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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