Brain Vulnerability to Postnatal Protein Calorie Deficiency in Infant Rhesus Monkeys[1] (35120)

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According to current estimates, one-half of the human infants of the world are likely to be affected by protein-calorie malnutrition (1). Industralization of a country may aggravate rather than reduce the extent of protein-calorie malnutrition in infancy. Breast feeding becomes less frequent and protein deficiency may occur earlier, coinciding with periods of greater infant vulnerability (2). Although evidence for an association between protein-calorie malnutrition and growth appears convincing, questions of vulnerable periods and prospects for reversibility remain unresolved. Inadequate diets in infancy are generally confounded with social and cultural deprivations and may also be related to genetic factors (3).

Since studies with human infants are less feasible, animals have been used for investigating the effects of nutritional deficiencies on growth (4). However, sequences and rates of early growth in lower species differ from higher primates and man. Serious questions always remain concerning the generalization of findings from lower species to humans. More recently, studies have used primate infants for establishing the effects of various deficiencies on early development (5–7). The growth of higher primates appears more comparable to man and these studies may provide an experimental "model" for future investigations of environmental influences on development (8, 9).

Animal experiments have suggested a critical period of organ vulnerability to protein deficiency during growth (10). Early protein-calorie malnutrition may produce irreversible damage to organs that are undergoing a period of maximal growth. According to this hypothesis, if dietary or other deficiencies occur during the most rapid growth of an organ, they may affect its growth and determine its ultimate structure and function (11). However, the rates of growth differ considerably from one organ to another in fetal and early postnatal periods (5, 6). Also, the adult brain of some species appears to differ significantly from other organs in its greater resistance to even severe protein deficiency or starvation (12). Since the brain plays such a critical role in behavior, one of the crucial problems for establishing the severity and reversibility of postnatal protein-calorie deficiency is to determine whether the "sparing" of the brain observed in the adult also occurs in infancy. The specific aims of this study were as follows: (1) Compare the effects of a high (25%), standard (12.5%) or low (3.5%) isocaloric protein diet on weight gain of the body, brain, pituitary, adrenals, pancreas, and liver of infant rhesus monkeys assigned to the 3 diets from 3 to 9 months of age. (2) Test the hypothesis of differential organ vulnerability or "sparing" of the brain or other organ by comparing organ/body ratios among the 3 groups of infants. (3) Compare rank-order correlation coefficients (ρ) between the weight of the body and each of the selected organs among infants on the 3 protein diets.

Materials and Methods. The subjects for this study were 18 infant rhesus monkeys (*Macaca mulatta*). Each infant was left with its mother from birth until 30 days. It was separated from its mother at 30 days, housed individually and maintained on a milk formula from 30 to 60 days. From 60 until 90

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TABLE I. Statistical Summary and Ranking of Organ and Body Weights and Body-Organ Correlations of Infant Rhesus Monkeys Assigned to a High (25%), Standard (12.5%) or Low (3.5%) Isocaloric Protein Diet from 3 to 9 Months of Age.

	Protein in diet (%)			Analyses				
Organ	25	12.5	3.5	Unit	p^a	$(\%)^b$	ρ^c	p
Pancreas	1.6	1.9	0.8	g	.01	48	.653	.01
Body	1323	1412	944	g	.01	69	—	—
Adrenal L	180	168	124	mg	.01	71	.680	.01
R	137	143	104	mg	.01	74	.564	.01
Liver	38.3	40.3	30.3	g	.05	77	.587	.01
Brain	76.7	78.6	67.3	g	.05	87	.660	.01
Pituitary	23.1	23.8	22.0	mg	NS	94	.212	NS

[a] Differences among the 3 groups were evaluated by analyses of variance and then by a multiple range test for combinations of group means; NS, not significant.

[b] The mean body and organ weights of the low protein diet infants were expressed as a percentage of the mean of the high and standard diet means.

[c] A separate rank-order coefficient (ρ) was computed between the body and each of the organ weights.

days of age, all infants were assigned to the standard (12.5%) protein diet which was fed to each infant in liquid form at 4-hr intervals. At 90 days, 6 infants were assigned to a high (25%), 6 to a standard (12.5%) and 6 low (3.5%) isocaloric protein diet. Each group included 3 male and 3 female infants. The 3 protein diets consisted of 25, 12.5, or 3.5% casein by weight. The other constituents were dextrin, primex, fat soluble vitamins, B vitamins, choline, alpha gel, and ascorbic acid (13). With increasing age, each infant received from 100 to 150 kcal/kg/day of the diet to insure growth. The diets were administered in liquid form to provide accurate measurement of total calorie intake. The infants were tested daily on visual discrimination learning problems for 6 months (14). They were sacrificed by decapitation at 9 months of age. The brain, pituitary, adrenals, liver, and pancreas were removed from each infant; weighed; and prepared for biochemical, histochemical, and electron microscopic evaluation. For this study, the statistical evaluations consisted of separate analyses of variance of the effects of the 3 protein diets on body and on organ weights, on organ/body ratios and of rank-order correlation coefficients (ρ) for establishing the relationship of organ to body weight.

Results. The low (3.5%) protein diet resulted in a significantly lower increase in body weight ($F = 13.73$; df 2/15; $p < .01$); brain weight ($F = 6.00$; df 2/15; $p < .05$); liver weight ($F = 4.09$; df 2/15; $p < .05$), weight of pancreas ($F = 8.58$; df 2/15; $p < .01$) and the weight of the left and right adrenals ($F = 9.90$; df 2/30; $p < .01$). Pituitary weights did not differ among infants on the 3 protein diets. The means of the body and organ weights of the low protein diet infants were next expressed as a percentage of the mean of the high and standard group means. The most dramatic effect of the low protein diet was the reduction of the pancreas of the infants on the low protein diet to 48% of the weight of the mean of the high and standard protein groups. By 9 months of age, the body weight of the low protein diet infants increased only to 69%, the left adrenal to 71%, the right adrenal to 74%, the liver to 77%, the brain to 87% and the pituitary to 94% of the mean of the high and standard protein groups. There were significant correlations between the weight of the body and the weights of the pancreas, adrenals, liver, and brain. The correlation between body and pituitary weights was not significant. Body and organ weights of male infants were generally higher, but differences among group means did not attain significant levels of confidence. Table I contains a summary of

TABLE II. Statistical Summary and Ranking of Organ/Body Ratios of Infant Rhesus Monkeys Assigned to a High (25%), Standard (12.5%), or Low (3.5%) Isocaloric Diet from 3 to 9 Months of Age.

Organ	Protein in diet (%)			Analyses		
	25	12.5	3.5	Unit	p[a]	(%)[b]
Body	1323	1412	944	g	.01	—
	Organ/body ratios					
Pancreas	.0013	.0013	.0009	g/g	NS	69
Adrenal L	.000139	.000119	.000132	g/g	NS	102
R	.000106	.000101	.000111	g/g	NS	107
Liver	.029	.028	.032	g/g	NS	112
Brain	.059	.056	.071	g/g	.05	123
Pituitary	.000018	.000017	.000023	g/g	.01	131

[a] Differences among the 3 groups were evaluated by analyses of variance and then by a multiple range test for combinations of group means; NS, not significant.

[b] The means of the organ/body ratios of the low protein diet infants were expressed as a percentage of the means of the high and standard diet means.

the effects of the 3 protein diets on increases of body and organ weights of the infant monkeys.

To determine if "sparing" occurred of the brain or any other organ, organ/body ratios were calculated for the 6 organs. The brain/body and pituitary/body ratios of the low protein infants were significantly higher than those of the standard and high protein infants. The liver, pancreas, and adrenal/body ratios did not differ significantly among the 3 groups. Table II contains a summary of the effects of the 3 diets on organ/body ratios of the monkeys.

Discussion. Previous studies have suggested that the severity and prospects of reversibility produced by protein-calorie deficiency may depend upon age, protein value of the diet and duration of deficiency (15). To estimate when the greatest organ vulnerability occurs in the rhesus monkey, the body and organ weights of the 9 month old monkeys of the present study need to be compared with growth rates of these organs from conception or birth to their adult values. Some allometric information has become available recently on the brain and on some other organs in relation to body weight of several primate species, including adult rhesus monkeys (16–19). Prenatal growth rates for the brain and other organs have also been reported from 50 to 175 days gestation or birth (5, 6). Growth rates of major organs in the fetal rhesus monkey show a rapid decrease with advancing age (20). Most organs continue to grow at slower rates after birth by cell division and cell growth. Cell division in the brain stops at or shortly after birth, making the postnatal growth of the brain more comparable to man. At birth, the average body weight is 544 g and the brain 58 g (5, 6). Body weight of adult males has been reported as 6 kg and of females as 5 kg. The young adult brain ranges from 87 g in the male to 82 g in females (5, 6). At birth, the brain has reached 60%, but the body less than 10%, of adult weight. Growth curves for the liver, lungs, heart, and kidneys before birth are linear. The brain, adrenals, and thymus follow a different growth curve shortly before birth (5, 6). From the reported prenatal organ growth curves and adult values, it may be inferred that the growth of the brain, pituitary, and adrenals continues, but at a slower rate, during the first postnatal year. The present findings indicate that protein calorie deficiency can influence organ growth significantly even after maximal growth.

The findings of this study also indicate significant differences in organ vulnerability to protein-calorie deficiency in the rhesus monkey during the first year of postnatal

life. The effects on the brain and pituitary differed significantly from those on the body, liver, adrenals, and pancreas. The higher brain and pituitary/body ratios of the low protein infants may be interpreted either as a "sparing" of these organs, similar to the adult brain, or that these two organs had simply passed their most rapid growth when the infants were exposed to the diets from 3 to 9 months of age (10, 11). The lesser effects on brain weight need to be interpreted with caution. The brain is composed of many different chemical constituents with considerable variation in morphological, physiological, and chemical growth rates of neurons and glial cells among different regions (21). Small differences in regional vulnerability of the neocortex may be of considerable importance for intellectual development and behavior (22).

Although the growth of rhesus monkeys appears more comparable to man, there are differences in organ growth rates. In man, the brain grows most rapidly from approximately 350 g at birth to about 1000 g by the end of the first year (23). By puberty, the brain weights approximately 1250 g in females and 1350 g in males (24). At birth, the infant brain has reached only 30%, and the body only 5%, of adult weight (23, 25). If organ vulnerability is greatest during periods of maximal growth rate, it seems prudent to assume that the severity would be greatest and the prospects of recovery the least likely if protein-calorie deficiency occurred during the first year in man. Less information is available on postnatal growth rates of other major organs in man (26).

There is considerable current interest in population density and malnutrition in infancy in relation to mental and physical development (27). Studies with human infants have been undertaken only very recently and the authors acknowledge difficulties in interpreting the data since protein deficiencies generally include combinations of cultural deprivations (28, 29). The findings of this study suggest that the effects of early postnatal protein-calorie malnutrition on development and the conditions essential for reversibility can be identified more readily in controlled studies with primate infants.

Summary. Comparisons were made of increases in brain, body, and other organ weights and of organ/body ratios of 18 infant rhesus monkeys assigned to a high (25%), standard (12.5%), or low (3.5%) isocaloric protein diet from 3 to 9 months of age. Significantly lower increases in body, brain, liver, pancreas, and adrenal weights were observed in the low protein monkeys. Pituitary weights did not differ among infants in the 3 diets. Significantly higher brain/body and pituitary/body ratios of low protein infants indicated that the brain and pituitary were significantly less affected by the low protein diet than the body, pancreas, liver, and adrenals. The higher brain and pituitary/body ratios of low protein infants may be interpreted as a "sparing" of these organs similar to that of adults, or that the organs had passed their most rapid growth when the infants were exposed to the 3 protein diets from 3 to 9 months of age.

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