1973

Recovery from malnutrition: food preference and neophobia

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RECOVERY FROM MALNUTRITION:

FOOD PREFERENCE AND NEOPHOBIA

By

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B.A., University of San Diego, 1971

Presented in partial fulfillment of the requirements for the degree of

Master of Arts

UNIVERSITY OF MONTANA

1973

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Aug. 2, 1973

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ACKNOWLEDGEMENTS

The author would like to express his appreciation to the members of his thesis commitee for their assistance and consideration: Dr. Robert Zimmermann (Chairman), Dr. Laurence Berger, Dr. Odin Vick, and Dr. Robert Gambs (Social Work).

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CHAPTER 1

INTRODUCTION

Animal research has indicated that several of the more pronounced behavioral abnormalities resulting from early protein-calorie malnutrition appear to be motivational in nature. Nutritionally deprived rats have been reported to exhibit heightened reactivity to stress (Barnes, Moore, Reid, & Pond, 1967), loss of inhibition (Frankova & Barnes, 1968), over-sensitivity to the environment (Barnes, Moore, & Pond, 1969; Frankova & Barnes, 1968), and altered levels of activity (Barnes, Neely, Kwong, Labadan, & Frankova, 1968). Such deficiency-produced alterations in behavior have been reported to persist, even following nutritional rehabilitation. Research with rehabilitated pigs has indicated that nutritional recovery does not appear sufficient to alleviate behavioral abnormalities developed during a period of early protein-calorie malnutrition (Barnes, Moore, & Pond, 1970). These authors report that behavioral differences continue to be manifest for months following the initial nutritional insult, despite extensive periods of nutritional rehabilitation.

Neophobia

Recent research with protein-malnourished rhesus monkeys has pointed out additional motivational aspects of behavior which appear to be significantly affected by early malnutrition. These include "neophobic" reactions in response to novel stimuli (Zimmermann, Strobel, & Maguire, 1970), and apathy toward manipulatory situations (Zimmermann & Strobel, 1969; Strobel & Zimmermann, 1971, 1972). In the latter test, the intro-

duction of food reward immediately brought manipulatory responses to control levels (Aakre, Strobel, & Zimmermann, 1973), suggesting that a salient variable governing manipulative tendencies in the malnourished animal concerns the food-reward contingencies.

Geist, Zimmermann, and Strobel (1972) have noted that malnourished rhesus monkeys also display certain physiological manifestations of early dietary history. These investigators found that protein-malnourished monkeys showed a marked reduction in weight gain when compared to adequately nourished controls. Analysis of blood serum components revealed values of albumin and total protein which consistently reflected the levels of dietary protein fed. These findings are consistent with those of Ordy, Samorajski, Zimmermann, and Rady (1966), who noted that protein-malnourished rhesus monkeys showed lower rates of weight gain, and displayed decreased levels of serum proteins, albumin, cholesterol, and alkaline phosphatase enzyme activity.

Food Preference

Peregoy, Zimmermann, and Strobel (1972) have reported marked differences in the protein preferences of malnourished and adequately nourished monkeys. These investigators found that malnourished animals, when presented with a choice between high and low protein foods, displayed a distinct preference for food high in protein, while tending to avoid foods of lower protein content. Adequately nourished controls, however, did not exhibit this preference for high protein food, selecting all foods at random. Also, the response of protein-malnourished monkeys to novel stimuli was observed to differ from that of controls. In this test, control animals selected more toy-like objects than did

the low protein raised animals, confirming previous findings that malnourished monkeys tend to be less responsive to, or avoid strange stimuli.

Thus, part of the behavioral syndrome associated with protein-malnutrition in the developing rhesus monkey appears to be the acquisition of a preference for high protein food, and the development of an aversion, or apathy toward novel or strange objects. However, evidence regarding whether or not these behavioral abnormalities persist following nutritional rehabilitation has not been reported. The purpose of the present investigation was to examine the effects of nutritional rehabilitation on responsiveness toward diets containing different amounts of protein and reactions to novel stimuli present in the food preference test.

CHAPTER 2

METHOD

Subjects and Diets

Subjects were 10 laboratory-born rhesus monkeys which were approximately 3.5 years of age at the beginning of the experiment. The animals were separated from their mothers at 90 days of age and weaned to a diet containing 25% protein (casein) by weight. At 210 days of age six monkeys (210-low protein group) were transferred to a low protein diet, equivalent in calories to the previous diet, but containing only 3.5% protein. The remaining four monkeys (210-high protein group) were designated as high protein controls and were retained on the 25% protein diet. Weight gain was minimized in the low protein monkeys by shifting them from the 3.5% diet to a 2% protein diet at 728 days of age. The 25%, 3.5%, and 2% protein diets were color-coded green, white, and red, respectively, with non-nutritive food coloring. Details of diet preparation, feeding procedure, and weight gain are given in Geist, et al. (1972).

At 1500 days of age, the malnourished 210-LP animals began nutritional rehabilitation. Rehabilitation consisted of removing the animals from their 3.5% protein diet and gradually transferring them to a high protein (25%) diet. Transition from the low to the high protein diet was extended over a period of eight weeks. During this time period the percentage of protein in the diet was increased from 3.5 to 25 in steps of approximately 4% per week. Diet color coding remained the same with an increasing amount of green (25%) diet being added in biscuit form each week. The first test (at 60 days post rehabilitation) was conducted

when subjects began receiving the 25% protein diet exclusively.

Apparatus

The apparatus for testing preferences (preference wall) was identical to the one described by Peregoy, et al. (1972). This apparatus consisted of 54.6 x 58.4-cm. plywood board replacing one wall of a steel cage which measured 54.6 x 54.6 x 58.4-cm. Mounted on the board were eight bins, 25.-cm. apart and arranged in two rows separated by 5.1-cm. Each bin consisted of a 10.2-cm. square ledge extending horizontally away from a 10.2-cm. square plexiglas window. The window and ledge combinations were hinged at the bottom. Stimuli were placed on the ledges and were visible to the monkey through the window. The monkey made a response by pulling a knob located at the top of a window causing the stimulus to drop from the ledge onto the level window where the subject had access to it. A 0.32-cm. thick opaque Masonite partition was interposed between the animal and the preference wall for the "baiting" of the bins and raised to allow for the presentation of discrete trials.

Procedure

All animals had been trained to open the bins for a piece of sugar-coated cereal (Peregoy, et al., 1972). Each animal showed complete transfer to this apparatus at the first test in this experiment which was conducted 60 days following the initiation of nutritional rehabilitation.

The experiment employed four categories of food and object stimuli: green 25% protein diet, white 3.5% protein diet, red 2% protein diet, and approximately 200 objects constructed of miscellaneous toys ranging up to 7.6-cm. in length. The monkey was placed in the cage with the

opaque door blocking his view of the preference wall. Two 0.5-gm. portions of each of the three diets and two of the objects were individually loaded into the eight bins, employing an 8 x 8 Latin square for randomization. A trial was initiated by raising the partition, and terminated by lowering the partition after one minute. If the monkey opened all of the bins in less than one minute, the partition was lowered after the last bin was opened. Each monkey was given five sessions consisting of 9 one-minute trials. The number of each of the different stimuli removed from the bins was recorded at the termination of each trial.

Subjects were fed 150 to 200 gm. of diet each day late in the afternoon. All food remaining was removed from the cage and drop pan the following morning. Testing was conducted just prior to the afternoon feeding.

Testing

All animals had been tested previously in this apparatus during a period of malnutrition (Peregoy, et al., 1972). In this study the animals were tested 60 and 180 days after the initiation of rehabilitation. Control animals followed an identical schedule of testing. The performance of each animal during rehabilitation was compared to its performance during malnutrition, to determine the effects of the nutritional rehabilitation program.

As part of the standard laboratory procedure, animals were weighed daily as a measure of response to the new diet. Blood samples were taken periodically to evaluate the effect of the rehabilitation program. For details see Geist, et al. (1972).

CHAPTER 3

RESULTS

Weight gain data obtained in this experiment indicates that the initiation of the rehabilitation program resulted in a marked increase in the rate of weight gain of the rehabilitated animals with the previously malnourished Ss gaining 2268 gm within 180 days post-rehabilitation (Figure 1). Immediately prior to the beginning of the rehabilitation period, weight values for the Ss, then malnourished, were approximately 44% of those of adequately nourished controls. However, 60 days following the implementation of a high protein diet, weight values for the rehabilitated animals had reached 54% of those of controls. At 180 days post-rehabilitation, this figure had increased to 65%. Although a significant discrepancy remained between the weights of the two groups following 180 days of rehabilitation, an examination of the sitting heights of the rehabilitated animals revealed that their height-weight ratio was equal to that of adequately nourished controls at an identical height.

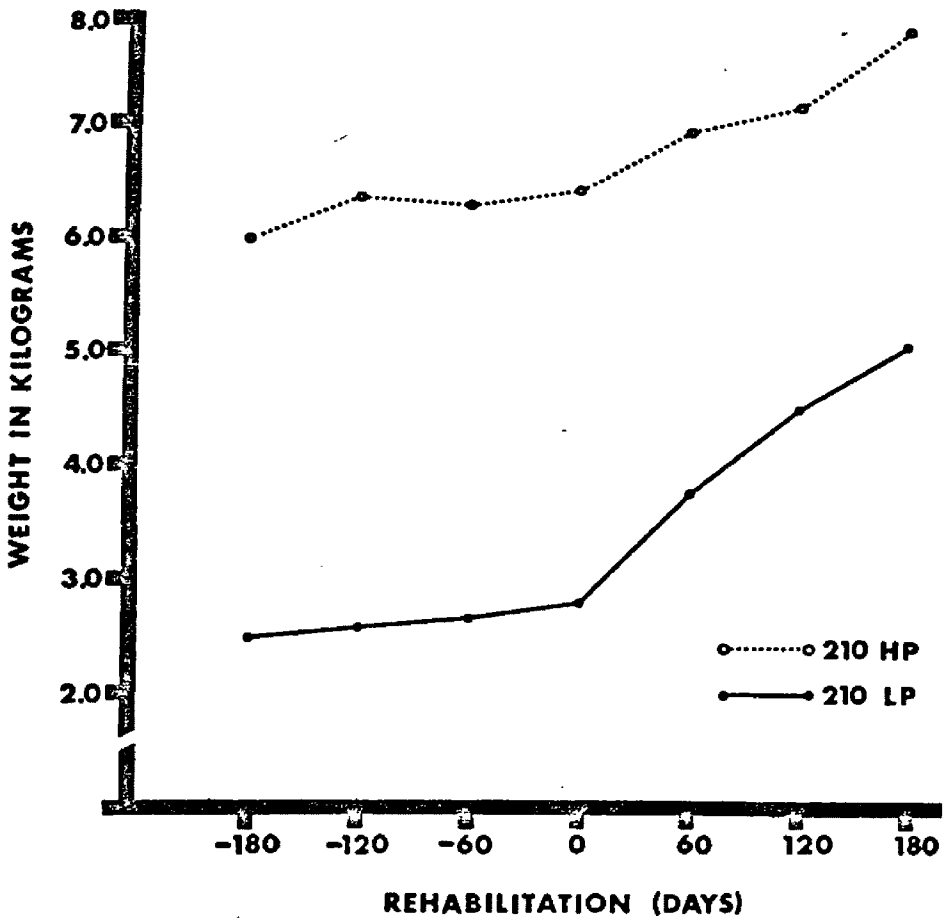
Altered levels of blood serum albumin and total protein were also observed to return to normal expectations following 180 days of rehabilitation. Due to an apparatus failure discovered at approximately 120 days into rehabilitation, the blood sample analysis taken prior to that time were found to be unreliable and were discarded. Samples taken during malnutrition and at 180 days rehabilitation were reliable.

Four separate 2 x 3 analyses of variance employing the least squares method for repeated measures and unequal subject numbers (Winer, 1962)

FIGURE 1

Average Weight Gain of

Rehabilitated and Control Groups



were computed from the total quantity of test items selected by each subject. The statistical procedures were employed to determine the relationships between dietary conditions (high protein and low protein) and treatment levels (malnourished, 60 days post-rehabilitation, and 180 days post-rehabilitation) under each of the test item categories (2%, 3.5%, 25% protein, and toy-like objects).

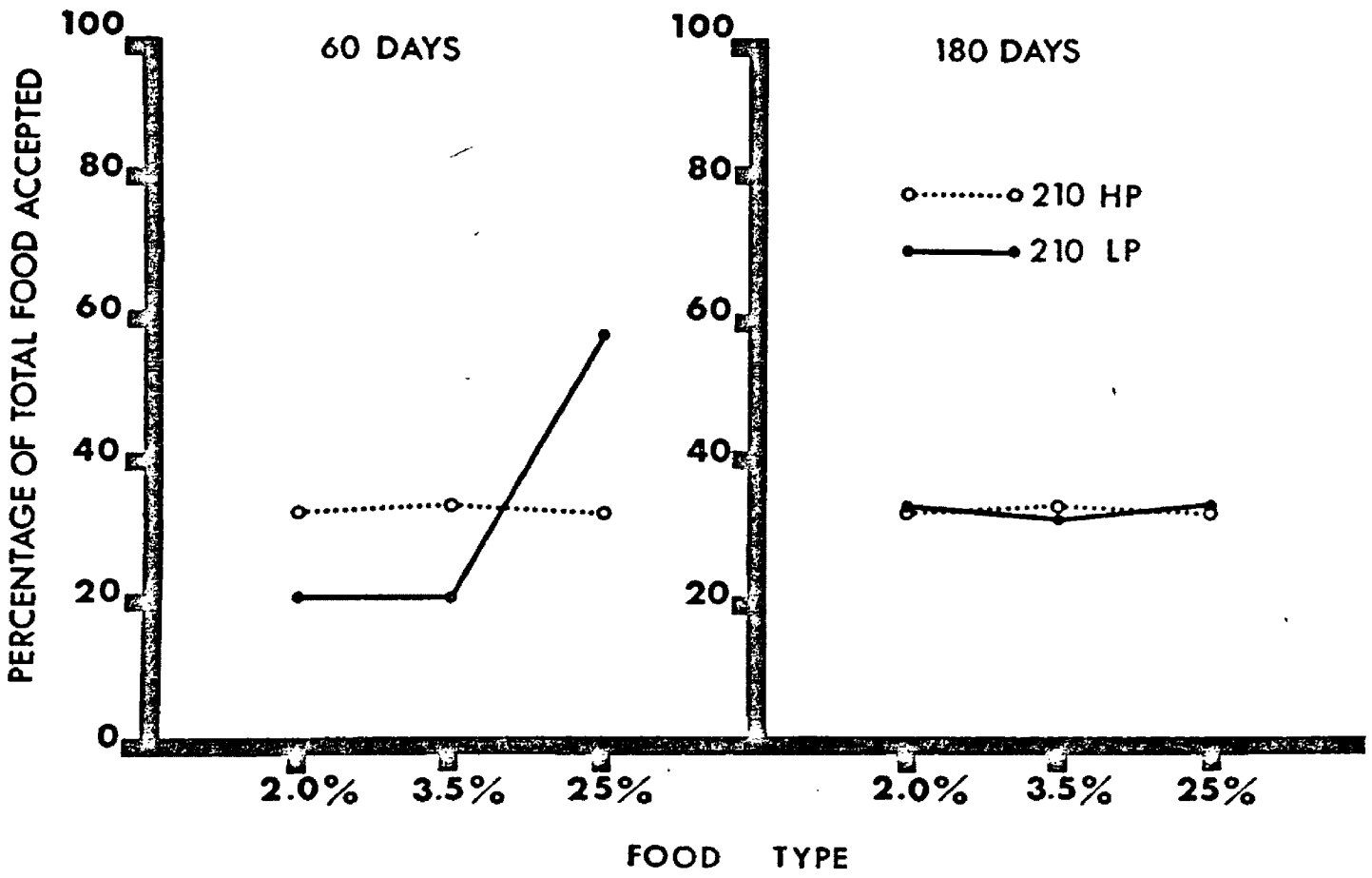
Significant differences were found with respect to the quantity of 2% protein accepted for diet ($F = 32.59$, $p < 0.01$), treatment level ($F = 20.45$, $p < 0.01$). Rehabilitated animals selected significantly smaller amounts of 2% protein when compared to high protein controls. However, as rehabilitation progressed from 60 to 180 days, previously deprived monkeys exhibited a marked increase in selection of the 2% diet until, at 180 days post-rehabilitation, no significant differences were present between the groups (Figure 2). Adequately nourished control animals consistently maintained an equivalent selection preference during the testing conditions, as reported by Peregoy, et al. (1972). Although no significant differences were found for the high protein animals between treatment times, a smaller amount of 2% protein diet was selected for each of the three testing periods. Further, the total quantity of diet chosen by the high protein subjects at 180 days was less than that chosen by the rehabilitated subjects. These differences were such that a diet X treatment interaction was manifest.

Selection of the 3.5% test diet also showed significant differences for all main effects and interaction components. Previously malnourished animals chose markedly less diet when compared to control animals ($F = 18.18$, $p < 0.01$). Further, a significant difference was extracted

FIGURE 2

Food Preferences at 60 Days

Post-Rehabilitation and 180 Days Post-Rehabilitation



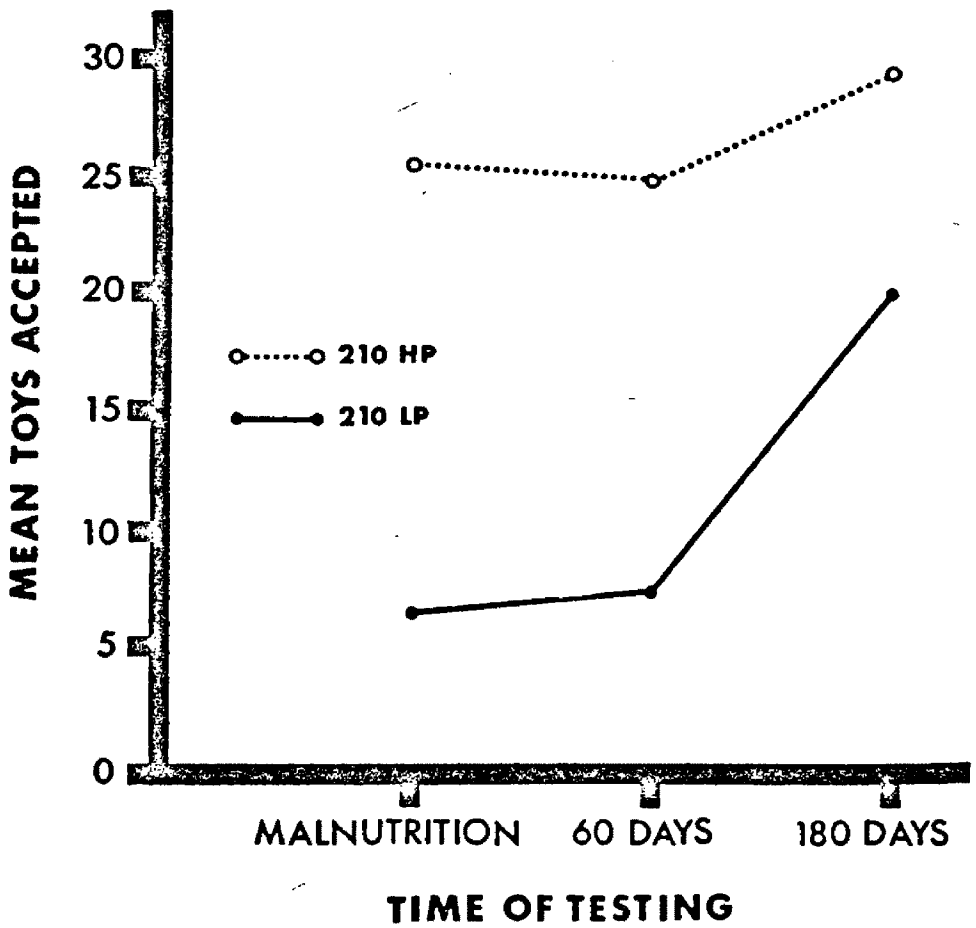
for treatment levels ($F = 7.81$, $p < 0.01$), with low protein subjects selecting similar amounts of diet during malnutrition and at 60 days post-rehabilitation, but significantly more diet at 180 days following rehabilitation (Newman-Keuls, $p < 0.01$). Contrariwise, high protein controls maintained the same preference throughout the treatment conditions. Although equivalent preferences were found during malnutrition and 60 days post-rehabilitation, a significantly increasing linear trend was found from 60 to 180 days post-rehabilitation for all rehabilitated low protein subjects. In contrast, high protein subjects exhibited a decreasing trend throughout these treatment times, resulting in the diet X treatment interactions.

With respect to the selection of 25% protein diet, no significant differences were extracted. Under both of the rehabilitation treatment levels (60 and 180 days), both control and rehabilitated subjects exhibited no specific preference for the 25% diet.

The analysis of variance of the response to toy objects revealed no significant main effects or interaction. The failure to find significant effects appears to be the result of the large amount of variability across test periods by the control animals. However, a non-parametric analysis (Wilcoxon signed-ranks test for related measures) of the net change from the malnourishment to 180 day test conditions for the two groups revealed that the rehabilitated animals showed a significant increase in response to toy objects in the 180 day condition ($p < 0.05$). This increase in responsiveness by rehablitated subjects is shown in Figure 3.

FIGURE 3

Selection of Toy-Like Objects During Malnourishment, 60 Days Post-Rehabilitation, and 180 Days Post-Rehabilitation



CHAPTER 4

DISCUSSION

The results indicate that several of the physiological correlates of early protein malnutrition, including reduced rate of weight gain and decreased levels of blood serum albumin and total protein, return to control levels following the period of nutritional rehabilitation measured. Although several factors are apparently involved in the recovery of the malnourished animal, such as the length of nutritional deprivation, severity of insult, and time when imposed (i.e. pre- or post-weaning), one of the most critical variables appears to be the length of the rehabilitation period. In the present study, rehabilitated animals continued to manifest marked increases in rate of weight gain even following 180 days of nutritional rehabilitation. This fact raises the possibility that prolonged nutritional rehabilitation may be necessary in order to alleviate abnormalities in weight gain resulting from nutritional deprivation.

During the malnutrition condition, developing monkeys have been reported to demonstrate abnormalities in manipulatory responsiveness (Zimmermann & Strobel, 1969; Strobel & Zimmermann, 1971), curiosity (Zimmermann & Strobel, 1969), dominance behavior (Wise, 1972), reaction to novel stimuli (Zimmermann, et al., 1970; Strobel & Zimmermann, 1971; Peregoy, et al., 1972), and food preference (Peregoy, et al., 1972). An important question regarding such nutritionally induced behavioral characteristics involves whether these deviant behavior patterns are maintained during nutritional rehabilitation. The results of the present

investigation suggest that altered food preference and aversion to novel stimuli tend to disappear during six months of nutritional rehabilitation. A significant trend toward the emergence of equivalent food preference behavior between rehabilitated and control animals was noted only 60 days following the imposition of a nutritionally adequate diet. The neophobic response of the malnourished rhesus monkey, as manifest in its aversion to novel stimuli, was found to persist for 60 days of rehabilitation, but disappeared by 180 days, indicating that the reappearance of adient responses to novel stimuli may be dependent upon an extensive period of nutritional recovery. Further research is required to determine whether nutritional rehabilitation, and not time alone, resulted in this change in responsiveness to novel stimuli.

The results of this study indicate that at least part of the behavioral syndrome associated with malnutrition, as well as some concomitant physiological parameters, are corrected by six months of nutritional rehabilitation. Similar results have been reported in studies with humans. Pollitt (1972), in a review of research dealing with the effects of kwashiorkor on mental functioning in children, concludes that this form of malnutrition does not necessarily result in irreversible intellectual impairment. The diet used in this experiment was similar to that used by Ramalingaswami (1968) to induce a kwashiorkor-like syndrome in rhesus monkeys. Thus, as the children reported by Pollitt (1972) appear to have recovered mental function after nutritional rehabilitation. The monkeys in the present experiment appear to recover normal motivation for food and novel stimuli following nutritional rehabilitation, similar to the recovery of mental function in children reported by Pollitt (1972).

CHAPTER 5

SUMMARY

Six protein malnourished monkeys were nutritionally rehabilitated by placing them on diets high in protein content. At 60 and 180 days following the initiation of the rehabilitation program experimental animals, along with four dietary controls, were run in a preference test designed to look at the reactions of the two groups to novel stimuli and to foods containing various amounts of protein. Each monkey was presented a choice situation involving diets containing 2%, 3.5%, and 25% protein, along with non-food objects. In addition, weight gain was monitored and blood samples taken periodically to determine the response of the animals to the rehabilitation program. The results indicated that 180 days of nutritional rehabilitation were sufficient to restore total body weight and blood serum level to normal expectations. Also, the preference for high protein food and the aversion to novel stimuli (neophobia) previously reported in protein malnourished monkeys were not found to persist following the period of rehabilitation measured. The results appear to indicate that at least part of the behavioral syndrom associated with malnutrition, as well as some concomitant physiological parameters, are corrected by six months of nutritional rehabilitation.

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