming and in many more attacks on other animals. While in the rearing cage the deprived rats spent less time in play with the mother and in active aggregation, more time in passive protection, and less time playing with littermates. After weaning the protein calorie deprived rats showed a higher level of exploratory activity except in the presence of a second animal in which case its activity was reduced.

In the following studies nutritional deprivation was induced during gestation, gestation and lactation, or lactation only. Zimmerman and Zimmerman (1972) studied the effects of a low protein diet from birth to seven weeks of age on responses to novel stimuli and behaviour in the open field. They found increased activity with novel stimuli among rats fed a high

protein diet whereas rats fed low protein diets showed decreased activity when novel stimuli were present. Simonson and Chow (1970) deprived the dams of 50% of the usual ad libitum intake during gestation and lactation. The $2\frac{1}{2}$- to 14-month-old male progeny were tested for learning on an elevated T-maze. The progeny of the restricted mothers had longer starting and running times than the controls and erred more frequently on the last 12 sessions of the first testing period. In a second testing period, two months later, the deprived group still showed longer starting and running times, as well as longer times to reach extinction criterion and significantly more defecation than the control group. These results indicate long term behavioural effects of nutritional restriction during gestation and lactation. Simonson, Stephan, Hanson and Chow (1971) also found that rats from dams malnourished during pregnancy showed more variable and longer reaction times, a higher activity level indicated by more squares crossed, to spend less time in the center squares and to defecate more in an open field apparatus than rats from mothers well nourished during pregnancy.

Ottinger and Tanabe (1968), who restricted the diets of Purdue Wistar rats during the preweaning periods, reported that the adult progeny of dams restricted during lactation showed long-term body weight deficits. Although no differences were found on either the level of activity or the amount of defecation in the open field, the progeny of restricted

lactation dams erred more frequently on the Hebb-Williams maze. Vore and Ottinger (1970), who restricted diets of the dams to 50% ad libitum from 20 days prior to mating through gestation and lactation, have reported reduced learning ability in the progeny. Wells and Ottinger (1970), who deprived the dams prior to conception, during gestation, lactation, or gestation and lactation, tested learning performance of the adult progeny on a shock escape T-maze. Again the adult progeny showed learning deficits, with the most severe consequences among those deprived during the dam's lactation. Hsueh, Simonson, Chow, and Hanson (1974) manipulated diets in the same manner as Ottinger and Tanabe (1968). They found that dams restricted during gestation alone produced progeny with a low birth weight, slight but permanent stunting of body growth, and impaired learning in an elevated T-maze whereas dams restricted during lactation only produced severely and permanently stunted offspring with no impairment of learning ability. Restriction during both gestation and lactation effected similar but more severe consequences in the progeny.

Cowley and Griesel (1959, 1963) studied the effects of a low protein diet on exploratory behaviour, intelligence, and growth patterns of the first-filial and second generation Wistar rats. The first-filial generation showed no difference in exploratory behaviour but made more errors on the Hebb-Williams maze and took longer to reach the goal box. The

second generation continued to make more errors on the Hebb-Williams maze. Both first and second generation rats were retarded in growth.

In summary, dietary deficits produced in the dam result in retarded growth of the offspring. Exploratory behaviour and intelligence are generally reported to be reduced and social behaviour to develop more slowly and in a different direction than normal.

Human studies of protein-calorie deprivation generally demonstrate retardation in physical and mental development, but can only implicate nutrition as the cause. Table 1 presents Winick's (1970) summary of the functional changes in the developing brains of malnourished animals and humans.

Iron Deficiency Research

Iron deficiency literature has dealt primarily with the metabolism (McCall, Newman, O'Brien, Volberg & Witts, 1962b; Underwood, 1971), the physiological changes accompanying iron deficiency (Anderson & Barkve, 1970; Ericsson, 1970 a & b; Giorgio, 1970; McCall et al, 1962 b; The Ross Conference 1972; Underwood, 1971; Vellar, & Hermansen, 1971), and the assessment of iron status in the population (Blix, 1968).

Underwood (1971) states that the most frequently occurring deficiency disease clinically manifest in humans is iron deficiency. Nutrition surveys support his statement

TABLE 1

Functional Changes in the Developing Brains
of Malnourished Subjects

Functional Changes	Animals	Humans
Neurologic symptoms	Transient: tremors, hobble skirt gait, convulsions	Transient: apathy, lethargy, or hyperirritability
EEG changes	Increased slow wave activity (transient)	
Behavioural	Decreased "exploratory" behaviour Poor ability to extinguish a response	Exaggerated response to certain stimuli Poor ability to extinguish a response
Intellectual	Difficulty in maze learning	Decrease in cognitive and perceptual development*

*Malnutrition has not been entirely isolated as the sole cause of these changes.

Note: From Winick, M., Nutrition and Mental Development. Medical Clinics of North America, 1970, 54(6), p. 1413.

(Blix, 1968; Nutrition Canada Survey 1975; Underwood, 1971). Despite the incidence of iron deficiency few investigations have focused on the effects it has upon the "health" and well-being of the individual. "Despite clinical belief that anemia causes symptoms, there seems to be little valid evidence of an increase in symptoms with low levels of circulating hemoglobin, or of a beneficial effect of iron therapy upon well-being" (Elwood, 1973, p. 958).

Generally the symptoms of iron deficiency among human adults are said to include listlessness, fatigue, headache, dizziness, breathlessness, palpitation on exertion, sore tongue, angular stomatitis, dysphagia, and koilonychia (Elwood, Waters, Greene & Sweetnam, 1969; Giorgio, 1970; Underwood, 1971). Research reported by Wood and Elwood (1966), Elwood and Wood (1966), Elwood, Waters, Greene, Sweetnam, and Wood (1969), and Elwood and Hughes (1970) indicated no consistent relationship between hemoglobin levels and behavioural symptoms. However Elwood (1973) did find high levels of circulating Hb to be positively correlated with high mortality rates. Beutler, Larsh and Gurney (1960) reported iron therapy to have fatigue alleviating effects in non-anemic women but to have no consistent effects amongst an anemic group. Anderson and Barkve (1970) observed that the cardio-respiratory system of anemic subjects took longer to return to a resting level of function after exercise than did the cardio-respiratory system of normal subjects. Similarly, Ericsson (1970) found physical work capacity in males increased with an increase in Hb levels.

Symptoms of iron deficiency in children include anorexia, depressed growth, and reduced resistance to infection (Underwood, 1971). While the investigations of iron deficiency effects upon children and adolescents do indicate subtle changes in attitude (Diamond, 1970), energy level (Diamond, 1970), and possibly intelligence (Sulzer, Hansche and Koenig, 1973), the relationships are not well established.

In rats, iron deficiency anemia is characterized by specific physiological symptoms such as slow weight gain, rough coats, white incisor teeth, paleness of normally pink ears, paws and eyes, enlargement of the heart, spleen and caecum, and a characteristic sequence of changes in the blood chemistry (McCall, et.al. 1962 b). However, the behavioural effects have seldom been described in terms more explicit than lethargy, lassitude, fatigue, and low level of energy output.

Bernhardt (1936a) reported not only that iron deficient rats failed to gain weight and showed lower resistance to infection but that their learning of a water maze tended to require more time and trials and that they covered more distance in doing so. Edgerton, Bryant, Gillespie, and Gardner (1973) induced anemia in a group of male Sprague-Dawley rats by diet and by injection of a hemolytic agent. They found a positive relationship between Hb level and endurance on an exhaustive sprint run test. Anemic rats ran for a shorter time during their deprivation than the controls. However, after iron repletion the performance capacity of the two groups was

virtually the same. They also found voluntary activity of the anemic animals, as measured by amount of use of an activity wheel adjoining the home cage, to be at a lower level than that of the control group. Again the difference disappeared with nutritional rehabilitation.

Elwood (1973) suggested that although human and animal studies of this last type are important theoretically, they have little practical significance. Seldom is the average person or animal required to work to capacity, or until exhaustion. It is perhaps study of the more subtle behavioural effects and more moderate levels of deficiency that may have more practical application.

Statement of the Problem

Though there appear to be relationships between malnutrition and physical and psychological performance, difficulties are encountered in specifying the behavioural effects. From the review of literature it becomes apparent that behavioural correlates of malnutrition are frequently reported in gross descriptive terms such as fatigue, lassitude, exploratory behaviour, and intelligence. Generally the descriptive terms and constructs have been intuitively inferred from sets of specific behaviours without a systematically obtained empirical base. A consequent of this is inconsistency in the referents labelled by the same term or construct. For example, exploratory behaviour, a frequently used psychological construct, has been defined as (a) the time it takes a rat to

enter the room off an open field apparatus (Levitsky & Barnes, 1972), (b) the number of squares entered in the open field (Denenberg, 1969), and (c) vertical behaviour exhibited by the animal in conjunction with the number of squares crossed in a box situation (Frankova & Barnes, 1968). On the other hand, some researchers use number of squares crossed as an indicator of activity (Bolles, 1963; Cowley & Griesel, 1959, 1960; Hsueh et.al.1974) and others as an indicator of the animal's nonspecific excitability level (Kendrick, 1972).

From her systematic observation of exploratory behaviour of rats under different nutritional or stimulation conditions, Frankova (1973) concluded

> it was necessary to evaluate horizontal as well as vertical components of exploratory behaviour. In previously malnourished rats the horizontal component, namely, the number of squares traversed or units entered, was not influenced as much as number (or duration) of standing-up reactions. Intensity of standing up reactions appears to be a better measure of exploratory drive (p. 483).

Shettleworth (1975) also emphasizes the importance of the vertical component of behaviour when studying exploratory behaviour in hungry animals. However, definitions of exploratory behaviours generally include only the horizontal aspect. Winick's (1970) statement that "The weakest link in the chain of animal experiments is the lack of standardization of the behavioural tests employed (p. 1426)" also expresses this basic problem of nutrition/behaviour research.

The challenge of research is to produce findings which can be replicated. Systematic observational

analysis such as that used by Bolles (1963), Frankova and Barnes (1968), and Shettleworth (1975) may provide a means of doing so. Behaviour units operationally defined in terms of identifiable physical acts of the organism comprise the basis of this type of analysis. The final score attained (number of panel rears, number of face washes, etc.) is then not confounded by an operationally defined construct. Rather it provides data in terms of behaviour units that can be analyzed for variety, frequency, and duration of each behaviour or for all behaviours in relation to each other.

In this research the association of five behaviours with three nutritional states of rats were examined in familiar and unfamiliar environments. The behaviours included vertical, horizontal, grooming, maintenance, and activity categories. Specifically the exploratory questions posed were:

a) At different points during the induction of a protein-calorie malnutrition or an iron-copper deficiency are the five categories of behaviours exhibited by the malnourished in a familiar environment the same as those exhibited by an adequately nourished group of rats?

b) After 24 days of nutritional deprivation do the three groups show differences in the five behaviours when placed in an unfamiliar situation, the open field?

c) At different points during subsequent nutritional rehabilitation are the five categories of behaviour exhibited by the previously malnourished in a familiar environment the same as

those exhibited by the previously adequately nourished?

d) After 24 days of nutritional rehabilitation do the three groups show differences in the five behaviours when placed in the open field?

CHAPTER II

METHOD

Subjects

Thirty-six female, Wistar rats, obtained from the Woodlyn Laboratories at 21 days of age, were grouped on the basis of body weight into three categories: light (42.5-47.1 gm), medium (47.8-51.2 gm), and heavy (51.3-55.1 gm). The rats in each weight group were then randomly assigned to one of three nutritional treatments; iron-copper deficient (ICD), protein-calorie malnourished (PCM), or adequately nourished (ADN). The original sample consisted of 36 rats with 12 in each group. However, with the death of one PCM rat the data of one ICD and one ADN rat of matching initial weights were also excluded from the sample in order to maintain equal sample size. Therefore, the data-producing sample consisted of 33 rats with 11 in each treatment group.

Apparatus

Familiar Environment. The home cages in which the animals were housed for the duration of the experiment constituted the familiar environment. The cages were 23x17.5x12.5 cm stainless steel hardware cloth front and back panels. Sterilized aspen shavings were used for bedding. Food was provided in removable ceramic dishes, and water in bottles attached to the front hardware cloth panel.

The 12-hr. day-night cycle began at 3:00 a.m. with illumination provided by two fluorescent tubes. An air-

conditioning system and two oscillating fans provided the ventilation as well as a continuous masking noise. The ambient temperature of the colony room was 23-25° C. All apparatus was kept and all testing was done in the colony room.

Novel Environment. The open field apparatus was adapted from Denenberg (1969) with modifications in size. For both Phases A & B the scale of the apparatus was determined by the average body length (excluding tail) of the rats. At the end of Phase B the apparatus was increased from a 75 cm. square plywood base divided into 36 12.5 cm. squares and 30 cm. walls to a 84 cm. square divided into 36 16.5 cm. squares with 52.5 cm. walls. The field was treated with Liquitex Matte Varnish which sealed the wood, and prevented urine from soaking into the wood.

Other Apparatus. The seven activity wheels used to measure activity levels were rotating drums with automatic rotation counter mechanisms and detachable hardware cloth holding cages. A Spencer Hemoglobinometer was used to assess blood samples for hemoglobin (Hb) content. To determine body weight (BW) a gram balance scale equipped with a removable holding basket was used.

Procedure

Following assignment of the rats to their respective treatment groups the experimental regimen began. The daily routine consisted of: (a) removal of food dishes from the cages, (b) determination of the amount of food left, (c) preparation of fresh diet, and (d) placement of food rations in the cages. The other procedures which included assessments

of Hb, BW and activity level, and behavioural observations in the familiar and novel settings were based on a six day cycle (Figure 1). Every fifth day of the cycle Hb levels and BW were taken prior to returning the food dishes to the cages. Every third and sixth day of the cycle the animals were observed in their home cages. Following observation on the sixth day they were placed in activity wheels while the cages were cleaned.

The experiment consisted of eight six-day cycles. The first four cycles constituted Phase A, during which diets were manipulated to produce three nutritional conditions; an iron-copper deficiency (ICD), a protein-calorie malnutrition (PCM), and adequate nutrition (ADN). The second four cycles constituted Phase B, during which time all animals were given adequate amounts of standard laboratory rat diet. On the fourth and fifth days of the fourth cycle in each phase, observations were made in a novel situation and Hb, BW and activity measures were taken after the observation on the sixth day instead of during their usual periods.

Nutritional Treatments and Monitors. There were two phases of nutritional treatment (Phase A and Phase B) each lasting for a period of 24 days. During Phase A, the period of nutritional deprivation, the rats were given a diet composed of 50% dry skim milk with supplements of Vitamins A and D (Lucerne Dry Skim Milk), 7.5% corn oil, 40.5% corn starch and 2% Alphacel. The milk powder ration of the ADN

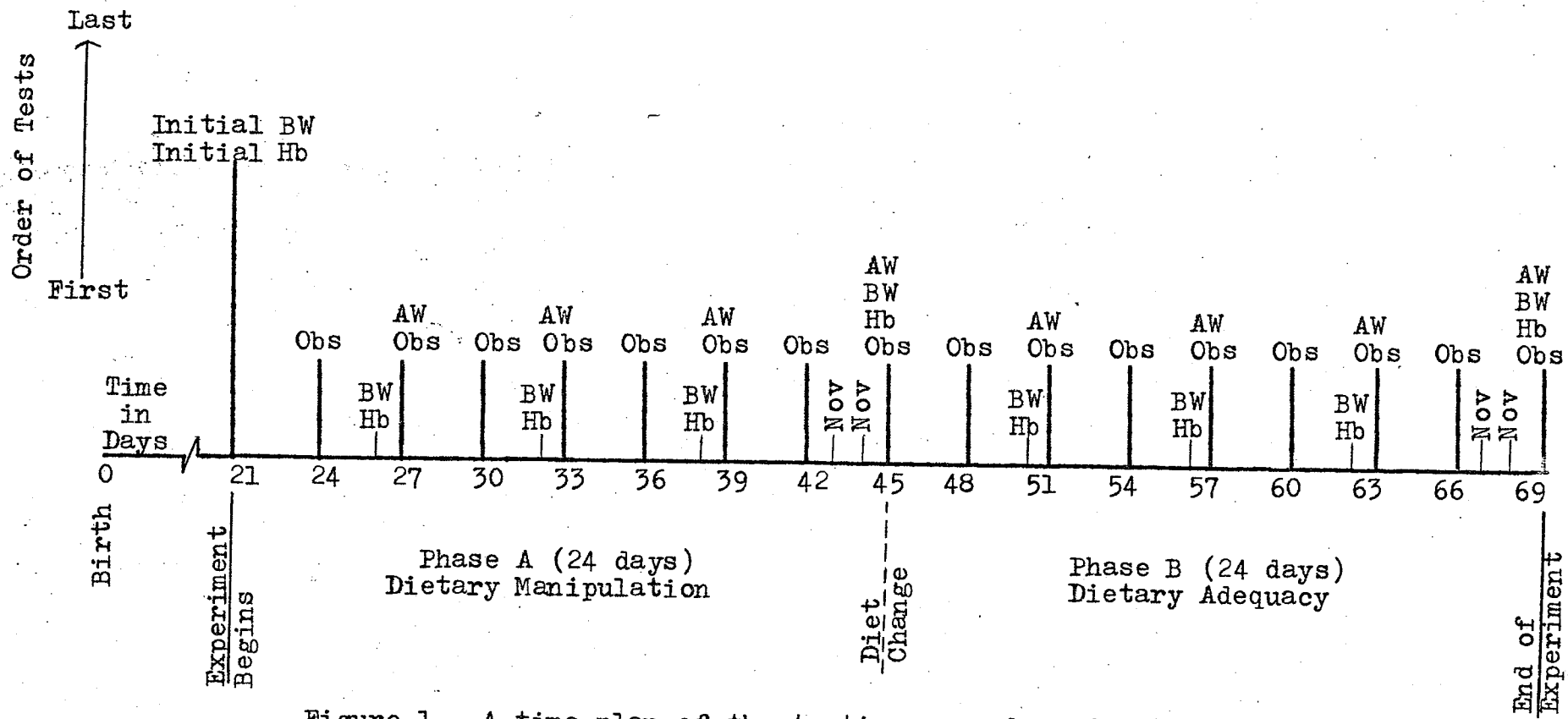

Figure 1. A time plan of the testing procedure for the physiological monitors of body weight (BW), and hemoglobin (Hb), for the behavioural observations in the familiar environment (Obs) and the novel situation (Nov), and for activity in the activity wheel (AW). On days where more than one measure was taken the testing order proceeded from bottom to top.

group was mixed with supplements of 10 ml of each a 25mg $FeSO_4 7H_2O$ and a 5mg $CUSO_4 5H_2O$ solution to form a paste. The milk powder given to the ICD rats was mixed with 20 ml of distilled water. The PCM group received exactly the same diet as the ADN group but was given only 50% of the amount.

Initially, the ICD and the ADN rats were given 6 gm of the milk diet daily while the PCM rats were given 3 gm. A record of food intake was kept. When the ADN rats began to eat their complete ration, the amount of milk powder was increased by 2 gm for the ADN and ICD and by 1 gm for the PCM.

During Phase B, the period of nutritional rehabilitation, all treatment groups were given equal and adequate amounts of Purina laboratory rat chow. Distilled water was available to all rats ad libitum throughout Phase A and Phase B.

The effects of the nutritional treatments upon body iron status and growth were monitored by regular checking of circulating blood hemoglobin (Hb) levels and body weight (BW). A measure of Hb was taken between 11 a.m. and 1:30 p.m. every sixth day during both Phase A and Phase B (Figure 1). The Hb content of a blood sample taken from the tail vein of the animal was assessed by means of Spencer Hemoglobinometer. Two readings of a sample were taken by each of two observers. If any of these readings differed by more than 0.99, a second set of four readings was taken. The average of the four or the eight readings was accepted as the animal's Hb level. Of 288 readings taken throughout the experiment, only 6 required

a second reading. Following determination of Hb levels the body weights of the subjects were assessed by means of a gram balance scale.

Observational Procedure and Measures Used in the Familiar Environment. Between 10:30 a.m. and 1:30 p.m. every third day observations of home cage behaviour followed removal of the food dishes from the cages. Observations in the home cage were made for a three min. period. Every five sec. the observer recorded the behaviour shown by a rat for at least three of the five sec. Therefore during a three min. period, 36 judgements of behaviour were made. The order in which animals were observed differed in each of the 16 home cage observations. The only criterion for observation was that the animal show some signs of being awake. Those animals which slept during the entire 2-3 hour observation period were recorded as sleeping.

The behaviour categories used in this study (Table 2) were a modification of those used by Shettleworth (1975) with golden hamsters (Appendix A). Shettleworth's (1975) behaviour categories were pretested with rats (one iron-copper deprived, two iron deprived, two copper deprived, and two adequately nourished. The observations indicated that some of the categories either were not shown by rats or were restricted by apparatus. Behaviour categories not included were hind kick, pick up sawdust, and scent mark. However, some rat behaviours originally not defined by Shettleworth's categories

TABLE 2

Operational Definitions of the Specific Behaviours Which Comprise the Behaviour Categories of Vertical, Horizontal, Grooming, and Maintenance and Which Were Used in Coding Behaviour in the Home Cage and in the Open Field.

Behaviour Category	Behaviour	Definition	Not Used in Open field
Vertical	Climb	Standing on one leg and pulling the body up the mesh portions of the cage with the forepaws Or moving about the mesh portion of the cage with no paws touching the flooring	x
	Hang	Clinging, without movement of any paws to the mesh portions of the cage and with no paws touching the flooring	x
	Panel rear	Standing on hind legs with at least one forepaw touching the front or back mesh panel of the cage	x
	Wall rear	Standing on hind legs with at least one forepaw touching a wall	
	Open rear	Standing on hind legs with both forepaws off floor and walls	
	Scrabble	Scraping with forepaws against a wall while standing erect. Sometimes moving along wall, hopping up and down while doing this. Distinguished from digging	

TABLE 2 (continued)

Behaviour Category	Behaviour	Definition	Not Used in Open field
	Scrabble (continued)	at the wall by the fact that each stroke is directed to the side, the head is oriented up rather than toward the wall, and the animal is usually stretched to its full extent. Also includes climbing where situation permits animal to lift his hind feet clear of the floor.	
Horizontal	Walk/sniff	Walking around or staying still and sniffing with at least three feet touching the floor	
	Dart and hop	Moving suddenly and very fast (darting), sometimes leaving floor with all four feet at once (hopping)	
	Freeze	Standing motionless, not sniffing, with at least three feet on the floor	
	Sit	Sitting on haunches or on all four paws, sniffing, with eyes open perhaps head swaying from side to side but remaining very still. Carrying on no other activity.	
	Shake	Sudden convulsive shaking of whole body	
	Dig	Scraping with forepaws directed in front of face at sawdust, bare floor, or wall	
Groom	Groom	Behaviours which are definitely either face washing or grooming belly and sides but because of position or alternation of one to the other cannot be stated as one or the other	

TABLE 2 (continued)

Behaviour Category	Behaviour	Definition	Not Used in Open field
	Groom belly and sides	Scratching and/or biting belly, sides, or legs with mouth and/or forepaws	
	Wash face	Rubbing forepaws over any part of head. Includes interspersed licking of forepaws.	
	Scratch with hind leg	Scratching any part of body with hind foot. Includes interspersed licking of the scratching foot.	
Maintenance	Gnaw	Biting at wall, corner, or edge of any surface.	
	Sneeze	Sudden sneezing by the animal	
	Eat something other than food	Biting and gnawing anything other than food, e.g. sawdust, mesh, water spout, feces, flies.	
	Yawn and stretch	Self-explanatory. Usually occur together, but stretching can occur alone.	
	Sleep	Lying relatively motionless with eyes closed	
	Eat	Biting and chewing food	x
	Drink	Licking at water spout	x
	Hoard	Depouching or dropping food onto food pile. Pushing food around with paws, nose or mouth. Carrying chow pellet in teeth.	x

TABLE 2 (continued)

Behaviour Category	Behaviour	Definition	Not Used in Open field
	In nest	Standing or lying wholly on the nest paper	x
	Manipulate nest paper (arrange nest)	Holding, carrying or pushing nest paper with paws and/or mouth. Pouching or depouching nest paper. Standing or lying Wholly on the nest paper.	x
	Defecate	Assuming defecation posture.	
	Urinate	Assuming urination posture.	

were added. The additions included climb, hang, sit, wall rear, and groom. The final list used in this study consisted of 28 mutually exclusive behaviour categories for the home cage observations.

Observational Procedure and Measures Used in the Novel Situation. Each animal was observed in a novel situation, the open field, for a five-min. period on each of two days at the end of Phase A and Phase B. Video-tape records were made of all open field behaviour. The subsequent coding of the behaviours from the video-tape was done in the same manner as that in the familiar environment. The PCM group was observed first, the ICD second, and the ADN third with the order of animals observed within the group counter-balanced from the first to the second day. In the novel environment, the apparatus precluded use of some categories such as climb, hang, panel rear, manipulate nest, in nest drink, hoard and eat. Therefore, analysis of behaviour in the open field involved a subset of 20 behaviour categories (Table 2).

Activity. Three measures of activity were obtained: frequency of behaviour changes, number of rotations made in an activity wheel, and number of squares crossed in the open field. A change from one behaviour category to another provided the measure of frequency of behaviour changes which was used as an indicator of activity level in the home cage. The number of rotations made during a 15-min. period in the activity wheel provided a measure of voluntary running. The

number of squares crossed during the five minutes in the open field and the length of time it took the animal to leave the center four squares (latency), measured activity in the novel environment.

CHAPTER III

RESULTS

This chapter is divided into three sections. The first deals with physiological monitors of the diets, reliability of the data, and a description of the data analysis. The results obtained from analyses of behaviours and activities in the familiar environment and in the novel situation during Phase A are presented in the second section and the results of Phase B analyses in the third section.

Monitors of Nutritional Treatments

Hemoglobin (Hb) and body weight (BW) were the physiological measures used to monitor the effects of the diets. Nutritional treatment group differences were assessed by paired *t*-tests.(See Appendix B for means, t-values, and significance levels.) Changes in mean Hb values during Phases A and B are illustrated in Figure 2. After only six days on the iron and copper deficient diet (27 days of age) the mean Hb level of the ICD animals was significantly lower than those of the PCM and ADN rats and remained lower throughout Phase A. The high mean levels of Hb evident in the PCM group may be attributable to their smaller body size and therefore smaller blood volume during Phase A. With provision of Purina laboratory rat chow in Phase B the mean Hb level of the ICD animals increased to normal (13-15 gmHb/100 ml blood) and the mean Hb level of the PCM animals decreased with a concomitant increase in their BW's.

Figure 3 illustrates the changes in mean BW values for each group throughout the experiment. Paired *t*-tests of

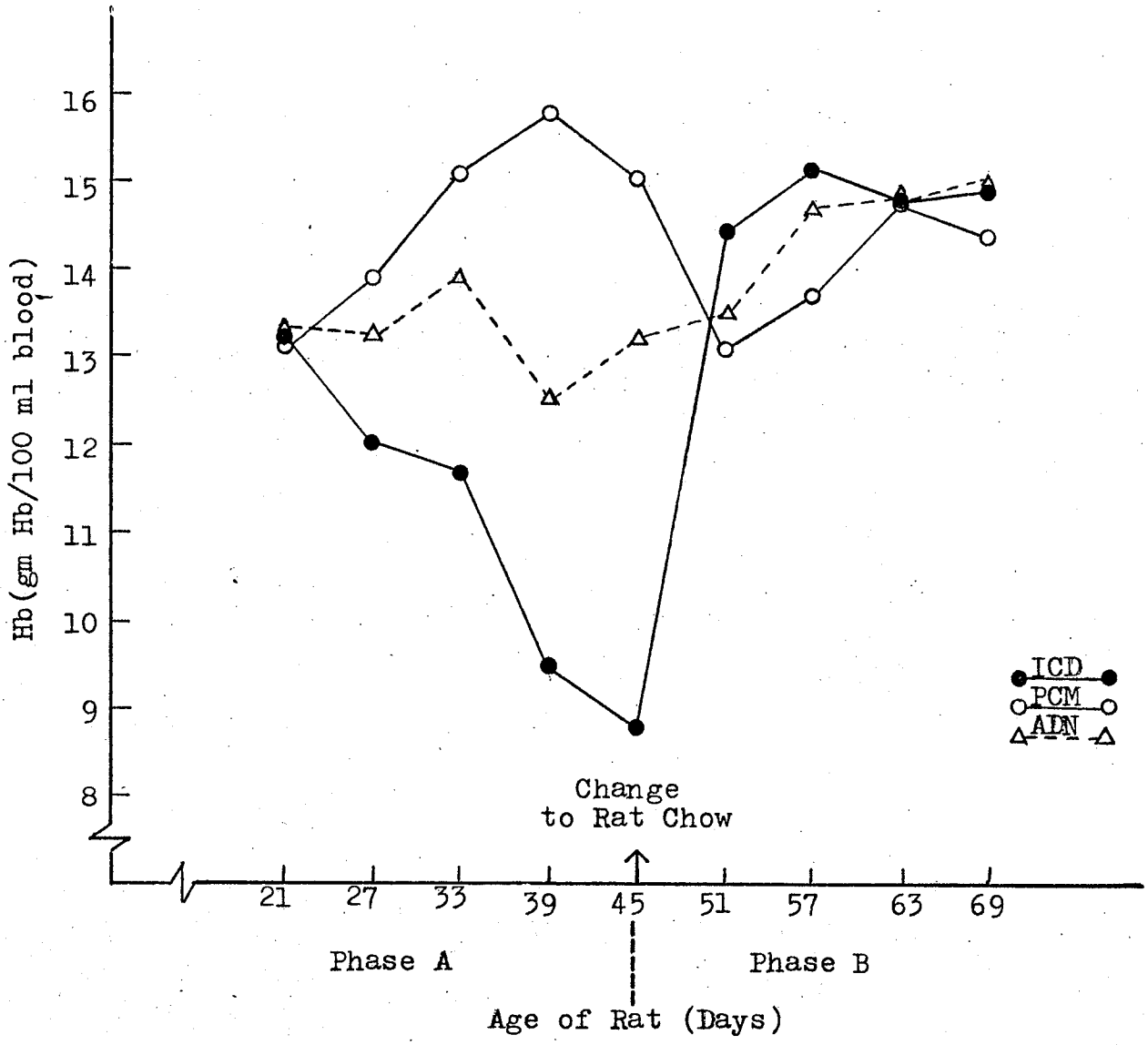

Figure 2. Mean hemoglobin (Hb) of rats in the ICD (●), PCM (O), ADN (△) groups on each day of testing in Phase A and Phase B of the experiment.

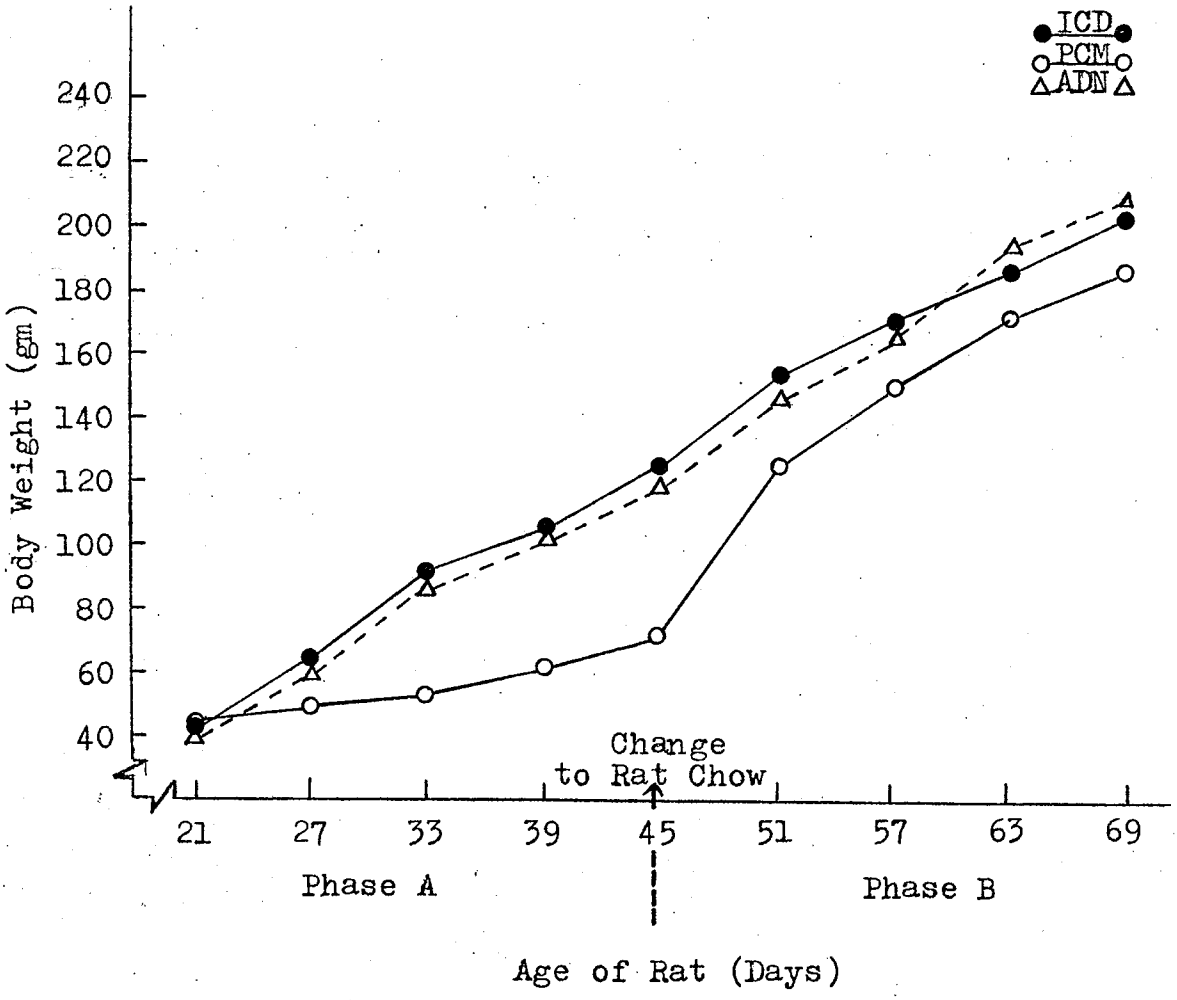

Figure 3. Mean body weights of rats in the ICD(●), PCM(○) and ADN (△) groups on each day of testing in Phase A and Phase B of the experiment.

BW showed that by 27 days of age, after only six days on the restricted diet, the mean BW of the PCM rats was significantly less than either the ICD or the ADN groups. The PCM animals remained at a significantly lower BW level throughout Phase A and Phase B while the ICD and ADN animals did not differ from each other during either phase.

Reliability of the Dependent Variable Measures

All behavioural recording and coding was done by one observer. Video-tape records permitted assessment of intraobserver reliability on a sample of six animals selected from each of the four days of open field observation. The percentages of agreement based on the frequency of the 20 open field behaviours for Phase A were 93.6% on Day 1 and 92.8% on Day 2 and for Phase B, 94.8% and 93.9% on Days 1 and 2 respectively. The mean difference between the first and second recordings of the number of squares crossed in the open field was 3.95 with a standard deviation of 3.44. For latency, that is, the amount of time the animals took to leave the centre four squares of the open field, the mean difference was .17 sec. with a standard deviation of .28 sec.

Data Analysis

To facilitate analysis the 28 specific behaviours were logically grouped into four larger behaviour categories, namely, vertical, horizontal, grooming, and maintenance (Table 2). As indicated in Table 2 three vertical and five maintenance behaviours do not apply in the open field. In addition to the four behaviour categories, two measures of activity level (p. 29) were obtained for each of the home

cage and open field situations. The activity measures of square crossing and latency were transformed to produce integer data which was required for the computer program.

Split-plot two-way analyses of variance were used to test the effect of nutritional treatment (3) and of day in treatment (home cage 8, activity wheel 4, open field 2) on behaviour. Each set of behaviours (vertical, horizontal, grooming, and maintenance) and each activity level indicator (frequency of behaviour changes, activity wheel rotations, latency scores, and rate of square crossing) for each phase of the experiment (A and B) in each environmental setting (familiar and unfamiliar) were analyzed separately. Because of the exploratory nature of this study only analyses which produced results significant at a .05 probability level or better are presented. Where there are significant results a summary of the analysis of variance and appropriate figures are included in the body. All other pertinent information, such as complete tables of means and t-values, if not provided in the text, is provided in Appendix C[1]. Because the effect of nutrition on behaviour was the focus of this study, results from the Day factor which reflected behavioural changes occurring with maturation, are also presented in Appendix C.

Phase A: Nutritional Deprivation

Behaviours and activity in the familiar environment. A summary of the F-ratios of the analyses of variance

of behaviours observed in the home cages during the period of nutritional deprivation is presented in Table 3. During this phase nutritional treatment was associated with differences in vertical and grooming behaviours and frequency of behaviour changes. The length of time in nutritional treatment (Days) was also associated with differences in vertical behaviour as well as with differences in maintenance behaviour, frequency of behaviour change, and activity wheel scores (Appendix C). Significant nutritional treatment with time in treatment interactions were noted for vertical and maintenance behaviour, frequency of behaviour changes, and activity wheel scores.

Figure 4 provides a summary of the mean frequency of vertical behaviour of rats in each of the nutritional treatment groups when observed in their home cages during Phase A. This figure suggests that the amount of vertical behaviour shown was related to differences in diet. The analysis summarized in Table 4 did indicate a significant nutrition effect, $F(2,20)=19.46, p<.001$. Significantly more vertical behaviour was observed in the PCM rats than either the ICD, $t(164)=3.78, p<.001$, or the ADN rats, $t(164)=6.17$, $p<.001$. The ICD animals exhibited less vertical behaviour than the PCM, $t(164)=3.78, p<.001$, but more than the ADN rats, $t(164)=2.38, p<.05$. The ADN rats showed the least amount of vertical behaviour. The day-to-day changes in frequency of vertical behaviour over the eight days of observation, which

TABLE 3

Summary of F-ratios for Behaviour Category Scores and Activity Level Scores in the Familiar Environment during Nutritional Deprivation

Behaviour	Nutrition (df=2,20)	Day (df=7,210)	Nutrition x Day (df=14,210)
Vertical	19.46***	17.53***	2.36**
Horizontal	0.22	1.27	1.18
Grooming	7.07**	1.88	1.51
Maintenance	0.10	12.39***	1.86*
Activity			
Frequency of Behaviour Changes	9.20**	14.79***	2.13*
Activity Wheel	1.23	18.73*** (df=3,90)	5.10** (df=6,90)

*$p<.05$
**$p<.01$
***$p<.001$

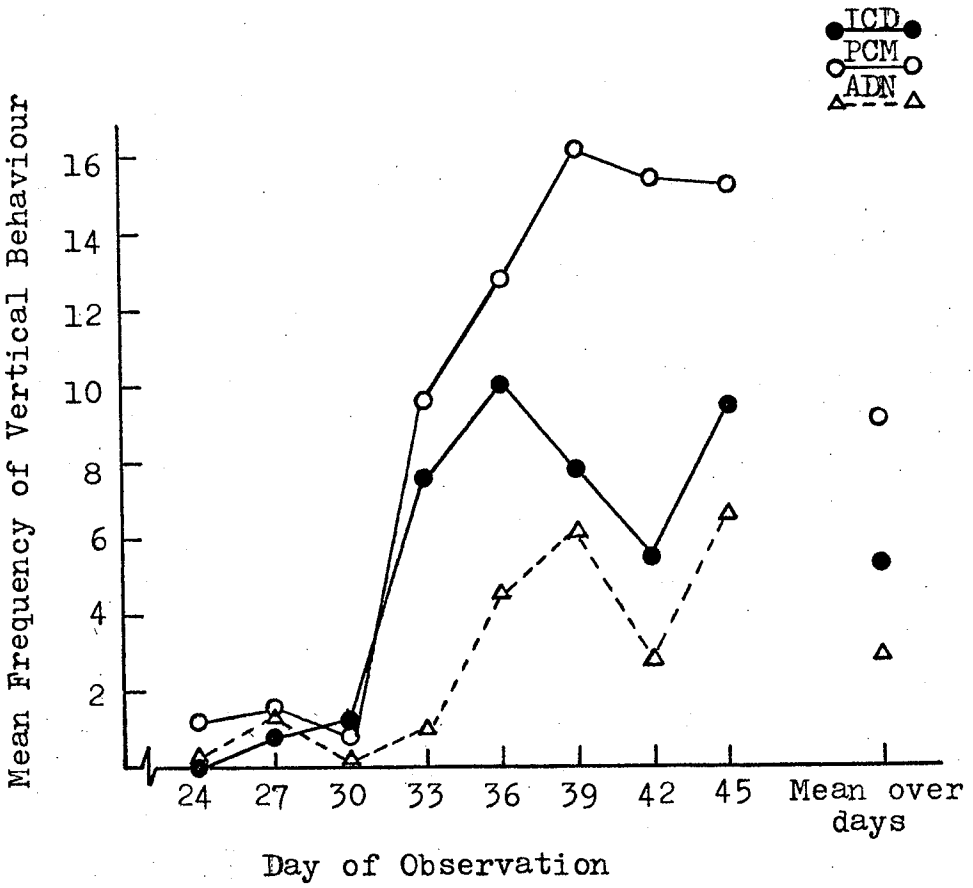

Figure 4. Mean frequencies of vertical behaviour (based on 5-sec. observational units) exhibited by each nutritional treatment group for each day of observation in the familiar environment during nutritional deprivation. Significant group differences were indicated for:

Day 33	ICD > ADN	PCM > ADN	
36	ICD > ADN	PCM > ADN	
39	ICD < PCM	PCM > ADN	
42	ICD < PCM	PCM > ADN	
45	ICD < PCM	PCM > ADN	
Mean over Days	ICD < PCM	ICD > ADN	PCM > ADN

TABLE 4

Summary of ANOVA of Vertical Behaviour
in the Familiar Environment
during Nutritional Deprivation

Source	df	SS	MS	F
Subjects	10	311.27	31.13	
Nutrition (N)	2	1735.38	867.69	19.46***
$Error_b$	20	891.77	44.59	
Days (D)	7	4296.88	613.84	17.53***
N x D	14	1156.43	82.60	2.36**
$Error_w$	210	7355.66	35.03	
Total	263	15747.41		

** $p < .01$
*** $p < .001$

also implies the age of the rats, was significant, $F(7,20)=17.53, p<.001$. Nutritional treatment also interacted with day, $F(14,210)=2.36, p<.01$, which suggests a differential effect of nutrition upon the frequency of vertical behaviour. Paired t-tests indicated no differences in frequency of vertical behaviour between nutritional groups before Day 33, that is, during the first 12 days on the deficient diet. From Day 33 to Day 45 the PCM rats showed significantly more vertical behaviour than the ADN group and from Day 39 to 45 also more than the ICD rats. More vertical behaviour was observed among the ICD than the ADN rats only on Days 33 and 36.

Analysis of grooming behaviour (Table 5) indicated differences among nutritional groups, $F(2,20=7.07, p<.01$. The PCM rats groomed less ($M=5.42, SD=9.79$) than the ICD rats ($M=2.35, SD=5.16$; $t(20)=2.24, p<.05$) and also less than the ADN rats ($M=7.45, SD=11.92$; $t(20)=5.73, p<.001$).

The mean frequencies of maintenance behaviour observed in the home cages over the eight days of nutritional deprivation are illustrated in Figure 5. The analysis summarized in Table 6 indicated no differences among nutritional treatment groups but significant changes from day to day, $F(7,210)=12.39, p<.001$, and a significant interaction of nutritional treatment with day of observation, $F(14,210)=1.86, p<.05$. However, t-tests indicated significant differences between only the ICD and the PCM animals on Days 30

TABLE 5

Summary of ANOVA of Grooming Behaviour in the Familiar Environment during Nutritional Deprivation

Source	df	SS	MS	F
Subjects	10	659.99	66.00	
Nutrition (N)	2	1161.14	580.57	7.07*
$Error_b$	20	1641.35	82.07	
Days (D)	7	1114.49	159.21	1.88
N x D	14	1794.85	128.20	
$Error_w$	210	17802.28	84.77	1.51
Total	263	24174.11		

* $p < .05$

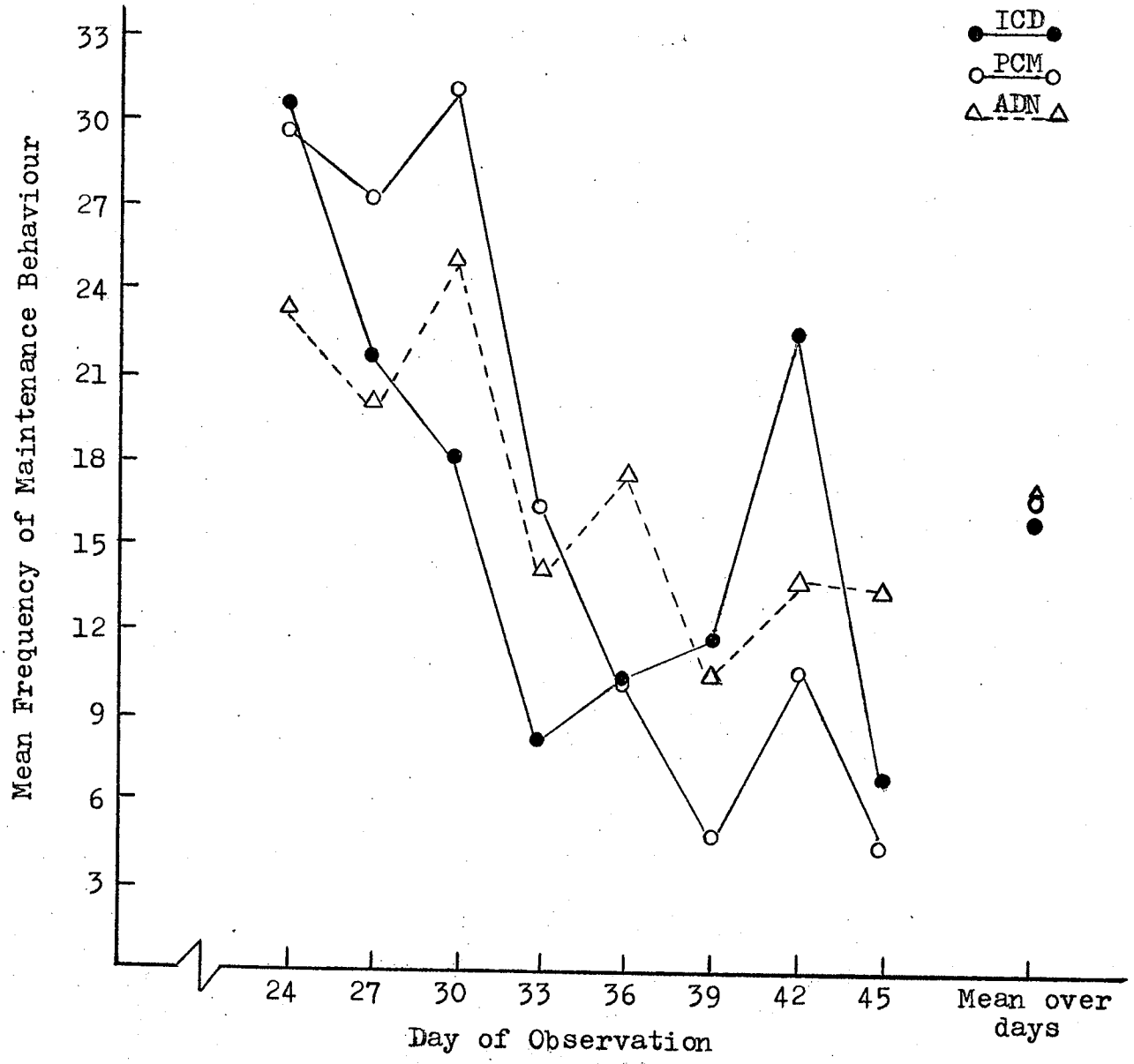

Figure 5. Mean frequencies of maintenance behaviour (based on 5-sec. observational units) exhibited by each nutritional treatment group for each day of observation in the familiar environment during nutritional deprivation. Significant group differences were indicated for:

Day 30	ICD < PCM
42	ICD > PCM

TABLE 6

Summary of ANOVA of Maintenance Behaviour in the Familiar Environment during Nutritional Deprivation

Source	df	SS	MS	F
Subjects	10	1104.90	110.49	
Nutrition (N)	2	47.05	23.53	0.101
$Error_b$	20	4649.03	232.45	
Days (D)	7	13024.05	1860.58	12.39***
N x D	14	3920.34	280.02	1.86*
$Error_w$	210	31542.98	150.20	
Total	263	54288.36		

* $p<.05$
** $p<.01$
*** $p<.001$

and 42. Less maintenance behaviour was observed among the ICD than the PCM animals on Day 30 while on Day 42 the PCM showed less than the ICD animals.

Figure 6 illustrates how frequently the rats in each treatment group changed from behaviour to behaviour during the 3-min. observation periods. The analysis of behaviour changes summarized in Table 7 demonstrated significant differences among nutritional treatment groups, $F(2,20)=9.20, p<.01$. During the period of nutritional deprivation the PCM group changed behaviour more frequently than the ICD, $t(164)=2.04, p<.05$, and the ADN group, $t(164)=3.66$, $p<.001$. The day to day differences in frequency of behavioural changes over the eight days of observation was significant, $F(7,210)=14.79, p<.001$. However, the significant interaction of nutritional treatment with day of observation, $F(14,210)=2.13, p<.01$, suggests diet influenced the frequency of behaviour changes differently at different times during the course of treatment. Group differences were not indicated until Day 33 at which time the ICD and the PCM animals changed behaviour more frequently than the ADN. The PCM also showed more behaviour changes than the ADN on Day 39 and 45 and than the ICD on Day 45.

Activity wheel measures were obtained on only four days during the period of nutritional deprivation (Figure 7). The analysis summarized in Table 8 indicated no differences between nutritional treatment groups in activity wheel running

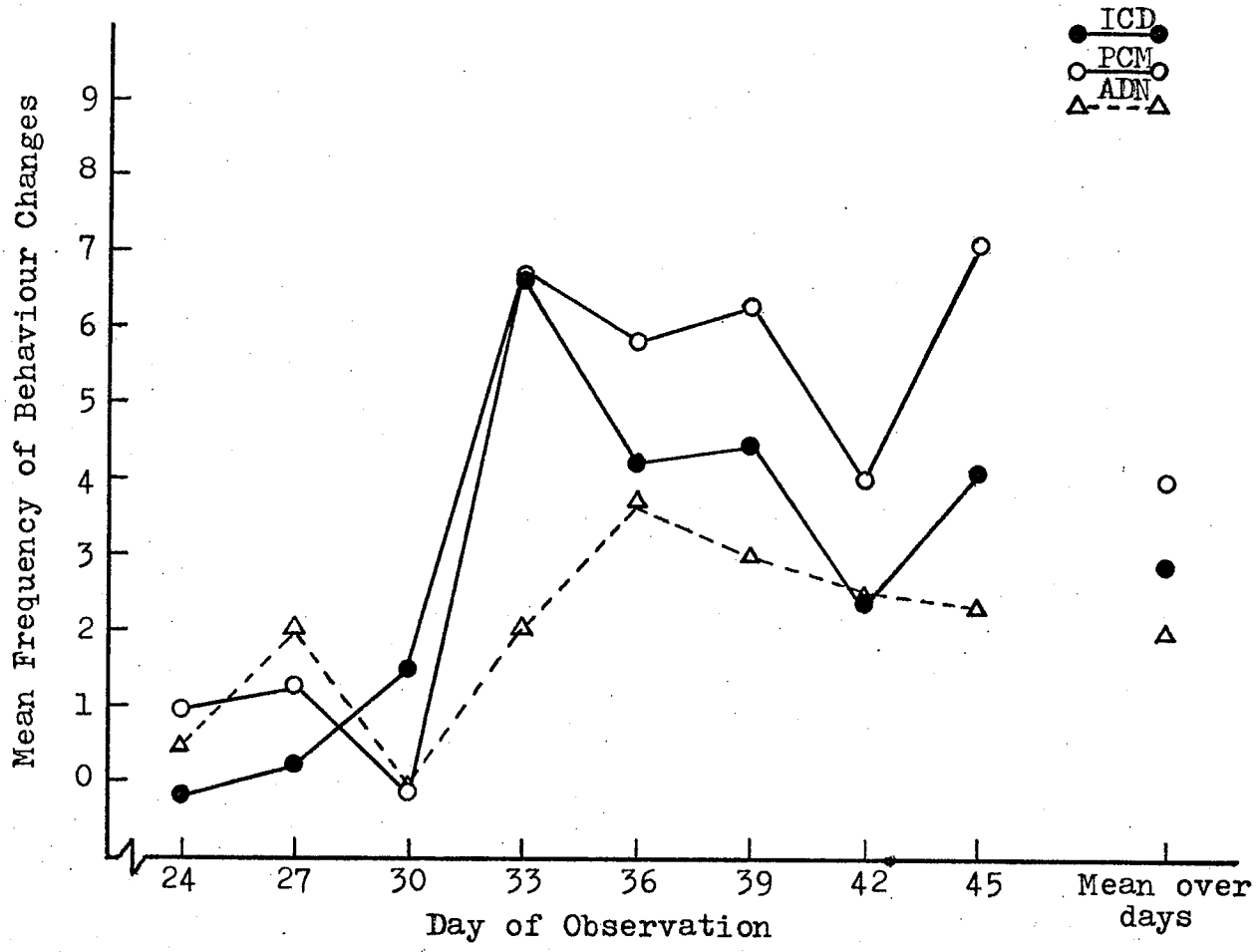

Figure 6. Mean frequency of behaviour changes (based on 5-sec. observational units) exhibited by each nutritional treatment group for each day of observation in the familiar environment during nutritional deprivation. Significant group differences were indicated for:

Day 33	ICD > ADN	PCM > ADN
39	PCM > ADN	
45	ICD < PCM	PCM > ADN
Mean over days	ICD < PCM	PCM > ADN

TABLE 7

Summary of ANOVA of Frequency of Behaviour Change in the Familiar Environment during Nutritional Deprivation

Source	df	SS	MS	F
Subjects	10	477.36	47.74	
Nutrition (N)	2	679.16	339.58	9.20**
$Error_b$	20	738.00	36.90	
Days (D)	7	3727.90	532.56	14.79***
N x D	14	1072.54	76.61	2.13**
$Error_w$	210	7560.88	36.00	
Total	263	14255.84		

* $p<.05$
** $p<.01$
*** $p<.001$

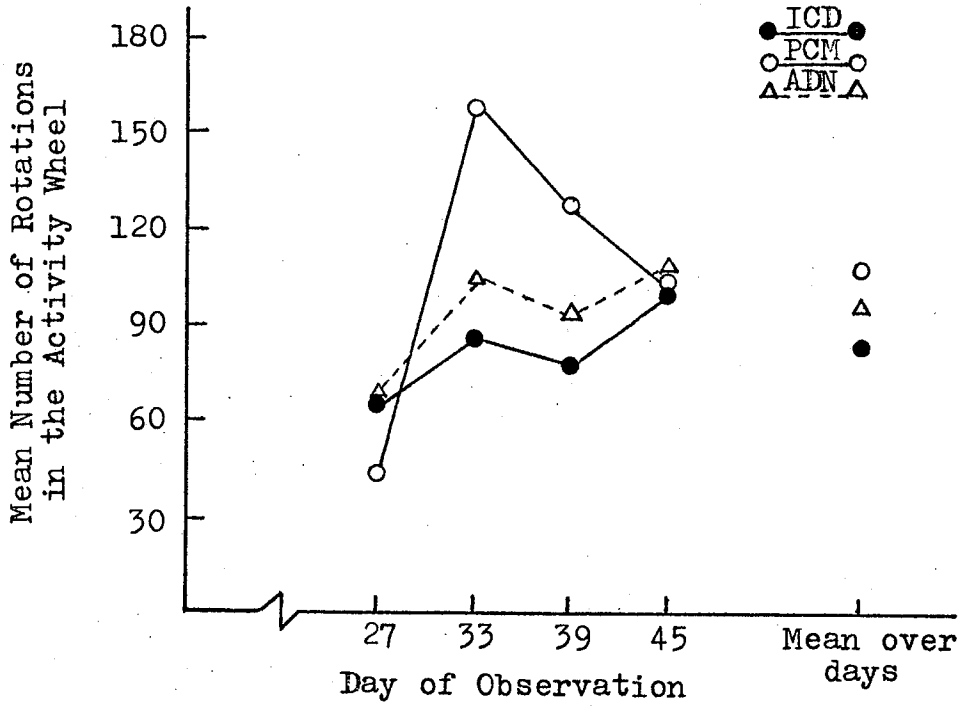

Figure 7. Mean number of activity wheel rotations (based on a 15-min. period) exhibited by each nutritional treatment group in the familiar environment during nutritional deprivation. Significant group differences were indicated for:

Day 27	ICD > PCM	PCM < ADN
33	ICD < PCM	PCM > ADN
39	ICD < PCM	PCM > ADN

TABLE 8

Summary of ANOVA of Activity Wheel Rotations during Nutritional Deprivation

Source	df	SS	MS	F
Subjects	10	80713.13	8071.31	
Nutrition (N)	2	14416.55	7208.27	1.23
$Error_b$	20	117.06.06	5855.30	
Day (D)	3	6.609.18	20536.39	18.73***
N x D	6	33543.69	5590.61	5.10***
$Error_w$	90	98666.25	1096.29	
Total	131	406054.94		

* $p<.05$
** $p<.01$
*** $p<.001$

but a significant difference from day to day, $F(3,90)=18.73$, $p<.001$, during the period of nutritional deprivation. The Nutrition x Day interaction, $F(6,90)=5.10, p<.001$, indicated less activity wheel running among the PCM rats than the ICD and ADN on Day 27. However, on Days 33 and 39 the PCM rats ran more than either of the other two groups.

Behaviours and Activity in the Novel Situation. Generally, analyses of the data from the novel situation at the end of Phase A indicated that differences in vertical, horizontal, and grooming behaviours as well as in the number of squares crossed in the open field were associated with nutritional treatment (Table 9). Latency, that is, the length of time taken to leave the four centre squares of the open field, was longer on the first than on the second day of exposure for all three nutritional treatment groups (Appendix C). However, the amount of maintenance behaviour shown on the first and second day of exposure to the open field differed among nutritional treatment groups.

Specifically, analysis of variance of the frequency of vertical behaviour (Table 10) indicated differences among nutritional groups, $F(2,20)=11.19, p<.001$, attributable to the PCM rats ($M=19.41$) who showed more vertical behaviour than the ICD, ($M=11.09$; $t(42)=3.32, p<.001$). In contrast, the significant nutritional treatment effect on horizontal behaviour indicated by analyses of variance (Table 11; $F(2,2)=12.08, p<.001$) was due to the ICD group ($M=46.68$) that

TABLE 9

Summary of F-ratios for Behaviour Category Scores and Activity Level Scores in the Novel Situation during Nutritional Deprivation

Behaviour	Nutrition (df=2,20)	Day (df=1,30)	Nutrition x Day (df=2,30)
Vertical	11.19***	0.17	3.23
Horizontal	12.08***	0.82	1.41
Grooming	6.06**	3.45	3.21
Maintenance	0.65	0.68	4.44*
Activity			
Latency	0.48	19.98***	1.60
Total Squares Crossed	5.21*	2.38	2.38

*$p<.05$
**$p<.01$
***$p<.001$

TABLE 10

Summary of ANOVA of Vertical Behaviour
in a Novel Situation
during Nutritional Deprivation

Source	df	SS	MS	F
Subjects	10	1071.69	107.17	
Nutrition (N)	2	813.30	406.65	11.19***
$Error_b$	20	727.03	36.35	
Days (D)	1	3.41	3.41	0.17
N x D	2	127.18	63.59	3.23
$Error_w$	30	590.91	19.70	
Total	65	3333.52		

* $p<.05$
** $p<.01$
*** $p<.001$

TABLE 11

Summary of ANOVA of Horizontal Behaviour
in a Novel Situation
during Nutritional Deprivation

Source	df	SS	MS	F
Subjects	10	1027.79	102.78	
Nutrition (N)	2	816.64	408.32	12.08***
$Error_b$	20	676.02	33.80	
Day (D)	1	17.52	17.52	0.82
N x D	2	60.57	30.29	1.41
$Error_w$	30	644.92	21.50	
Total	65	3243.45		

* $p<.05$
** $p<.01$
*** $p<.001$

exhibited more horizontal behaviour than the PCM (M=38.14; $t(42)$=4.89, $p<.001$) and ADN groups (M=41.45; $t(42)$=2.99, $p<.01$). The analysis summarized in Table 12 indicates that frequency of grooming behaviour also varies with nutritional treatment, $F(2,20)$=6.06, $p<.01$ More grooming behaviour was observed amongst the ADN (M=4.77) than ICD (M=1.45, $t(42)$=3.02, $p<.01$) or PCM animals (M=1.45; $t(42)$=3.02, $p<.01$). Similar to vertical behaviour, the significant differences among nutritional groups in the rate of crossing squares in the open field (Table 13, $F(2,20)$=5.21, $p<.05$) resulted from the PCM rats crossing more squares per second (M=.62) than either the ICD (M=.45; $t(42)$=2.94, $p<.01$) or the ADN groups (M=.47; $t(42)$=2.60, $p<.01$).

The only interaction of nutritional treatment with day of exposure to the open field was indicated for the analysis of maintenance behaviour (Table 14; $F(2,30)$=4.44, $p<.05$). On the first day more maintenance behaviour was observed in the PCM (M=1.09) than the ICD (M=0.18; $t(20)$=2.39, $p<.05$) and ADN groups (M=0.18; $t(20)$=2.39, $p<.05$). On the second day there were no differences among nutritional groups in amount of maintenance behaviour observed.

Phase B: Nutritional Recovery

Behaviours and Activity in the Familiar Environment. The summary of analyses of behaviours observed in the familiar environment during nutritional recovery (Table 15) suggests that these behaviours were not associated with nutritional

TABLE 12

Summary of ANOVA of Grooming Behaviour
in a Novel Situation
during Nutritional Deprivation

Source	df	SS	MS	F
Subjects	10	101.76	10.18	
Nutrition (N)	2	161.48	80.74	6.06**
$Error_b$	20	266.51	13.33	
Day (D)	1	16.50	16.50	3.45
N x D	2	30.64	15.32	3.21
$Error_w$	30	143.36	4.78	
Total	65	720.26		

* $p < .05$
** $p < .01$
*** $p < .001$

TABLE 13

Summary of ANOVA of Total Number of Squares Crossed per Second in a Novel Situation during Nutritional Deprivation

Source	df	SS	MS	F
Subjects	10	380240.00	38024.00	
Nutrition (N)	2	387680.00	193840.00	5.21*
$Error_b$	20	744768.00	37238.40	
Day (D)	1	29120.00	29120.00	2.38
N x D	2	54032.00	27016.00	2.21
$Error_w$	30	367152.00	12238.40	
Total	65	1962992.00		

*$p<.05$
**$p<.01$
***$p<.001$

TABLE 14

Summary of ANOVA of Maintenance Behaviour in a Novel Situation during Nutritional Deprivation

Source	df	SS	MS	F
Subjects	10	9.79	0.98	
Nutrition (N)	2	1.12	0.56	0.65
$Error_b$	20	17.21	0.86	
Day (D)	1	0.55	0.55	0.68
N x D	2	7.18	3.59	4.44*
$Error_w$	30	24.27	0.81	
Total	65	60.12		

*$p<.05$
**$p<.01$
***$p<.001$

TABLE 15

Summary of F-ratios for Behaviour Category Scores and Activity Level Scores in the Familiar Environment during Nutritional Recovery

Behaviour	Nutrition (df=2,20)	Day (df=7,210)	Nutrition x Day (df=14,210)
Vertical	0.27	2.94**	0.81
Horizontal	1.80	1.92	1.95*
Grooming	0.84	1.01	1.75*
Maintenance	1.50	1.80	0.98
Activity			
Frequency of Behaviour Changes	2.25	4.66***	0.60
Activity Wheel	2.10	9.36*** (df=3,90)	1.78 (df=6,90)

*$p<.05$
**$p<.01$
***$p<.001$

treatment. Vertical behaviour, the frequency of behaviour changes, and rotations in the activity wheel differed from day to day during nutritional recovery (Appendix C). However, the Nutrition x Day interactions indicated for the horizontal and grooming categories suggests an influence of nutritional treatment.

Figure 8 illustrates the nutrition x day interaction of horizontal behaviour (Table 16; $F=(14,210)=1.95$, $p<.05$). Paired t-tests indicate the group differences on Day 48 resulted from the ICD showing significantly more horizontal behaviour than either the PCM or ADN animals. The means for grooming behaviour that illustrate the nutrition x day interaction, (Table 17; $F=(14,210)=1.75, p<.05$) are presented in Figure 9. Significantly more grooming behaviour was observed in the PCM than the ADN group on Day 54 while on Day 60 the ICD group exhibited more grooming than either of the other two groups.

Behaviours and Activity in the Novel Situation. In Table 18 the summary of analyses of variance indicates that after 24 days of adequate nutrition the main effect of nutritional treatment indicated no differences among the groups. However, the day of exposure does have some influence on vertical and horizontal behaviour as well as on latency and the number of squares crossed per sec.

The only effect of nutritional treatment on behaviour in the novel situation at the end of the period of

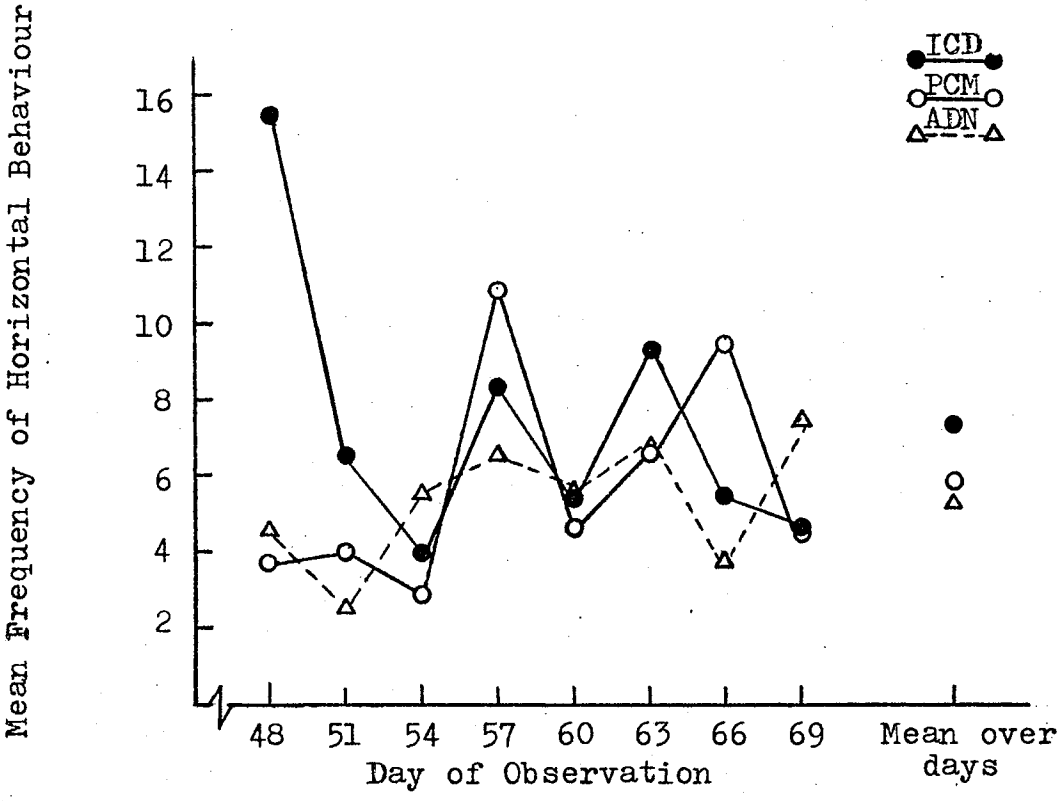

Figure 8. Mean frequency of horizontal behaviour (based on 5-sec. observational units) exhibited by each nutritional treatment group on each day of observation in the familiar environment during nutritional recovery. Significant group differences were indicated for:

Day 48	ICD > PCM	ICD > ADN

TABLE 16

Summary of ANOVA for Horizontal Behaviour in the Familiar Environment during Nutritional Recovery

Source	df	SS	MS	F
Subjects	10	242.23	24.22	
Nutrition	2	187.87	93.93	1.80
$Error_a$	20	1042.88	52.14	
Days	7	644.36	92.05	1.92
Nutrition x Days	14	1312.07	93.72	1.95*
$Error_b$	210	10084.78	48.02	
Total	263	13514.19		

*$p < .05$

TABLE 17

Summary of ANOVA for Grooming Behaviour in the Familiar Environment during Nutritional Recovery

Source	df	SS	MS	F
Subjects	10	1172.28	117.23	
Nutrition	2	210.48	105.24	0.839
Error	20	2507.60	125.38	
Days	7	892.02	127.43	1.012
Nutrition x Days	14	3083.82	220.27	1.749*
Error	210	26448.58	125.95	
Total	263	34314.78		

*$p < .05$

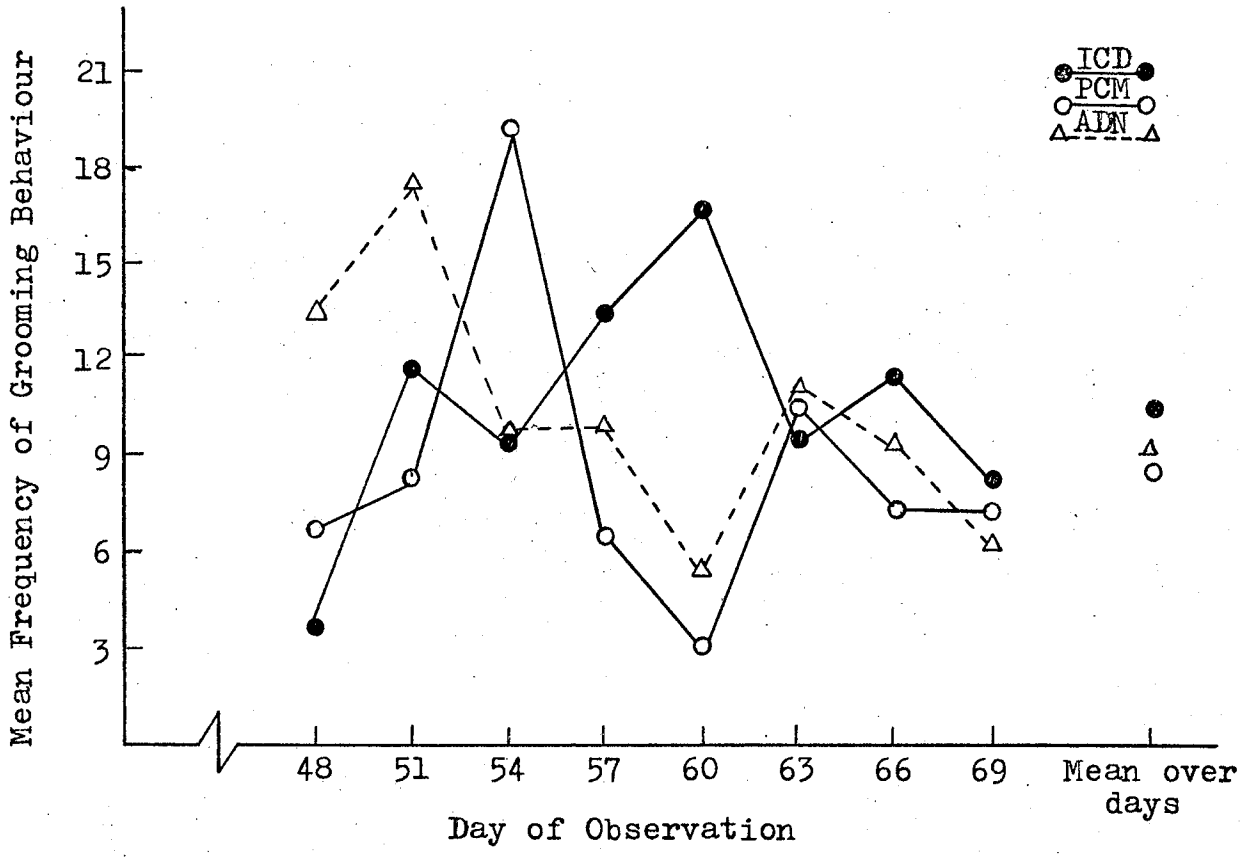

Figure 9. Mean frequency of grooming behaviour (based on 5-sec. observational units) exhibited by each nutritional treatment group on each day of observation in the familiar environment during nutritional recovery. Significant group differences were indicated for:

Day 54	PCM > ADN		
60	ICD > PCM	ICD > ADN	

TABLE 18

Summary of F-ratios for Behaviour Category Scores and Activity Level Scores in the Novel Situation during Nutritional Recovery

Behaviour	Nutrition (df=2,20)	Day (df=1,30)	Nutrition x Day (df=2,30)
Vertical	0.35	6.74*	1.38
Horizontal	0.45	7.50*	0.86
Grooming	0.78	0.48	1.33
Maintenance	3.10	3.11	1.78
Activity			
Latency	0.52	6.65*	0.77
Total Squares Crossed	0.53	44.77***	3.99*

*$p < .05$
**$p < .01$
***$p < .001$

nutritional recovery appeared in the Nutrition x Day interaction (Table 19) of rate of crossing squares, $F(2,30)= 3.99, p<.05$. This interaction, illustrated in Figure 10, was due to the ICD rats crossing more squares per sec. than the PCM on the first day of exposure to the open field. On the second day both the ICD and the PCM crossed more squares than the ADN.

In summary the analyses of the results indicated the differences in behaviour among the treatment groups were greater during nutritional deprivation than during nutritional recovery in both the familiar environment and the novel situation. Furthermore, during each of the nutritional treatment periods different behaviours were affected.

TABLE 19

Summary of ANOVA for Number of Total Squares Crossed per Second in a Novel Situation during Nutritional Recovery

Source	df	SS	MS	F
Subjects	10	558592.00	55859.20	
Nutrition	2	46976.00	23488.00	0.53
$Error_a$	20	886160.00	44308.00	
Days	1	349840.00	349840.00	44.77***
Nutrition x Days	2	62368.00	31184.00	3.99*
$Error_b$	30	234416.00	7813.00	
Total	65	2138352.00		

*$p<.05$
**$p<.01$
*** $p<.001$

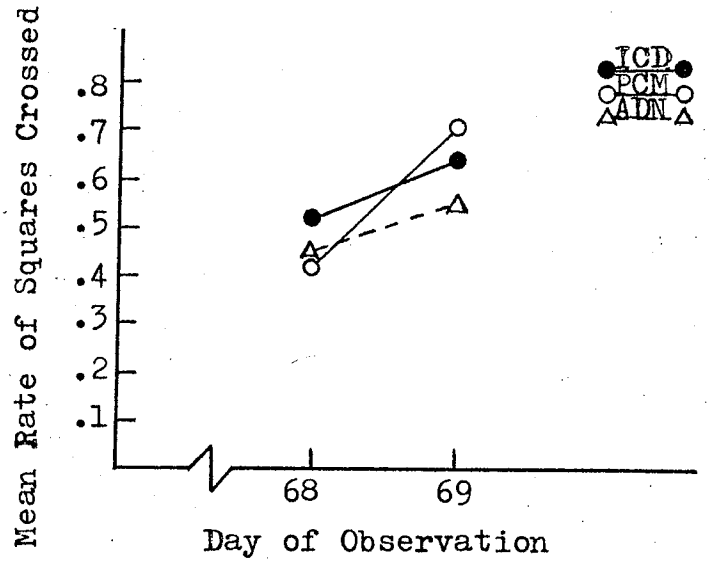

Figure 10. Mean rates of total squares crossed in the open field by each nutritional treatment group during nutritional recovery.

CHAPTER IV

DISCUSSION AND SUMMARY

Differences in behaviour were found to be related to nutrition in both the familiar environment and the novel situation. In the familiar environment both of the nutritionally deficient groups exhibited more vertical behaviours during the first few days of deprivation. The PCM animals engaged in more vertical behaviours (jumping, climbing, hanging and rearing) and showed a higher level of activity (frequency of behaviour changes and activity wheel running) than the other groups throughout Phase A. It may be this greater than usual amount of activity and vertical behaviour which accounts for the use of terms as 'restless, hyperactive, irritable' in descriptions of hungry or protein-calorie malnourished animals. On the other hand, if restlessness is inferred from the frequency of behaviour changes, activity wheel running, and vertical behaviour, then the term also applies to animals of the ICD group because they too showed an increase in these behaviours during the first 15 days of iron-copper deprivation, even though the mean hemoglobin level of the group was falling during this same time. These are not, however, the behaviours one would expect to indicate the usual symptoms of anemia which include 'fatigue and lassitude.' Furthermore, although a reduction in the

occurrence of these behaviours coincided with further drops in Hb levels, the ICD continued to engage in a higher mean frequency of vertical and grooming behaviours, and frequency of behaviour changes than the ADN animals. However, lassitude and fatigue would be expected to imply lower than normal levels of these behaviours.

Examination of the behaviour differences between groups in the familiar environment shows differences to be more frequent between the ICD and the PCM or between the PCM and ADN groups and much less common between the ICD and the ADN groups. Results from this research suggest that anemia can be identified from behavioural symptoms but these symptoms occur early in the development of the deficiency. After the ICD animals reached a mean Hb level of 9.58 their behaviour became very similar to that exhibited by the adequately nourished animals and, hence, no longer distinguishable from normal behaviour. The initial phase of increased activity followed by a return to a 'normal' level of activity found amongst the anemic animals of this study contradicts the general description of anemic animals appearing easily fatigued. Perhaps the difficulty in pinpointing the symptomatic correlates of anemia which Elwood (1973) discussed stems in part from different behaviours being exhibited at different times in the development of the deficiency.

After 24 days on the deficient diet the ICD animals did not differ from the ADN animals on any category of behaviour

and the PCM animals showed less difference in activity wheel running. However, when placed in a novel situation at this time, behaviour differences among nutritional groups again became clearly delineated. A greater degree of 'restlessness' in the PCM rats could be inferred from the greater amount of vertical behaviour and crossing of squares per second shown by them than by the other two groups. On the other hand, the ICD animals, who spent more time than the other groups in horizontal behaviours could be described as showing a sign of 'fatigue,' since the category includes behaviours in which the animal remains on all fours, close to the floor, and not necessarily moving. Finally, the ADN animals spent more time grooming in the open field than the other two groups. Shettleworth (1975) suggested that the tendency to groom in an unfamiliar setting is an indication of a relaxed animal. From the grooming behaviour of the adequately nourished rats they could be described as 'relaxed."

The changes in behaviour found during nutritional deprivation in this experiment are similar to those observed by Bolles (1963), Frankova and Barnes (1968) and Shettleworth (1975). Bolles reported that adult rats redistribute their daily behaviour patterns after nine days of insufficient diet. Hung ry animals were generally more active, they slept less but rested more and groomed less than sated animals. The PCM group in this study groomed less and both deficient groups were more active than the adequately fed group. Shettleworth (1975) found that hungry animals in

a familiar environment showed an increase in frequency of walking and rearing, and a decrease in the frequency of grooming. As with the PCM animals of this study, Fankova and Barnes (1968) reported that female rats that were protein-restricted during preweaning and calorie restricted during postweaning groomed less, crossed more squares, and engaged in more standing up reactions than control animals. They also observed that malnourished females were more active than males and differences between the malnourished and the well-nourished females tended to be less evident.

Analyses of behaviour during nutritional recovery indicated no effects of nutritional treatment on behaviour in the familiar environment except on a few specific days. It is possible as Denenberg (1968) has pointed out and Zimmerman and Zimmerman (1972) have reported that the handling involved in carrying out the experimental procedure in this study provided additional stimulation which may have lessened the effects of the nutritional treatment on behaviour. For example, the experimental procedures may have influenced vertical behaviour and frequency of behaviour changes. A six-day cycle of increased activity was observed in which activity level peaked on each day following assessment of blood hemoglobin.

On the other hand, it is also possible that there are no long-term effects on these behaviours with such mild and short-duration deficiencies. Other research supports this view. Edgerton et.al. (1972) reported that differences in

capacity for activity disappeared between normal and anemic animals after seven days of diet repletion. Bolles (1963) also found a rapid return to normal behaviour distribution patterns with the provision of an adequate diet.

After 24 days of nutritional recovery the carry-over effect was evident only for the rate of crossing squares in the open field. It could be argued that the open field no longer held a novel effect. However, since the rate of square crossing was consistent with the effect of novelty reported by Denenberg (1968) it would seem that the open field continued to provide a novel stimulus. The rats that crossed a great number of squares on the first day of open field exposure Denenberg described as showing low emotionality in the novel situation whereas rats crossing many squares on the second day as exhibiting high emotionality. He also reported that generally rats cross more squares on the first than the second day. The fact that the Day effect was not present in Phase A but was present in Phase B, may indicate a hampering of normal behavioural responses to a novel situation with poor nutrition.

It should be pointed out that though behaviour changes were associated in part to maturation, in this study no attempt was made to assess differences in trends nor to partial out statistically the age factor from the main effect of nutritional treatment. It is recognized that behavioural changes that occur with maturation may have produced variability in the data that could have masked differences between nutritional treatment groups.

The results do demonstrate that the observational technique is sensitive enough to discriminate changes in behaviour. With the adaptation of Shettleworth's (1975) behaviour categories data were obtained which indicated subtle differences in behaviour associated with diet. However, difficulties were encountered with analysis of such detailed data. To make the data more manageable the 28 behaviours were logically grouped into four categories. Consequently, for the sake of manageability, detail was sacrificed. This raises the question of whether these behaviour categories present the same type of weaknesses attributed earlier to psychological constructs. Vertical and grooming categories appeared to contain homogeneous behaviours, but the categories of horizontal and maintenance behaviours may have contained meaningful subcategories. The horizontal category included all behaviour in which the animal's body remains parallel to the ground which included very passive behaviour such as walking, as well as very active behaviours such as darting, hopping and running. The additional breakdown of horizontal behaviours along a passive to active continuum may make the category more useful. Similarly, the maintenance behaviour category may be more meaningfully used if it were divided along an active-passive dimension. An alternative to logical grouping of the 28 behaviours is factor analysis.

Although the observational study of behaviour changes in relation to nutritional treatment has proven fruitful in

terms of data, the detailed results are difficult to explain without a theoretical framework. For example, group differences in vertical behaviour appeared coincident with significant group differences in Hb and BW but not consistently. Lower BW of the PCM animals and lower Hb of the ICD animals both coincided with a higher mean frequency of vertical behaviour in the two groups. With further mean Hb level drops the mean frequency of vertical behaviour also dropped, even though it was still at a level higher than that of the ADN group. Similarly, with continued significantly lighter BW of the PCM group the mean frequency of vertical behaviour remained higher. If the specific behaviours were investigated within the context of an appropriate theoretical framework as, for example, Selye's stress model, the progress of behaviour changes during a developing deficiency could be hypothesized beforehand and then tested.

SUMMARY

During the development of nutritional deficiency the rats receiving the protein-calorie deficient diet engaged in more vertical behaviour, more behaviour changes, more activity wheel running and less grooming behaviour than the other groups. Those rats receiving the iron-copper deficient diet similarly showed frequent behaviour changing and more vertical behaviour but only during the first half of the deprivation period. In the second half of the period the

frequency of behaviour changes and of vertical behaviours reduced to a level similar to that exhibited by the adequately nourished group. The adequately nourished group showed more grooming behaviour than either of the other two groups. In the novel situation of an open field the protein-calorie malnourished engaged in vertical behaviour more frequently than the other groups. The iron-copper deficient engaged in horizontal behaviour most frequently and the adequately nourished showed the most grooming behaviours.

During the period of nutritional recovery the lack of significant differences between groups of rats previously malnourished, except on specific days in the home cage and for the rates of square crossing in the open field, suggested long-term behavioural effects were not associated with the nutritional manipulations of this study.

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Footnote[1]

The reader may wish to exercise caution in attributing significance to the numerous t-tests reported in this section and in Appendix B and C. Various authors (Harris, 1975; Scheffe, 1959) have pointed out, the use of multiple t's may increase the changes of a Type I error occurring, i.e., indicating significant group differences where, in fact, none exist. On the other hand, Carmer and Swanson (1973) have reported that multiple t-tests seem to produce inferences which are as robust as more commonly favored multiple comparisons, such as the Tukey test. Thus, although the validity of some multiple comparisons reported in this thesis could be doubted, it is likely that the general pattern of comparisons, and the bulk of comparisons performed are substantive.

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APPENDIX A

SHETTLEWORTH'S BEHAVIOUR CATEGORIES

Definitions of Behavioural Categories

Action pattern	Description
Walk/sniff	Walking around or standing still and sniffing with at least three feet touching floor.
Open rear	Standing on hind legs with both forepaws off floor and walls.
Wall rear	Standing on hind legs with at least one forepaw touching a wall.
Panel rear	Wall rearing with at least one forepaw touching one of the panels holding feeder and lever.
Scrabble	Scraping with forepaws against a wall while standing erect. Sometimes moving along wall, hopping up and down while doing this. Distinguished from digging at the wall by the fact that each stroke is directed to the side, the head is oriented up rather than toward the wall, and the hamster usually stretches up to his full extent. Also includes climbing where situation permits animal to lift his hind feet clear of the floor (e.g., in wire mesh cage).
Dig	Scraping with forepaws directed in front of face at sawdust, bare floor, or wall.
Hind kick	Pushing backward at sawdust or bare floor with both hind feet at once.
Gnaw	Biting at wall, corner, or edge of any surface.
Freeze	Standing motionless, not sniffing, with at least three feet on floor.
Dart and hop	Moving suddenly and very fast (darting), sometimes leaving floor with all four feet at once (hopping).
Scent mark	Pressing scent gland in flank against (usually) a vertical surface with arched back, raised tail, and at high intensity, distinctive mincing steps.

Definitions of Behavioural Categories (continued)

Action pattern	Description	Action pattern	Description
Wash face	Rubbing forepaw(s) over any part of head. Includes interspersed licking of forepaws.	Pick up sawdust	Sitting in eating posture holding piece of sawdust in forepaws. Biting and pouching or depouching it.
Groom belly and sides	Scratching and/or biting belly, sides, or legs with mouth and/or forepaws.	Eat	Biting and chewing food. In food reinforcement experiments in open field includes pushing Noyes pellets into cheek pouches and depouching them.
Scratch with hind leg	Scratching any part of body with hind foot. Includes interspersed licking of the scratching foot.	Drink	Licking at water spout or dipper containing water.
Shake	Sudden convulsive shaking of whole body.	Hoard[a]	Depouching or dropping food onto food pile. Pushing food around with paws, nose, or mouth. Carrying Purina Chow pellet in teeth.
Yawn and stretch	Self-explanatory. Usually occur together, but stretching can occur alone.	Manipulate nest paper[a]	Holding, carrying, or pushing nest paper with paws and/or mouth. Pouching or depouching nest paper. Standing or lying wholly on the nest paper.
Urinate	Assuming urination posture, stiffened with tail up, usually backed into a corner. Same as lordosis posture of estrus females.	In nest[a]	Standing or lying wholly on the nest paper.
Defecate	Bending over, usually while sitting up, pulling bolus from anus, and depositing it on ground.		

Definitions of Behavioural Categories (continued)

[a] Additional categories used in observations in home cage and in open field when Purina Chow and/or nest paper were available.

Note: From Shettleworth, S. Reinforcement and the Organization of Behaviour in Golden Hamsters: Hunger, Environment and Food Reinforcement. J. Exper. Pshych. Animal Behaviour Processes 1975, 104(1), p. 59.

APPENDIX B

MEANS, T-VALUES AND SIGNIFICANCE LEVELS OF HEMOGLOBIN AND BODY WEIGHT MEASURES

Means and S.d.'s of (a) Hemoglobin and (b) Body Weight for Each Nutritional Treatment Group on Each Day of Testing in Phase A and Phase B

(a) Hemoglobin (Hb)

Phase A							Phase B						
Group	ICD		PCM		ADN		Group	ICD		PCM		ADN	
Day	X	S.d.	X	S.d.	X	S.d.	Day	X	S.d.	X	S.d.	X	S.d.
21	13.2	0.72	13.1	0.68	13.2	0.73	51	14.4	0.91	13.1	1.05	13.5	0.55
27	12.0	0.79	13.9	0.56	13.2	1.14	57	15.1	1.01	13.6	0.97	14.6	0.62
33	11.6	1.03	15.1	1.93	13.8	1.06	63	14.7	0.72	14.7	1.02	14.7	0.64
39	9.5	0.70	15.7	1.09	12.5	0.80	69	14.8	0.78	14.3	0.47	15.0	0.58
45	8.7	0.77	15.0	0.97	13.2	1.05							

(b) Body Weight (BW)

Phase A							Phase B						
Group	ICD		PCM		ADN		Group	ICD		PCM		ADN	
Day	X	S.d.	X	S.d.	X	S.d.	Day	X	S.d.	X	S.d.	X	S.d.
21	46.4	2.95	45.7	2.90	45.5	3.49	51	155.8	10.02	126.7	6.95	150.0	14.50
27	66.1	3.94	51.0	2.44	60.7	8.62	57	172.9	10.39	152.5	10.75	170.1	15.83
33	94.8	3.04	55.4	2.78	85.1	7.84	63	187.3	9.11	176.7	12.79	193.8	18.08
39	106.8	4.93	63.0	2.85	104.6	8.73	69	204.4	11.64	191.4	15.61	207.3	17.63
45	122.9	6.84	75.5	2.06	120.5	8.74							

Summary of Student's t tests of (a) Hemoglobin and (b) Body Weight for All Pairs of Nutritional Treatment Groups on Each Day of Testing in Phase A and Phase B

(a) Hemoglobin (Hb)

Phase A				Phase B			
Day	Treatment Group	PCM	ADN	Day	Treatment Group	PCM	ADN
21	ICD	0.20	0.03	51	ICD	3.04*	2.65
	PCM		0.21		PCM		1.19
27	ICD	6.32**	2.85*	57	ICD	3.33*	1.42
	PCM		1.78		PCM		2.59
33	ICD	5.14**	4.79**	63	ICD	0.03	0.06
	PCM		1.89		PCM		0.07
39	ICD	15.29**	9.07**	69	ICD	1.94	0.45
	PCM		7.49**		PCM		2.95*
45	ICD	16.15**	10.98**				
	PCM		3.97**				

(b) Body Weight (BW)

Phase A				Phase B			
Day	Treatment Group	PCM	ADN	Day	Treatment Group	PCM	ADN
21	ICD	0.54	0.57	51	ICD	7.54**	1.05
	PCM		0.09		PCM		4.57**
27	ICD	10.28***	1.81	57	ICD	4.32**	0.47
	PCM		3.41*		PCM		2.92*
33	ICD	24.19***	0.67	63	ICD	2.15	1.00
	PCM		11.29***		PCM		2.44
39	ICD	24.13***	0.71	69	ICD	2.12	0.44
	PCM		14.31***		PCM		2.14
45	ICD	20.99***	0.70				
	PCM		15.83**				

* $p < .05$
** $p < .01$
*** $p < .001$

APPENDIX C

MEANS, T-VALUES, SIGNIFICANCE LEVELS, AND ANALYSIS OF VARIANCE SUMMARIES FOR BEHAVIOURS WITH SIGNIFICANT ANALYSIS OF VARIANCE F-RATIOS

TABLE 1a

Mean Frequencies and Standard Deviations of Vertical Behaviour (based on 5-sec. observational units) for (a) Nutrition, (b) Day, and (c) Nutrition x Day in the Familiar Environment during Nutritional Deprivation

(a) Nutrition

ICD		PCM		ADN	
M	SD	M	SD	M	SD
5.31	7.19	9.13	8.91	2.90	5.48

(b) Day

Day	M	SD
24	0.45	2.28
27	1.27	2.97
30	0.76	2.72
33	6.09	6.97
36	9.15	7.42
39	10.03	8.83
42	8.00	8.80
45	10.45	10.45

(c) Nutrition x Day

0	0	1.18	3.92	0.18	0.60
0.82	2.71	1.55	3.01	1.45	3.39
1.18	3.92	0.82	2.71	0.27	0.65
7.64	8.58	9.64	5.97	1.00	1.00
10.00	8.46	12.82	6.59	4.64	4.82
7.82	7.85	16.18	7.82	6.09	7.91
5.55	4.95	15.55	9.73	2.91	5.54
9.45	8.21	15.27	7.66	6.64	8.91

TABLE 1b

Student's t-values for Vertical Behaviour for (a) Nutrition, Nutrition x Day and (b) Day in the Familiar Environment during Nutritional Deprivation

(a) Groups Compared

Day	ICD vs. PCM	ICD vs. ADN	PCM vs. ADN
24	0.47	0.07	0.25
27	0.29	0.25	0.04
30	0.14	0.36	0.22
33	0.79	2.63*	3.43**
36	1.12	2.13*	3.25**
39	3.32**	0.69	4.00***
42	3.97***	1.05	5.01***
45	2.31*	1.12	3.43**
Mean over Days	3.78***	2.38*	6.17***

(b) Days

	24 vs. 27	27 vs. 30	30 vs. 33	33 vs. 36	36 vs. 39	39 vs. 42	42 vs. 45
t-values	0.52	0.33	3.40**	1.95	0.56	1.30	1.37

* $p < .05$
** $p < .01$
*** $p < .001$

TABLE 2a

Mean Frequencies and Standard Deviations of Maintenance Behaviour (based on 5-sec. observational units) for (a) Day and (b) Nutrition x Day in the Familiar Environment during Nutritional Deprivation

(a) Day			(b) Nutrition x Day					
			ICD		PCM		ADN	
Day	M	SD	M	SD	M	SD	M	SD
24	27.97	13.25	30.55	10.88	29.82	11.53	23.55	16.67
27	23.15	15.28	21.82	16.98	27.18	14.37	20.36	15.11
30	24.73	14.71	18.09	15.15	31.00	10.84	25.09	15.92
33	12.82	10.46	8.00	7.68	16.27	8.78	14.18	13.17
36	12.88	10.87	10.55	10.04	10.45	8.08	17.64	13.22
39	9.00	10.25	11.82	12.21	4.73	6.13	10.45	10.88
42	15.67	14.20	22.64	12.86	10.55	12.97	13.82	15.02
45	8.21	11.33	6.82	7.64	4.36	7.28	13.45	15.83

TABLE 2b

Student's t-values for Maintenance Behaviour for (a) Nutrition x Day and (b) Day in the Familiar Environment during Nutritional Deprivation

(a) Groups Compared

Day	ICD vs. PCM	ICD vs. ADN	PCM vs. ADN
24	0.14	1.34	1.20
27	1.02	0.28	1.30
30	2.47*	1.35	1.13
33	1.58	1.18	0.40
36	0.02	1.36	1.37
39	1.36	0.26	1.10
42	2.31*	1.69	0.63
45	0.47	1.27	1.74

(b) Days

	24 vs. 27	27 vs. 30	30 vs. 33	33 vs. 36	36 vs. 39	39 vs. 42	42 vs. 45
t-values	1.61	0.53	3.95**	0.02	1.29	2.21	2.47

* $p<.05$
** $p<.01$
*** $p<.001$

TABLE 3a

Mean Frequencies and Standard Deviations of Behaviour Changes for (a) Nutrition, (b) Day, and (c) Nutrition x Day in the Familiar Environment during Nutritional Deprivation

(a) Nutrition

ICD		PCM		ADN	
M	SD	M	SD	M	SD
7.91	7.25	10.09	7.91	6.17	6.39

(b) Day

Day	M	SD
24	2.88	5.64
27	4.64	7.05
30	2.97	4.29
33	12.33	7.32
36	11.33	6.14
39	11.24	6.56
42	7.94	6.16
45	11.12	7.54

(c) Nutrition x Day

ICD		PCM		ADN	
M	SD	M	SD	M	SD
1.73	4.78	3.91	7.23	3.00	4.90
2.55	4.16	4.64	7.81	6.73	8.44
5.00	5.18	1.82	2.40	2.09	4.41
15.36	6.41	15.45	6.38	6.18	8.21
10.55	6.23	13.82	5.36	9.64	6.50
11.0	7.42	14.73	4.80	8.00	5.90
6.82	5.25	10.09	6.52	6.91	6.63
10.27	7.63	16.27	5.31	6.82	6.71

TABLE 3b

Student's t-values of Frequency of Behaviour Changes for (a) Nutrition x Day, Nutrition, and (b) Day in the Familiar Environment during Nutritional Deprivation

(a) Groups Compared

Day	ICD vs. PCM	ICD vs. ADN	PCM vs. ADN
24	0.85	0.50	0.35
27	0.82	1.63	0.82
30	1.24	1.14	0.11
33	0.07	3.69**	3.62**
36	1.28	0.36	1.63
39	1.46	1.17	2.63*
42	1.28	0.04	1.24
45	2.34*	1.35	3.69**
Mean over Days	2.04*	1.62	3.66***

(b) Days

	24 vs. 27	27 vs. 30	30 vs. 33	33 vs. 36	36 vs. 39	39 vs. 42	42 vs. 45
t-values	1.0	1.13	6.37**	0.68	0.06	2.25*	2.16*

* $p<.05$
** $p<.01$
*** $p<.001$

TABLE 4a

Mean Number and Standard Deviations of Activity Wheel Rotations for (a) Nutrition x Day and (b) Day during Nutritional Deprivation

(b) Day			(a) Nutrition x Day					
			ICD		PCM		ADN	
Day	M	SD	M	SD	M	SD	M	SD
27	58.42	38.10	63.82	43.87	43.55	37.05	67.91	32.57
33	115.97	65.86	86.91	61.39	157.36	53.88	103.64	65.02
39	99.39	52.01	78.18	37.41	127.09	61.10	92.91	46.25
45	103.48	47.64	99.18	38.37	102.27	53.38	109.00	53.72

TABLE 4 b

Student's t-values of Activity Wheel Rotations for (a) Nutrition x Day and (b) Day during Nutritional Deprivation

(a) Groups Compared

Day	ICD vs. PCM	ICD vs. ADN	PCM vs. ADN
27	3.08**	0.29	1.72*
33	4.99***	1.18	3.80**
39	3.46**	1.04	2.42*
45	0.22	0.69	0.48

(b) Days

	27 vs. 33	33 vs. 39	39 vs. 45
t-values	7.10***	2.04*	0.50

* $p < .05$
** $p < .01$
*** $p < .001$

TABLE 5

Mean Frequencies and Standard Deviations of Maintenance Behaviour (based on 5-sec. observational units) for Nutrition x Day in the Novel Situation during Nutritional Deprivation

	Nutrition x Day					
	ICD		PCM		ADN	
Day	M	SD	M	SD	M	SD
44	0.18	0.40	1.09	1.38	0.18	0.60
45	1.00	1.26	0.36	0.50	0.64	0.92

TABLE 5b

Student's t-values of Maintenance Behaviour for Nutrition x Day in the Novel Situation during Nutritional Deprivation

	Groups Compared		
Day	ICD vs. PCM	ICD vs. ADN	PCM vs. ADN
44	2.39*	0.00	2.39*
45	1.67	0.96	0.72

* $p<.05$

TABLE 6

Summary of ANOVA of Latency Scores
in a Novel Situation
during Nutritional Deprivation

Source	df	SS	MS	F
Subjects	10	41478.81	4147.88	
Nutrition (N)	2	4254.0	2127.00	0.48
$Error_b$	20	88726.0	4436.30	
Day (D)	1	24940.75	24940.75	19198***
N x D	2	3990.56	1995.28	
$Error_w$	30	37450.25	1248.34	1.60
Total	65	200840.38		

*$p<.05$
**$p<.01$
***$p<.001$

TABLE 7

Summary of ANOVA for Vertical Behaviour in the Familiar Environment during Nutritional Recovery

Source	df	SS	MS	F
Subjects	2	477.35	47.74	
Nutrition	10	38.76	19.38	0.270
$Error_a$	20	1436.57	71.83	
Days	7	1059.51	151.36	2.94*
Nutrition x Days	14	585.00	41.79	0.81
$Error_b$	210	10804.32	51.45	
Total	263	14401.52		

*$p<.05$
**$p<.01$
***$p<.001$

TABLE 7a

Mean Frequencies and Standard Deviations of Vertical Behaviour (based on 5-sec. observational units) Shown Each Day in the Familiar Situation during Nutritional Recovery

	Day	
Day	M	SD
48	4.09	6.92
51	5.33	6.72
54	4.94	6.21
57	9.61	7.50
60	4.73	6.07
63	7.94	7.45
66	4.61	6.04
69	8.61	9.98

TABLE 7b

Student's t-values for Vertical Behaviour Compating Days in the Familiar Environment during Nutritional Recovery

	Days						
	48 vs. 51	51 vs. 54	54 vs. 57	57 vs. 60	60 vs. 63	63 vs. 66	66 vs. 69
t-values	0.70	0.22	2.64*	2.76**	1.81	1.92	2.29*

* $p<.05$
** $p<.01$

TABLE 8

Summary of ANOVA for the Frequency of Behaviour Changes in the Familiar Environment during Nutritional Recovery

Source	df	SS	MS	F
Subjects	10	963.20	96.32	
Nutrition	2	203.96	101.98	2.25
$Error_a$	20	906.20	45.31	
Days	7	1508.48	215.50	4.66***
Nutrition x Days	14	386.58	27.61	0.60
$Error_b$	210	9708.32	46.23	
Total	263	13677.75		

*$p < .05$
**$p < .01$
***$p < .001$

TABLE 8b

Mean Frequencies and Standard Deviations of Behaviour Changes Each Day in the Familiar Environment during Nutritional Recovery

	Day	
Day	M	SD
48	7.79	8.20
51	7.48	7.29
54	7.82	4.87
57	13.85	6.55
60	8.15	6.88
63	12.91	6.81
66	7.79	7.20
69	8.52	6.90

TABLE b

Student's t-values for Mean Frequencies of Behaviour Changes Comparing Days in the Familiar Environment during Nutritional Recovery

	Days						
	48 vs. 51	51 vs. 54	54 vs. 57	57 vs. 60	60 vs. 63	63 vs. 66	66 vs. 69
t-values	0.18	0.20	3.62***	3.42**	2.86*	3.07**	0.44

* $p < .05$
** $p < .01$
*** $p < .001$

TABLE 9

Summary of ANOVA of Activity Wheel Rotations during Nutritional Recovery

Source	df	SS	MS	F
Subjects	10	58747.20	5874.72	
Nutrition	2	28851.46	14425.73	2.10
$Error_a$	20	137694.00	6884.70	
Days	3	34294.49	11431.50	9.36***
Nutrition x Day	6	13070.30	2178.38	1.78
$Error_b$	90	109912.13	1221.25	
Total	131	382569.69		

*$p < .05$
**$p < .01$
***$p < .001$

TABLE 9b

Mean Number and Standard Deviations of Activity Wheel Rotations for Each Day during Nutritional Recovery

	Day	
Day	M	SD
51	115.64	53.86
57	126.64	44.17
63	144.21	49.64
69	157.67	59.73

Student's t-values of Activity Wheel Rotations Comparing Days during Nutritional R covery

	Days		
	51 vs. 57	57 vs. 63	63 vs. 69
t-values	1.29	2.05*	1.57

* $p < .05$

TABLE 10

Mean Frequencies and Standard Deviations of Horizontal Behaviour (based on 5-sec. observational units) for Nutrition x Day in a Familiar Environment during Nutritional Recovery

	ICD		PCM		ADN	
Day	M	SD	M	SD	M	SD
48	15.55	11.41	3.73	6.18	4.64	7.39
51	6.55	7.55	4.00	5.64	2.55	3.70
54	4.00	3.87	2.91	2.88	5.64	8.55
57	8.27	4.08	10.91	8.64	6.64	4.43
60	5.55	7.26	4.73	4.10	5.73	7.71
63	9.36	7.54	6.64	7.76	6.82	6.10
66	5.55	6.64	9.55	10.29	3.91	5.49
69	4.45	4.20	4.55	4.66	7.64	10.07

TABLE 10b

Student's t-values of Horizontal Behaviour for Nutrition x Day in a Familiar Environment during Nutritional Recovery

	Groups Compared		
Day	ICD vs. PCM	ICD vs. ADN	PCM vs. ADN
48	4.00***	3.69**	0.31
51	0.86	1.35	0.49
54	0.37	0.55	0.92
57	0.89	0.55	1.45
60	0.28	0.06	0.34
63	0.92	0.86	0.06
66	1.35	0.55	1.91
69	0.03	1.08	1.05

** $p<.01$
*** $p<.001$

TABLE 11

Mean Frequencies and Standard Deviations of Grooming Behaviour (based on 5-sec. observational units) for Nutrition x Day in the Familiar Environment during Nutritional Recovery

	ICD		PCM		ADN	
Day	M	SD	M	SD	M	SD
48	3.64	5.66	6.73	11.85	13.45	15.55
51	11.64	11.24	8.18	13.46	17.55	14.61
54	9.18	9.89	19.00	10.79	9.64	12.55
57	13.55	9.93	6.64	5.52	10.09	8.84
60	16.82	15.80	3.09	5.58	5.73	10.84
63	9.55	9.21	10.64	10.24	11.09	12.72
66	11.64	13.17	7.27	10.02	9.64	10.64
69	8.27	11.59	7.18	10.41	6.27	10.93

TABLE 11b

Student's t-values for Grooming Behaviour for Nutrition x Day in a Familiar Environment during Nutritional Recovery

	Groups Compared		
Day	ICD vs. PCM	ICD vs. ADN	PCM vs. ADN
48	0.65	2.05	1.41
51	0.72	1.23	1.96
54	2.05	0.09	2.16*
57	1.44	0.72	0.72
60	2.87**	2.32*	0.55
63	0.23	0.32	0.10
66	0.91	0.42	0.49
69	0.23	0.42	0.19

* $p<.05$
** $p<.01$

TABLE 12

Summary of ANOVA for Vertical Behaviour Scores
in the Novel Situation
during Nutritional Recovery

Source	df	SS	MS	F
Subjects	10	1345.12	134.51	
Nutrition	2	42.64	21.32	0.35
$Error_a$	20	1220.69	61.03	
Days	1	122.73	122.73	6.74*
Nutrition x Days	2	50.27	25.14	1.38
$Error_b$	30	546.01	18.20	
Total	65	3327.45		

*$p < .05$
**$p < .01$
***$p < .001$

TABLE 13

Summary of ANOVA for Horizontal Behaviour Scores in the Novel Situation during Nutritional Recovery

Source	df	SS	MS	F
Subjects	10	1216.03	121.60	
Nutrition	2	54.03	27.02	0.45
$Error_a$	20	1189.96	59.50	
Days	1	128.24	128.24	7.50*
Nutrition x Days	2	29.48	14.74	0.86
$Error_b$	30	513.27	17.11	
Total	65	3131.02		

*$p < .05$
**$p < .01$
***$p < .001$

TABLE 14

Summary of ANOVA for Latency Scores
in a Novel Situation
during Nutritional Recovery

Source	df	SS	MS	F
Subjects	10	23166.13	2316.61	
Nutrition	2	3145.31	1572.66	0.52
$Error_a$	20	60754.75	3037.74	
Days	1	8030.13	8030.13	6.65*
Nutrition x Day	2	1862.88	931.44	0.77
$Error_b$	30	36208.19	1206.94	
Total	65	133167.38		

*$p<.05$
**$p<.01$
***$p<.001$

TABLE 15

Mean Number and Standard Deviations of Total Squares Crossed for (a) Day and (b) Nutrition x Day per Second in a Novel Situation during Nutritional Recovery

(a) Day			(b) Nutrition x Day					
			ICD		PCM		ADN	
Day	M	SD	M	SD	M	SD	M	SD
68	479.97	162.82	520.18	118.36	439.09	202.03	480.15	162.47
69	625.58	171.41	650.73	166.19	666.36	167.15	559.64	176.51

TABLE 15b

Student's t-values for Number of Squares Crossed per Second for Nutrition x Day in a Novel Situation during Recovery

	Groups Compared		
Day	ICD vs. PCM	ICD vs. ADN	PCM vs. ADN
68	2.15*	1.05	1.10
69	0.41	2.42*	2.83*

* $p < .05$