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ABSTRACT

Perceptual motor development, habituation, and learning in squirrel monkeys were studied under controlled rearing and diet history conditions to determine whether the animal's level of behavioral development was similar to well-nournished animals of his own age (agemates) or his own size (sizemates). From birth to 8 weeks of age, the animals were tested weekly on two items of perceptual motor development, visual locating of a click and visual following of an object. At 18 and 52 weeks of age, they were tested in a standard visual habituation-dishabituation paradigm, and from 44 to 52 weeks, they were testing using a two-choice color discrimination learning procedure. Results indicate that early protein deficiency had a strong retarding effect on early growth, and a significant but less extreme effect on perceptual motor development. Protein deficient animals were retarded compared with agemates but precocious compared with sizemates. The behavioral effects associated with protein deficiency persisted for at least 10 weeks after the diet restriction. (GO)

EFFECTS OF PROTEIN RESTRICTION ON PERCEPTUAL-MOTOR DEVELOPMENT, HABITUATION AND LEARNING

Read by Marjorie F. Elias

The possibility of deficiencies in cognitive development is a major concern of those who study or treat malnutrition in human infants. In order to assess characteristics of the monkey infant which might bear some relation to cognitive development in humans, three aspects of behavior related to taking in information and regulation of behavior were studied: perceptual-motor development, habituation and learning.

Since it was possible to control both rearing conditions and diet in our research design, we could study the impact of each factor separately on behavior both during the period of restriction and later during rehabilitation. Although squirrel monkeys have not been studied in this way before, some effects of isolated rearing and/or nutritional deprivation have been investigated in rhesus and cebus monkeys. Harlow and his associates have studied rhesus monkeys during and after isolated rearing and have found many effects on social and sexual behavior, but no consistent deficiency in learning. Acutely malnourished and rehabilitated malnourished rhesus were tested on learning rate by Zimmermann and Strobel at Montana and by Harlow and co-workers at Wisconsin. Neither group reported effects on simple two-choice discrimination learning but the Montana investigators did find slower learning when the task was made more complex among currently malnourished monkeys. In our own laboratory, in studying cebus monkeys under conditions of acute protein deficiency and isolated rearing, we have found delays in perceptual-motor development, more frequent occurrence of stereotypy, slower adaptation to a novel setting, and deficiencies in exploratory behavior. Those behavioral differences were associated more strongly with the rearing restriction than with the nutritional restriction, but were most extreme for animals who were restricted in both ways at once. The measures to be reported on today for squirrel monkeys have not been used with the cebus monkeys, but will be used with them during the coming year.

In looking at human studies, delayed development as measured by infant developmental scales is a common finding among children who are malnourished and suffer from multiple environmental problems. The developmental scales include many items of perceptual-motor development including those used in the present study. A habituation-dishabituation study, using a paradigm of heart rate deceleration to a repeated auditory stimulus, gave evidence that dishabituation or orienting to a change of pitch was delayed or absent in malnourished infants (lester, 1973). Visual habituation to a checkerboard is being studied at present in malnourished infants in Bogota, Colombia. Two-choice discrimination learning has not been used as a measurement technique in malnutrition studies with humans.

Neither in children nor in monkeys has the extent of retardation in behavior been compared with the extent of retardation in physical growth. When the growth of various organs of the body has been compared, growth deficiency has been found to be most severe in musculature and least in the brain. This finding has been described by the term "brain sparing." By comparing behavioral development of malnourished animals with controls of the same body size but younger age (sizemates) as well as with controls of larger body size but the same age (agemates), one can determine whether there is "behavioral sparing" with respect to deficiency of physical size;

whether an animal's level of behavioral development is similar to well-nourished animals of his own age or of his own size.

Method

From birth to 8 weeks of age the animals were tested weekly on two items of perceptual-motor behavior; visual locating of a click and visual following of an object. The tests were adapted from items used in human infant development tests.. (Slide 1 - click photo). The animal was picked up, bottle fed, and then laid down facing away from the tester. A metal click toy was held about 6 inches above his head and was clicked 5 times at 3 sec intervals. His response was scored on a 5-point scale ranging from "no response" to "locates by eye." The animal was then moved so that he faced the tester (Slide 2 - follow photo). The click toy was moved in an arc around his face about 3 inches from his eyes. In positioning and moving the object the tester did not attempt to be constant but tried to catch and hold the infant's gaze.

At 18 and 52 weeks of age the animals were tested in a standard visual habituation-dishabituation paradigm (Slide 3 - checkerboards photo). Animals were presented with one pattern for 10 45 sec trials with 10 sec intertrial intervals. On trials 11 and 12 a different pattern was presented. The patterns were both black and white checkerboard designs, differing only in number and size of squares. One contained 64 small squares, the other 4 large squares. The animals' direction of gaze was observed and the time spent looking at the pattern during each 45 sec trial was recorded (Slide 4- lazy susan photo).

From 44 to 52 weeks of age the animals were tested in a two-choice color discrimination learning procedure in a modified WGTA. Pellets of food of the regular diet were covered by wooden blocks which were half covered in one of four colors and half in white. The blocks and food wells were on a lazy susan--a rotating circular tray. For baiting the food weels, the experimenter rotated the tray until the wells were in front of her and hidden from the monkey by a small black curtain (Slide 5 - monkey view of blocks photo). She then turned the tray until the food wells came around the edge of the curtain and into position next to the wire partition on the animal's side. The rotating motion of approach of the tray was used because it produced less withdrawal in these monkeys than did the conventional WGTA with guillotine door or direct approach of a tray. Thirty trials were presented daily to a criterion of 27/30 trials correct. The learning paradigm was designed in 6 stages to test discrimination learning of a color, maintenance of the discrimination when the blocks were turned so that the white half of the block was toward the monkeys' fingers, and reversal of the reinforcement contingency.

Results

The weekly tests of perceptual-motor development revealed significant retardation in currently protein-deficient animals. (Slide 6- graph cumulative freq.). Data were analyzed in terms of age at which the items of behavior were acquired. Age of acquisition was defined as the week in which an animal first obtained the maximum score and subsequently obtained it every week except one. The one exception was allowed, to avoid giving undue weight to one episode of inferior performance. The figure shows the cumulative frequency of acquisition of the skills. Only one animal had already acquired the skill of locating a click by 2 weeks of age. All members of the control diet group acquired that skill by 6 weeks, whereas one member of the protein-restricted group had not yet acquired it at 8 weeks. The difference in mean age of acquisition was significant by t-test at the 0.01 level of significance. The animals acquired the skill of visual following about a week later than locating a click on the average. Ten of the 11 animals in the control diet group acquired it by 8 weeks, in contrast to 7 animals in the protein-restricted group. The difference in mean age of acquisition was significant here, too, at the 0.01 level of significance.

Within the protein-restricted group, age of acquisition of the skill of locating the click was significantly associated with weight at 8 weeks ($r = 0.65$; $P < 0.05$), indicating that the smallest were the most retarded in acquiring the skill. However, when compared to their sizemates who were only 3 weeks of age, the deficient animals were precocious in that many more of them had acquired the skills. Their retardation in physical growth was more severe than their retardation in acquisition of these behavioral skills.

(Slide 7 - Habituation - All). At 18 weeks of age, 10 weeks after the end of the nutritional restriction, habituation was tested and the expected decrease in visual attention was found over 10 repeated presentations of the pattern. As can be seen in the figure, the monkeys' looking time decreased from trial 1 to trial 10 and increased on trial 11 when a different pattern was presented. Both the decrease and subsequent increase were significant changes.

(Slide 8 - Habituation - Diet groups). When the 6 treatment groups were compared, it was found that previously protein-deficient animals had significantly less total looking time summed over the first 10 trials than had well-nourished animals ($P < 0.05$). Looking time on trials 6 and 7 was significantly less when each trial was analyzed separately. There were no differences in total looking time associated with rearing conditions or with the interaction of rearing and diet, so the rearing conditions are pooled in the figure.

(Slide 9 - Habituation - 52 weeks). At one year of age, the animals were retested with the same habituation procedure. Significant habituation and dishabituation were found again, as measured by looking time, but at this age--44 weeks after the nutrition restriction had ended--no differences were found between the treatment groups.

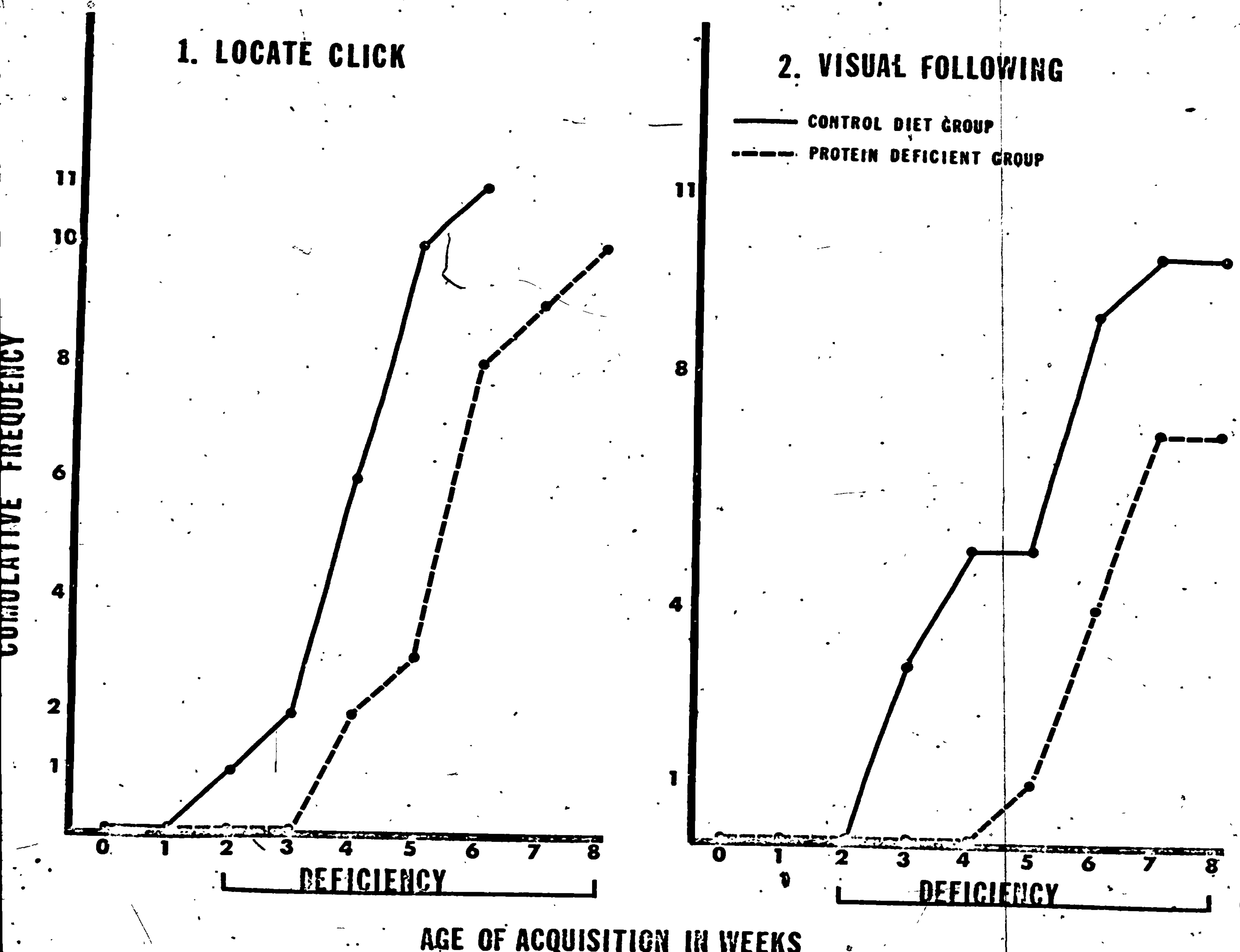
(Slide 10 - Learning - Handled only). The learning procedures were carried out between 10 and 12 months of age for each monkey. The data--number of trials to criterion--were transformed by a log + 1 transformation to normalize the distribution of scores and improve homogeneity of variance. Since within-group variability was still very large in the isolated rearing groups and the non-handled protein-deficient group had been reduced to one animal by deaths, however, statistical comparison could only be made within the handled-rearing group. Of the 8 animals reared in that condition, the 4 who had been protein restricted 8 months earlier were significantly slower to learn when all stages of learning were combined ($F = 10.8$; $P < 0.01$). When each stage of learning was examined separately, they were found to be significantly slower on the first reversal ($t = 2.94$; $P < 0.05$). They were not significantly slower in any other stage of learning.

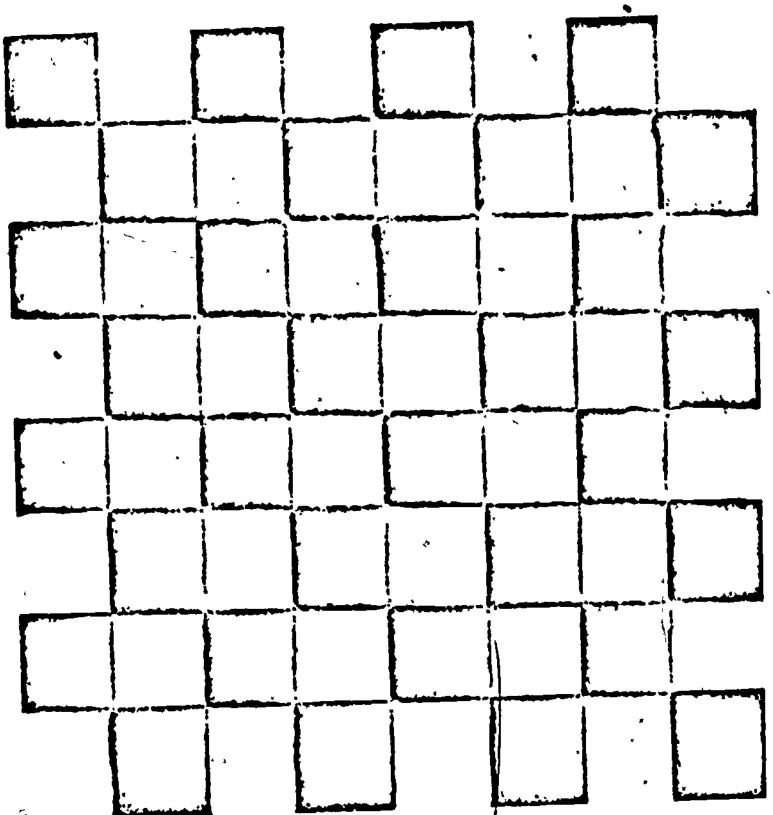
Discussion

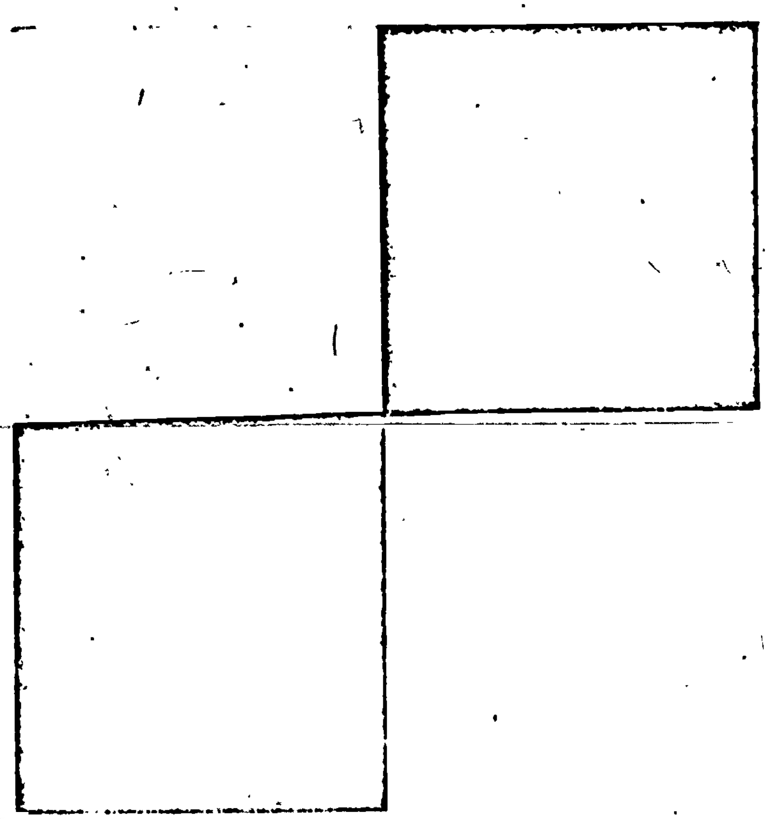
It has been shown that protein deficiency early in infancy increased mortality and had a strong retarding effect on physical growth. It had a significant but less extreme effect on perceptual-motor development during acute restriction. Protein-deficient animals were retarded compared to agemates but precocious compared to sizemates, so some behavioral sparing was found. It would be interesting to analyze developmental data from humans in terms of sizemate as well as agemate comparisons since growth retardation is the usual indicator of mild or moderate malnutrition. The children might be found to be much less severely affected behaviorally than physically.

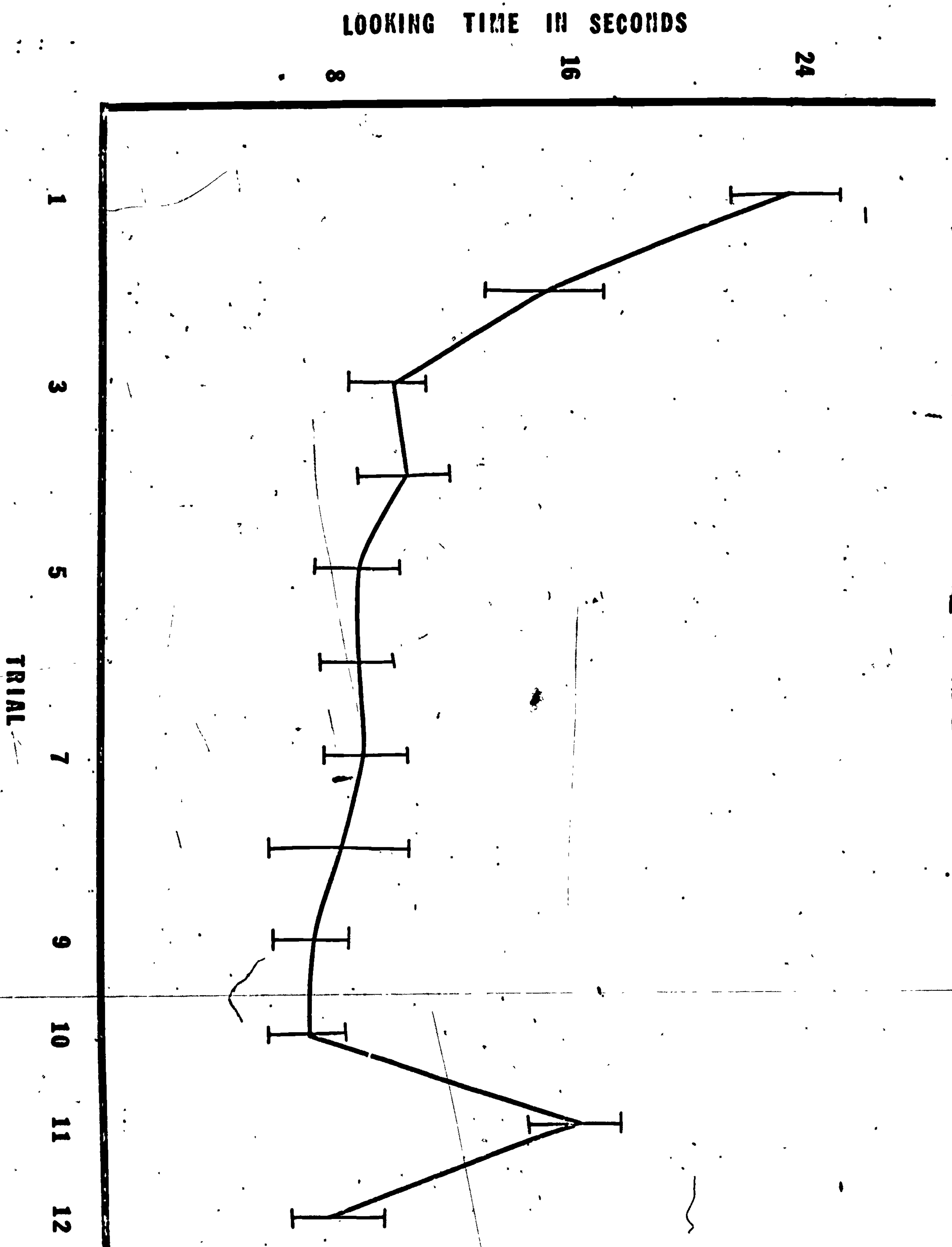
Behavioral effects were found to persist for at least 10 weeks after the end of the restriction, in that the previously restricted animals spent less time taking in visual information about the checkerboard pattern during the habituation tests. Unlike the acutely malnourished infants in Lester's study, however, our rehabilitated monkeys did show significant dishabituation when a different pattern was presented. No differences in habituation associated with the different rearing conditions were found. By one year of age all treatment effects on habituation had disappeared.

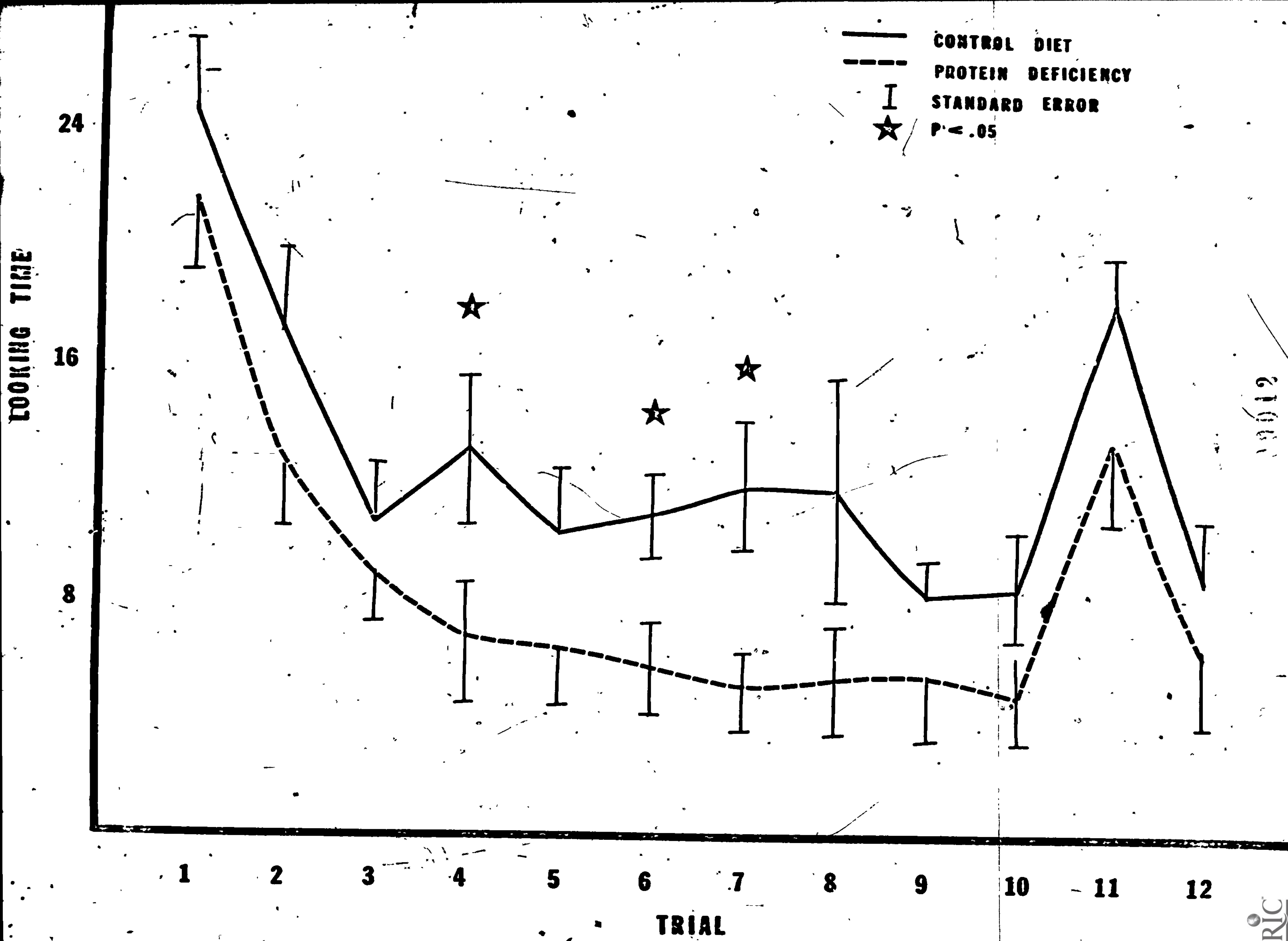
The difference in reversal learning in one rearing group 8 months after the nutritional restriction is too limited a finding to merit interpretation, and the possibility of differences in learning rate associated with rearing differences was not tested. If the effect associated with early protein deficiency should prove to be replicable in another group of animals, it would provide stronger evidence than has been found before that protein deficiency early in infancy did produce a persistent effect on learning ability many months later, but these findings can only suggest that. We can conclude on the basis of the findings reported today that the behavioral effects reported here associated with protein deficiency were found during and up to 18 weeks of age, 10 weeks after the period of restriction.


CUMULATIVE FREQUENCY
1. LOCATE CLICK
2. VISUAL FOLLOWING
CONTROL DIET GROUP
PROTEIN DEFICIENT GROUP
DEFICIENCY
DEFICIENCY
AGE OF ACQUISITION IN WEEKS






LOOKING TIME IN SECONDS
8
16
24
TRIAL
1
3
5
7
9
10
11
12


CONTROL DIET
PROTEIN DEFICIENCY
STANDARD ERROR
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LOOKING TIME
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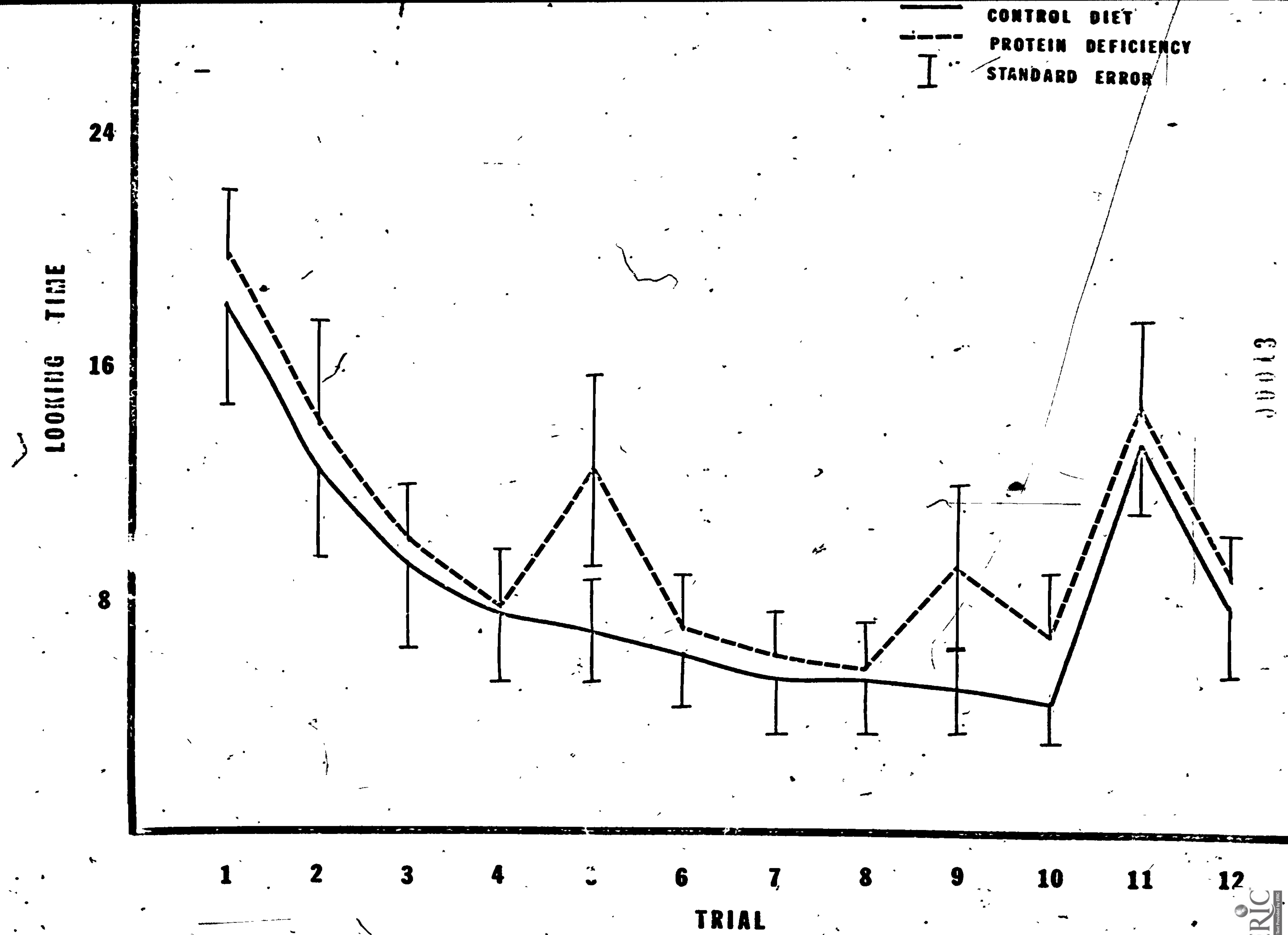
TRIAL



CONTROL DIET
PROTEIN DEFICIENCY
STANDARD ERROR
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LOOKING TIME
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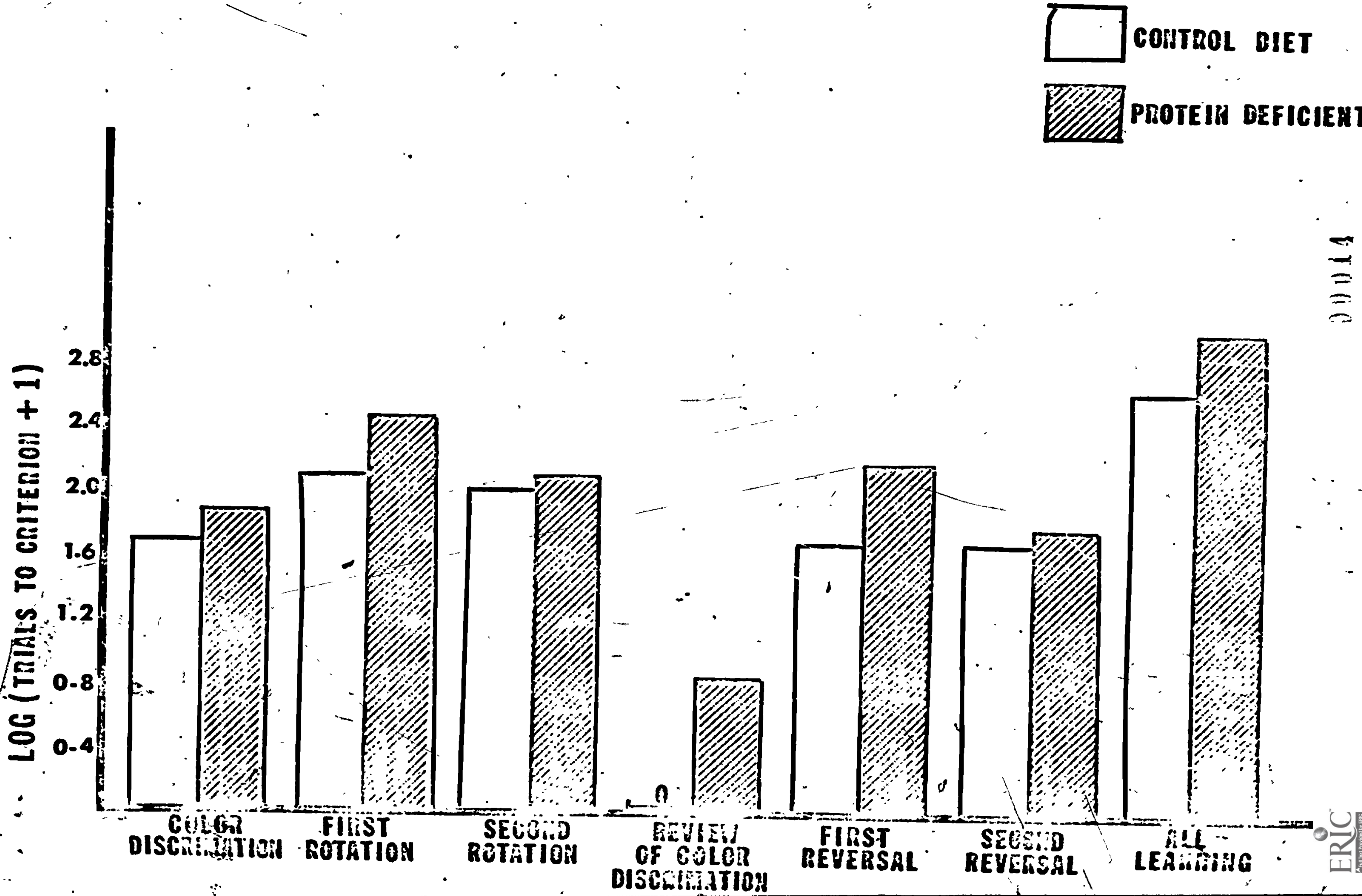
TRIAL



CONTROL DIET
PROTEIN DEFICIENT
LOG (TRIALS TO CRITERION + 1)
2.8
2.4
2.0
1.6
1.2
0-8
0-4
0
COLOR DISCRIMINATION
FIRST ROTATION
SECOND ROTATION
REVIEW OF COLOR DISCRIMINATION
FIRST REVERSAL
SECOND REVERSAL
ALL LEARNING