Bulletin of the Psychonomic Society
1989, 27 (4), 345-347

The effects of a tryptophan- and protein-deficient diet upon growth in rats

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The effects of a tryptophan- and protein-deficient diet (i.e., corn grits) upon growth in rats was investigated. The results indicated that animals exposed to this diet from birth until 75 days of age were significantly lighter than were animals exposed to a normal diet and animals exposed to the grits diet from weaning until 75 days of age. Administering the normal diet to the grits-fed animals resulted in weight gain. Thus, the effects of such dietary deficiencies appear to be reversible.

The experimental evaluation of the effects of low-protein diets upon retarded mental capacities has been of interest for nearly 25 years. Several earlier studies (e.g., Cravioto & Robles, 1965; Kaplan, 1972; Winick, 1976) reported data supporting the contention that if such diets are experienced during development, reduced mental functioning may result.

However, such data were based upon correlations gathered from field studies of human subjects. When such effects were subjected to more rigorously controlled experiments using animals as subjects, differences in learning ability were not always forthcoming. For example, Zimmerman, Geist, and Strobel (1975) failed to report differences in delayed-response, learning-set, object-discrimination, and simple reversal-learning tasks in monkeys. Likewise, Remley, Armstrong, Gilman, and Mercer (1980) reported that rats raised on a protein-deficient diet from weaning to maturity did not differ from normal littermates in terms of two-way active avoidance and black-white visual discrimination performance. Hence, the question of diminished cognitive capacities awaits further experimental verification.

Examination of the Remley et al. (1980) data yields a second point of interest—the growth data of their experimental animals. The general procedure employed by these investigators involved placing the selected animals on an exclusive diet of corn grits at weaning. A diet of corn grits was selected because it would simulate a more natural diet that was deficient in the desired nutrients. The control groups, each of which comprised littermates, were maintained on the normal diet of Purina Laboratory Chow. The results were dramatic. While no weight differences existed at 30 days of age, pronounced differences had developed by 60 days. The average weight of the normal males was 3.16 times more than that of the grits-fed males, while the normal female animals weighed 2.23 times that of their grits-fed counterparts. When the animals were weighed at 90 days of age, these differences were found to be more extreme.

Unfortunately, these were the only weighings reported by Remley et al. (1980). Hence, a complete picture of the growth curve of animals raised under these conditions has yet to be developed. Additionally, the effects of reversing the experimentally imposed dietary deficiency have yet to be reported. The present experiment was designed to address both of these issues.

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METHOD

Subjects and Apparatus

Four sperm-positive female rats were purchased from the Holtzman Company. Upon arrival in the laboratory, the pregnant dams were individually housed in a 10-gal aquarium with a floor of San-I-Cel animal bedding material. Purina Laboratory Chow and water were available during gestation. At birth, all litters were maintained in the aquariums until weaning (21 days old), at which time all animals were transferred to individual, suspended, wire-mesh cages in the animal vivarium.

The grits diet consisted of Quaker Instant Grits and warm water, mixed together into a thick, soupy consistency. Instant grits were used to avoid the ingestion of raw starch. To insure adequate and consistent consumption of the grits diet, .30 g of sodium saccharin were added to each 200 g of dry grits. According to Orr and Watt (1957), corn grits contain .053 g of tryptophan per 100 g and are composed of 8.70% protein. All foods were presented on a free-feeding basis during all phases of the experiment.

Procedure

At birth, one litter ($n = 11$) was randomly selected as the *grits/birth* group. By switching the diet of the nursing dam to grits at the birth of the pups and by providing this diet to the pups in their individual cages upon weaning, we maintained this group on the tryptophan- and protein-deficient diet from birth on.

The remaining 3 dams (and their litters) were maintained on Purina Laboratory Chow until weaning. At this time two groups, *grits/weaning* (8 males, 5 females) and *control* (7 males, 7 females), were randomly formed from these litters. The subjects in the grits/weaning group were placed on the grits diet when they were transferred to their individual cages at weaning. The subjects in the control group were maintained on laboratory chow for the duration of the experiment.

To evaluate the effects of reversing the growth defects engendered by the grits diet, all grits/weaning animals were placed on the normal, laboratory chow diet at 75 days of age. Due to the death of 9 of the 11 grits/birth animals between 85 and 110 days of age, it was not possible to produce comparable and reliable data concerning the effects of diet reversal for this group. The experiment was concluded when the animals were 183 days old. All animals were individually weighed every 2 days.

RESULTS

The mean weight of each litter from 3 days until 21 days (weaning) is shown in Figure 1. While all subjects gained weight, the grits/birth animals appeared to be significantly lighter than the animals that were nursed by dams maintained on a normal diet. Analysis of these data yielded significance for the groups [$F(3,34) = 2574.60$, $p < .001$], blocks [$F(8,272) = 1860.93, p < .001$], and groups × blocks [$F(24,272) = 149.37$, $p < .001$] effects. Subsequent Newman-Keuls tests, used to probe the interaction, indicated that while Litters 1, 2, and 3 did not differ reliably from each other, they were significantly ($p < .01$) heavier than the grits/birth animals from Block 5 through Block 9.

The group mean weights of the control and grits/weaning animals, as a function of gender, from weaning until 75 days of age are shown in Figure 2. While it is clear that all groups gained some weight during this period, it also is evident that the control animals gained more weight than did the grits/weaning animals. Moreover, the male control subjects appeared to gain more weight than did their female counterparts during this period. This male-female difference was suggested only slightly for the grits/weaning animals. Analysis of variance, which yielded significance ($p < .001$) for all factors, including the groups × gender × blocks interaction [$F(8,184) = 10.82, p < .001$], provided statistical support for the graphical impression created by Figure 2. The results of subsequent Newman-Keuls tests indicated that the control animals were significantly ($p < .01$) heavier than the grits/weaning animals by Block 3. Moreover, the control males were found to be significantly ($p < .01$) heavier than the control females by Block 5.

Figure 3 compares the growth of the grits/birth, grits/weaning, and control groups from weaning until 75 days of age. The grits/birth animals remained extremely

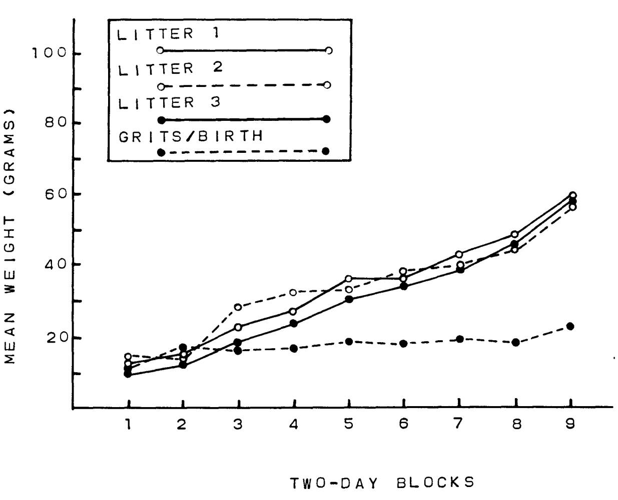

Figure 1. Group mean weight (grams) of the grits/birth and three normally maintained litters from 3 days of age until 21 days of age (weaning).

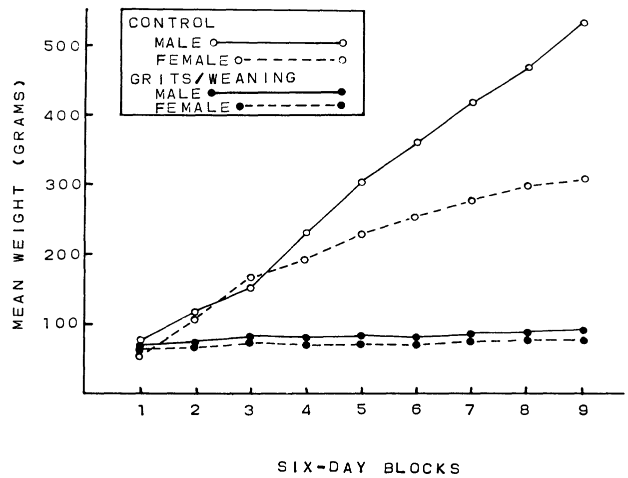

Figure 2. Group mean weights (grams), as a function of gender, for the grits/weaning and control animals from 21 days of age (weaning) until 75 days of age.

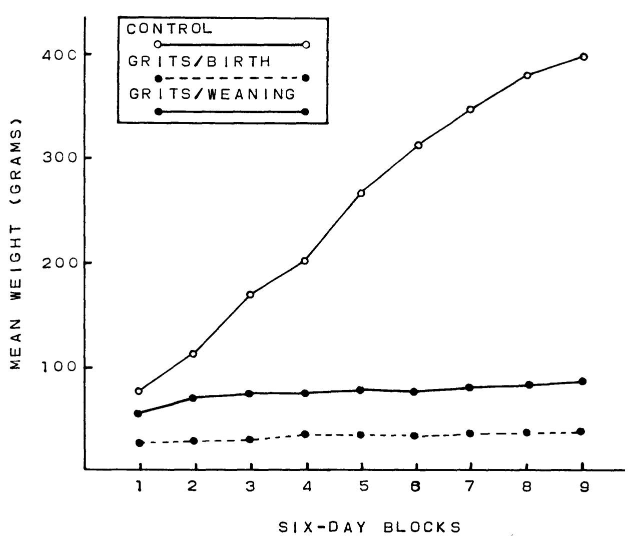

Figure 3. Group mean weight (grams) of the grits/birth, grits/weaning, and control groups from 21 days of age (weaning) until 75 days of age.

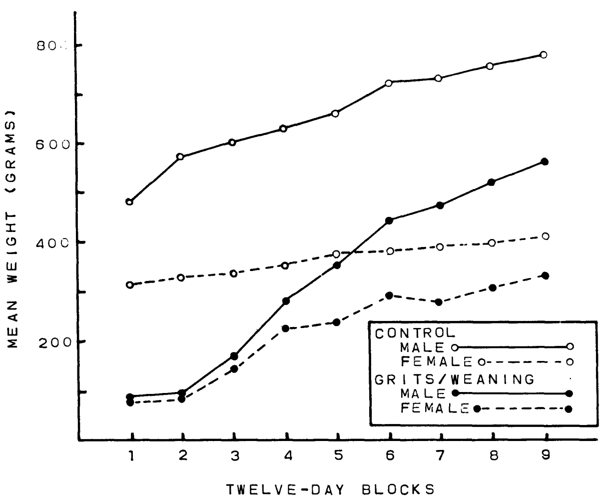

Figure 4. Group mean weight (grams), as a function of gender, of the control and grits/weaning groups from 75 through 183 days of age.

sexually immature during this period, which made it difficult to accurately sex them. Hence, the comparisons including these animals were collapsed over the gender factor. Despite the additional error variance created by pooling over gender, the analysis of these data yielded significance for the groups [$F(2,31) = 139.00, p < .001$], blocks [$F(8,248) = 52.99, p < .001$], and groups × blocks [$F(16,248) = 41.36, p < .001$] effects. Newman-Keuls tests indicated that the control animals were significantly ($p < .01$) heavier than the grits/birth animals on all blocks and the grits/weaning animals from Blocks 2-9. The grits/weaning animals were significantly ($p < .01$) heavier than the grits/birth animals on all blocks. Thus, the impression that curtailing the receipt of tryptophan and protein from birth on had a more pronounced effect upon growth than did the restriction of the substances from weaning on was statistically reliable.

In Figure 4, it clearly can be seen that the growth deficit induced by the grits diet was not permanent. Introduction of the normal diet led to a gradual increase in weight by both the males and the females in the grits/weaning group. Moreover, the anticipated male-female weight differences can be seen to develop during Blocks 4 through 9. Statistical support for these contentions was provided by analysis of variance, which yielded significance for all factors, including the groups × gender × blocks interaction [$F(8,184) = 4.94, p < .001$]. Newman-Keuls tests indicated that the control males were significantly ($p < .01$) heavier than all other subjects on all blocks. The control females were significantly ($p < .01$) heavier than the grits/weaning females on all blocks. While the grits/weaning males and females did not differ reliably on Blocks 1-3, the males were significantly ($p < .01$) heavier than the females on Blocks 4-9. On Blocks 6-9, the grits/weaning males were significantly ($p < .05$) heavier than the control females.

DISCUSSION

What conclusions are prompted by the present data? First, they are in agreement with those reported by Remley et al. (1980). In contrast to the growth exhibited by the control animals, the animals exposed to the grits diet showed substantial retardation in growth. In fact, our differences appear to be even more pronounced than those reported by Remley et al. Clearly, this dietary regimen produces major growth differences.

Second, the present data indicate that the development of sexual dimorphism was retarded in the grits/weaning group. Sexual dimorphism had begun to assert itself by approximately 40 days of age in the control animals, whereas it was a nonsignificant factor in the grits/weaning animals, even by 75 days of age.

Third, a comparison of the growth pattern of the grits/birth and grits/weaning groups indicates that the earlier this diet is imposed, the more severe its effects appear to be. The fact that the majority of the grits/birth animals did not survive past 80 days of age underscores this point.

The final phase of the experiment indicates that the growth deficit imposed by the grits diet is reversible. In fact, a projection of the trends depicted in Figure 4 suggests that the grits/weaning animals would have attained weights similar to those of their control counterparts if the duration of the experiment had been extended.

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(Manuscript received November 30, 1988.)