

EXPERIMENTAL STUDY ON RATS TO FIND THE USEFULNESS OF NUTRITIONAL SUPPLEMENTATION TO UNDERNOURISHED OFFSPRING OF PARENTS UNDERNOURISHED LIFE-LONG

B. RAJANNA, CAROL MASCARENHAS AND T. DESIRAJU

*Department of Neurophysiology,
National Institute of Mental Health & Neuro Sciences, Bangalore - 560 029*

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Summary : Undernourished parents getting only about half of normal feed requirement and whose body weights were deficient by 40–65% were mated and out of the resulting litters, the medium size (about 8) ones were culled to 8 per dam whose food supply was restricted to 10 g per day during the suckling (category M₂). Another category (M₃) was constituted out of large (over 12) litters born to similar undernourished parents and culling the litter size to 15 per dam besides restricting the dam's food to only 10 g per day during first week and to 15 g per day during the second and third weeks of suckling. Another category (M₁) was constituted like M₃ except that the parents were provided with normal, *ad libitum* nutrition throughout. Effects of post-weaning continuation of undernutrition or of rehabilitation with *ad libitum* food were studied in M₂ groups of pups till 425 days of age. Further, an additional protein-deficient type of undernutrition (M₄) was also superimposed in one group of M₂ category of pups between day 41 and 60 of age, and then rehabilitated on to normal diet to find the additional effect of this additional load of the protein-malnutrition. The control groups of normal pups were also reared along with the above groups for comparisons. The normal diet had: 22.8% protein, 10.6% fat, 61% carbohydrate, and vitamins and minerals.

By 21 days of age, the deficiencies of the M₁, M₂ and M₃ were about 28%, 64% and 77% respectively in body weights, and about 8%, 21% and 30% respectively in brain weights. Continuing the undernutrition after weaning on half of normal feed, the M₂ group of males and females stabilized at about 41% and 62% respectively of normal body weights by about 150 days of age.

Rehabilitation of M₂ or M₄ groups by providing *ad libitum* feed had never recovered their pre-rehabilitation body weight deficits even after the *ad libitum* feeding for as long as 425 days of age. On the contrary, the brain weights seemed to have partially recovered from the earlier deficits, but here also the general conclusion of permanency of deficits stood unequivocally.

The present study affirms that different degrees of gestational, lactational and post-weaning undernutrition can lead to different degrees of growth deficits and that supplemental feeding regimens introduced afterwards cannot bring out recoveries from such previous deficits which seem to remain permanently. However, the rehabilitatory feeding regimens will help to prevent the further additions to the deficits, from the time of the introduction of the rehabilitation.

Key words : chronic undernutrition
recovery from growth deficit

development of brain
body and brain growth

INTRODUCTION

Since the recognition of the "growth spurt" phenomenon and vulnerable periods in brain development (2), the subject of finding how much catch-up in undernutritionally retarded animals is possible in brain and body growth following nutritional rehabilitation has been attracting renewed interest (5,6,9,10).

Among the morphological studies of brain, it was reported that the deficits in dendritic branching and spine numbers of pyramidal cells of mouse cerebral cortex induced by undernutrition were not found to be recoverable following rehabilitation (3), whereas the synapse-to-neuron ratio in rat cerebral cortex was reported to be not only recovered, but also overshoot after rehabilitation in spite of the rat having had malnutrition during the gestation, lactation and in post-weaning upto 100 days of age (9).

Regarding the body mass, one view is that the body weight deficit produced by prenatal or postnatal undernutrition cannot be recovered by subsequent nutritional rehabilitation (1, 6, 10); the other and contrary view (5) is that the body weight deficits occurring due to undernutrition during gestation are recoverable by postnatal nutritional rehabilitation. In view of such differences in the literature, the possibilities of recovery of body weight deficits of undernourished rat following rehabilitation have to be investigated and further studied. The duration of rehabilitation may also have to be kept sufficiently long to draw inferences about the extents of recoveries.

Another important aspect of the undernutrition research is that most of the published studies have been made on short-term impositions of undernutrition during pregnant or lactating stages of the mother. It would be even more desirable to study the effects of rehabilitation of undernourished offspring born to parents who are also chronically undernourished life-long, just as undernutrition in human communities is life-long. Hence, the present study was conducted to find the extent of recovery of the retardation of the undernourished offspring of undernourished fathers and mothers who were continued to be undernourished also during gestational and lactational periods. Further, an additional protein-deficiency also was superposed during the on-going post-weaning undernutrition in some rat pups to increase the amount of undernutrition and to find the degree of their recovery by subsequent supplementation to the feeding.

MATERIAL AND METHOD

A. *Study of changes in body weight and brain weight due to undernutrition in gestational and lactational stages :*

Wistar rat pups with different categories of malnutrition were raised in the following manner, along with the groups of normally nourished pups (category N).

1. *Mother on ad libitum diet, but pups undernourished due to large size litter in gestation and lactation* : Mothers who happened to deliver large size (12 or more pups) litters were selected, and the pups culled to 15 per such dam for suckling. It was observed that the average body weight at birth of pups of large litters (of over 12) was about 13 to 15% smaller than of normal size litters (of about 8). The mothers were maintained on *ad libitum* normal food throughout (category M₁).

2. *Both parents were undernourished life-long, their offspring further subjected to gestational and lactational undernutrition* : Offspring obtained by mating undernourished parents (mothers having the body weight deficit of 40–50% and fathers having the deficit of 55–65%) were taken and further sub-divided into two categories for subsequent rearing from birth. (i) Pups born in normal size litters (of about 8) were culled at the rate of 8 per dam which was fed only with 10 g of diet per day throughout the suckling (category M₂). Such pups at birth were on an average about 15% less in weight than the normal controls. (ii) Pups from large litters (of over 12) of undernourished parents were culled to 15 per dam which was fed only at the rate of 10 g of diet per day during the first week and 15 g per day during the second and third weeks (category M₃). At other times, including during gestation, the mothers of both the groups were fed at the rate of only 8 g per day. The average weight at birth of pups of such large litters of undernourished mothers was about 20 to 25% less than the average weight of medium size litters (of about 8) of normally nourished mothers.

The rat diet consisted of 18% casein-wheat-oil-salts-vitamins (18 CW diet) (4). The diet had: 22.8% protein, 10.6% fat and 61% carbohydrate. The body weights of the above categories of undernourished pups and of the normally nourished control category of pups were measured daily in the pre-weaning period. The average daily food intake of normal *ad libitum* group in the post-weaning period for males and females respectively was: 6.8 g and 6.2 g in the first 10 days; 10.1 g and 8.7 g in the next 10 days; 14.8 g and 10.8 g in the third 10 days, and attaining by 60th day of age the values of 18 g and 14.4 g.

Groups of pups from the different categories were sacrificed on 1st, 5th, 10th, 15th and 21st day of age (day of birth is counted as '0' day) for obtaining the brain weight measurements. Thus, 20 groups of rat pups for each sex were reared for specific lengths of survival. The number structure of a group was not disturbed as the whole group of pups of a particular age was sacrificed at a time. Any isolated deaths of the pups occurring in the groups during rearing before the sacrificing day of the group were compensated by adding replacements to the group.

B. *Effects of continuing (post-weaning) undernutrition on body growth :*

Pups of the category M_2 mentioned above were weaned on to : 3 g of food per day during the initial 10 days; 5 g per day during the next 10 days and from then on 8 g per day till 425 days. The body weights of these rats growing on the reduced amounts of diet were measured daily. They were reared in individual cages. For comparison purpose, pups of the N category were simultaneously reared identically, except for the *ad libitum* diet.

Another group of M_2 which was continued to be undernourished in post-weaning as above, was given an additional infliction of undernutrition for 20 days (between 41st and 60th day of age) by giving low protein diet of 8% instead of the usual 22.8% (category M_4).

C. *Study of catch-up in body growth during post-weaning rehabilitation :*

The M_2 and M_4 categories of undernourished pups rehabilitated in post-weaning were designated respectively as R_2 and R_4 . R_2 : The pups of the category M_2 were rehabilitated by weaning them on to *ad libitum* normal diet from day 21 of age. Although the food was available *ad libitum* during rehabilitation, the previously undernourished male and female groups had consumed on an average about 9% and 4% respectively less than their normal controls. Normal pups of category N were also weaned identically and reared simultaneously for comparison. Their body weights were measured daily during the first 30 days; later on alternate days during the subsequent 150 days; and at weekly intervals later on. All the rats were reared in individual cages. R_4 : From day 61 of age, the pups of the M_4 category and control (C) groups were brought on to the normal protein diets (23%), *ad libitum*. The rats of these groups also were reared in individual cages and measurements of their body weights taken periodically.

The results were statistically tested using the methods of Student's t test and the analysis of variance (ANOVA) (7, 8).

RESULTS

(A) *Pre-weaning undernutrition*

The data of 1084 body weights and brain weights of 486 pups belonging to 40 groups (20 male and 20 female) is summarized in Fig. 1 for the three modes of undernutrition described in Section A of Methods.

Body weights : The body weights of the 3 categories of the undernourished pups were about 14–20% lower than the normal pups even on day 1 of age (Fig. 1). The amounts of the deficits of the body weights of the undernourished pups increased progressively till weaning, the less severely undernourished group (M_1) showing lower deficit than the more severely undernourished categories (M_2 , M_3). At weaning age (21st day), the average values of the weight deficit of the males and females combined were 28%, 64% and 77% for the M_1 , M_2 and M_3 categories respectively. The means of weights

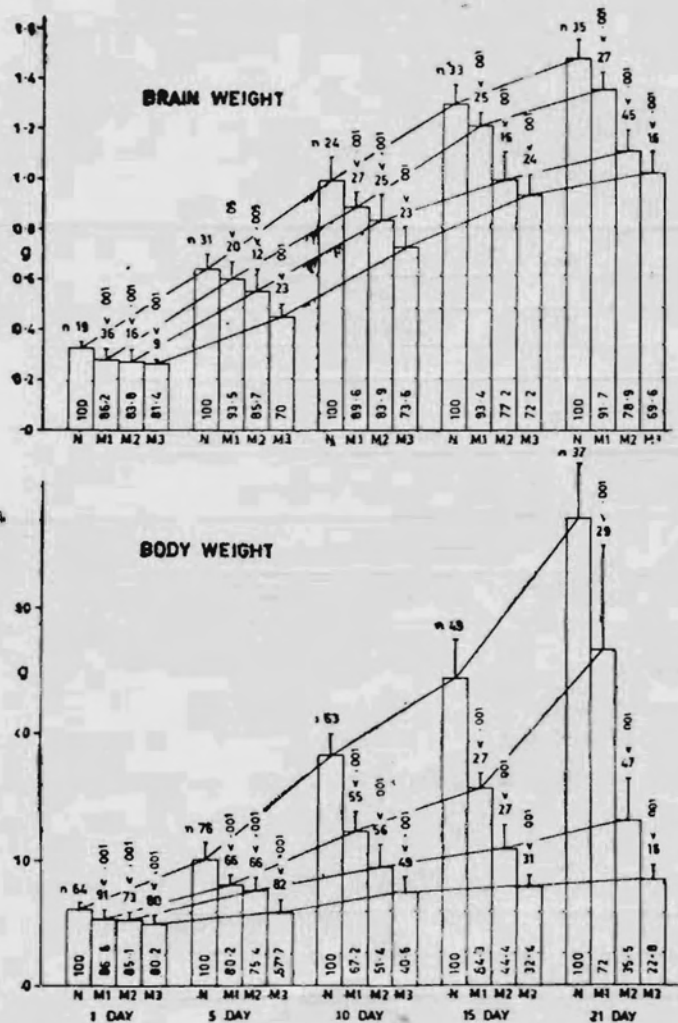


Fig. 1 : Mean brain weight and body weight histograms topped with standard deviation bars, numbers of samples, P values of malnourished categories (M_1 , M_2 , M_3) of rats compared with normal category (N), and percentile expressions (given inside the bars) of M_1 , M_2 and M_3 relative to N.

of the undernourished groups differed from the normals with a high statistical significance on Student's *t* test (Fig. 1) and also on the basis of analysis of variance (ANOVA) of weight gains (Table I).

TABLE I : Analysis of variance (ANOVA) of weight gain (5-day-wise) during pre-weaning (males and females).

Diet	Body weight gain (g)				Row Mean \pm S.D.
	Successive 5-day weight gains				
	5 D	10 D	15 D	21 D	
N	4.0	8.1	6.1	12.7	7.725 \pm 3.720
M ₁	2.8	4.2	3.3	11.0	5.330 \pm 3.830
M ₂	2.4	1.8	1.4	2.3	1.980 \pm 0.465
M ₃	0.9	1.6	0.5	0.5	0.875 \pm 0.519
					Row LSD (df 12) 4.94 (P < .05)

	Error	Rows	Columns
df	9	3	3
SS	45.0	118.0	41.8
MSS	5.0	39.3	13.9
F		7.9	2.8
P		<0.025	.100

Diet	Brain weight gain (mg)				Row Mean \pm S.D.
	Successive 5-day weight gains				
	5D	10 D	15 D	21 D	
N	317.0	353.3	305.2	183.3	289.7 \pm 73.83
M ₁	320.7	290.9	323.8	145.9	270.15 \pm 84.27
M ₂	269.8	285.0	169.4	166.7	222.73 \pm 13.45
M ₃	184.4	283.7	205.8	94.5	192.1 \pm 77.8*
					Row LSD (df 12) 84.4 (P < .05)

	Error	Rows	Columns
df	9	3	3
SS	13135.5	23709.6	54757.9
MSS	1459.5	7903.2	18252.6
F		5.4	12.5
P		<0.025	<.005

Brain weights : Brain weights of the categories M_1 , M_2 and M_3 on day 1 were significantly lower than normals by about 14%, 16% and 19% respectively (Fig. 1, Table I). The brain weights like the body weights have decreased progressively, but less conspicuously than the body weights, through the development till weaning. By day 21 of age, the brain weights of males and females of categories M_1 , M_2 and M_3 were lower than normals, by 8%, 21% and 30% respectively. The mean values of the brain weights of the undernourished pups of all ages between day 1 and 21 were lower than normal with a high statistical significance (Fig. 1, Table I). The brain weight/body weight x 100 computation revealed the lowest values for the normal pups and highest values for the M_3 , suggesting that the body growth is relatively more severely affected in the undernourished or that the brain in the undernourished is being spared as far as possible (Table II).

TABLE II : Brain weight/body weight ratio x 100.

Age days	Nutrition category			
	N	M_1	M_2	M_3
1	5.33	5.30	5.25	5.41
5	6.38	7.45	7.25	7.73
10	5.47	7.29	8.92	9.93
15	5.36	7.79	9.32	11.94
21	4.02	5.12	8.93	12.28

(B) *Continued undernutrition in post-weaning stage :*

Undernourished pups of M_2 category described above were continued on undernutrition as described in Section B of Methods. Continuing the undernutrition has resulted in adding a further deficit to the body weight. These rats (males) attained a body weight of only about 41% of control values by 150 days of age, i.e. a deficit of about 205 g in body weight, and stabilized at about that level till the age of 250 days (group M in Fig. 2). The female rats showed a better growth than the males, attaining about 62% of control values by day 150 of age, and stabilized at about that level without much further growth till 250 days of age (group M_2 in Fig. 3). The actual value of the deficit for the females was about 85 g in contrast to the 205 g of the males.

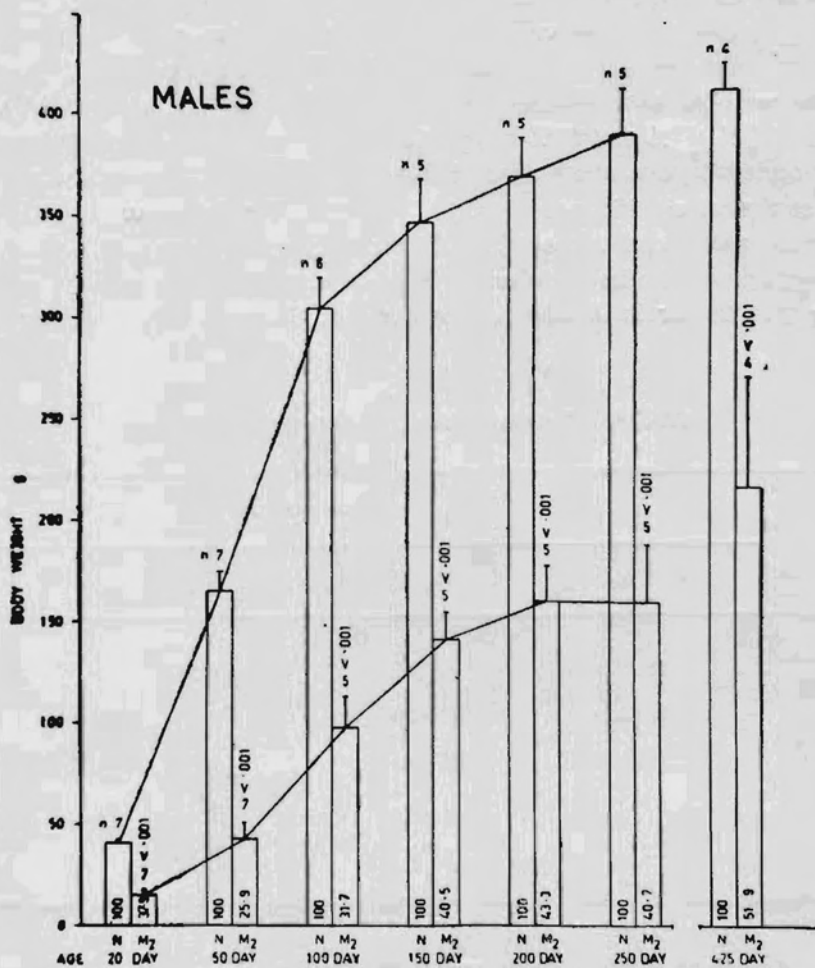


Fig. 2 : Mean body weight histograms of M₂ category male rats on continued post-weaning mainnutrition, and of normal rats (N).

(C) *Post-weaning rehabilitation of undernourished pups :*

Body weights : 2 categories of undernourished pups were used for the rehabilitation study.

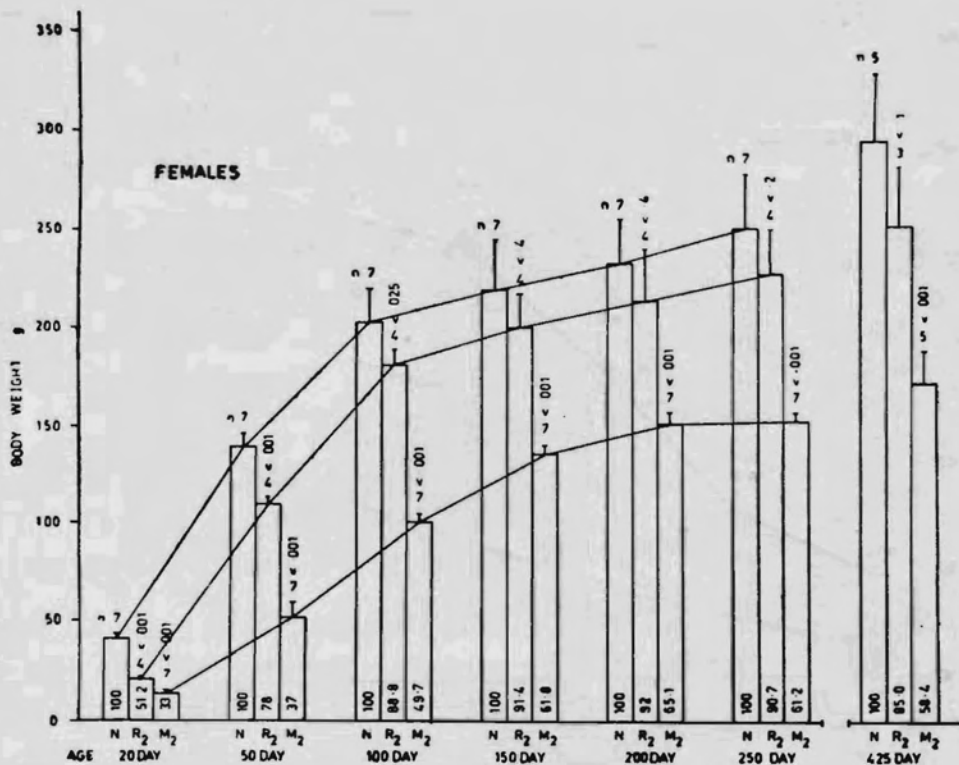


Fig. 3 : Mean body weight histograms of M₂ category of female rats rehabilitated by diet supplementation from 21st day of age (R₂), and of comparison groups N and M₂ as in Fig. 2.

One category (R₂) had suffered only a pre-weaning undernutrition having a mean deficit of body weight of 49% on day 20 (R₂ in Fig. 3). This category was rehabilitated on normal good *ad libitum* from day 21 onwards. By age of 100 days, the mean body weight of this group attained about 91% of control, i.e., the residual deficit was about 9%. The residual deficit did not diminish even by the age of 425 days. The actual value of the deficit on day 20 of age of this group, which is the day prior to weaning, was 21.1 g (Fig. 3). Even at the age of 425th day, i.e., after having a normal *ad libitum* nutrition for about 405 days, approximately this amount of pre-weaning deficit in body weight had been continued or carried forward permanently as was observed even on the 425th day of age. The normal *ad libitum* diet could contribute only to the growth of body occurring after the time of introduction of the normal feeding, but what was lost in the body weight in the earlier age had remained as a permanent loss. The above percentage an indication of a reduction of the deficit, a built inly and arge growth from

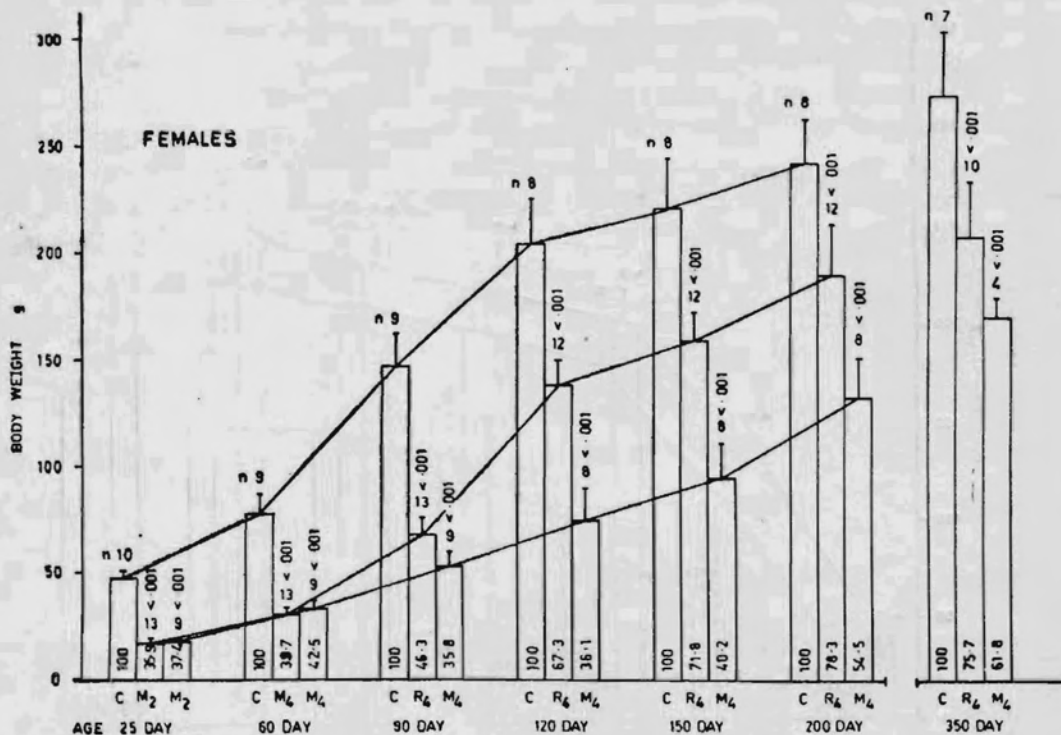


Fig. 4 : Body weight histograms of female rats (R_4) rehabilitated from 61st day out of M_4 group which suffered a second dose of undernutrition of protein deficiency between 41st and 60th day, and of the category (M_4) which continued on restricted amount of diet even after day 61, at the same level of diet as for M_2 of Fig. 3.

tion of the age-related subsequent growth occurring due to the introduction of the normal diet after weaning. In other words, the post-weaning nutritional restitution has not contributed to any catch-up of the previously lost weight, but has only enabled the expression of the growth potentialities of the later age after the restitution.

Another category (R_4) of malnourished pups was subjected to a different treatment as mentioned in Section C of the Methods. These pups were from M_2 group and subjected to an additional malnutrition of protein deficient type from day 40 to day 60 of age and later on to normal diet, *ad libitum*. The corresponding normal control groups (C in Fig. 4, 5) were also provided with the same kind of protein-deficient diets between day 40 and 60. The males of such an additionally undernourished group (R_4 in Fig. 5) grew by 200 days of age to about only 70% of control values and remained at that level even at 350 days of age. These rats showed deficits of 93.5 g – 108.4 g between

the ages of 90 days and 200 days. Thus, though the percentage expressions of body weights show an improvement from 41.7% to 70.5% from 90th day to 200th day of age, the absolute deficit of body weight continued till 350 days at the level of the above mentioned value of about 108 g, which thus remained as a residual value uninfluenced by restituting the normal nutrition. The female group also showed an average absolute deficit of 65.7 g between the 90th day and 200th day of age, while the percentage expressions indicate the new growth bringing up the body weight from 46.3% to 78.3% from 90th day to 200th day (R_4 in Fig. 4). This level of the unrecoverable deficit continued to be seen even at the 350th day. Thus, the females suffered less injury due to malnut-

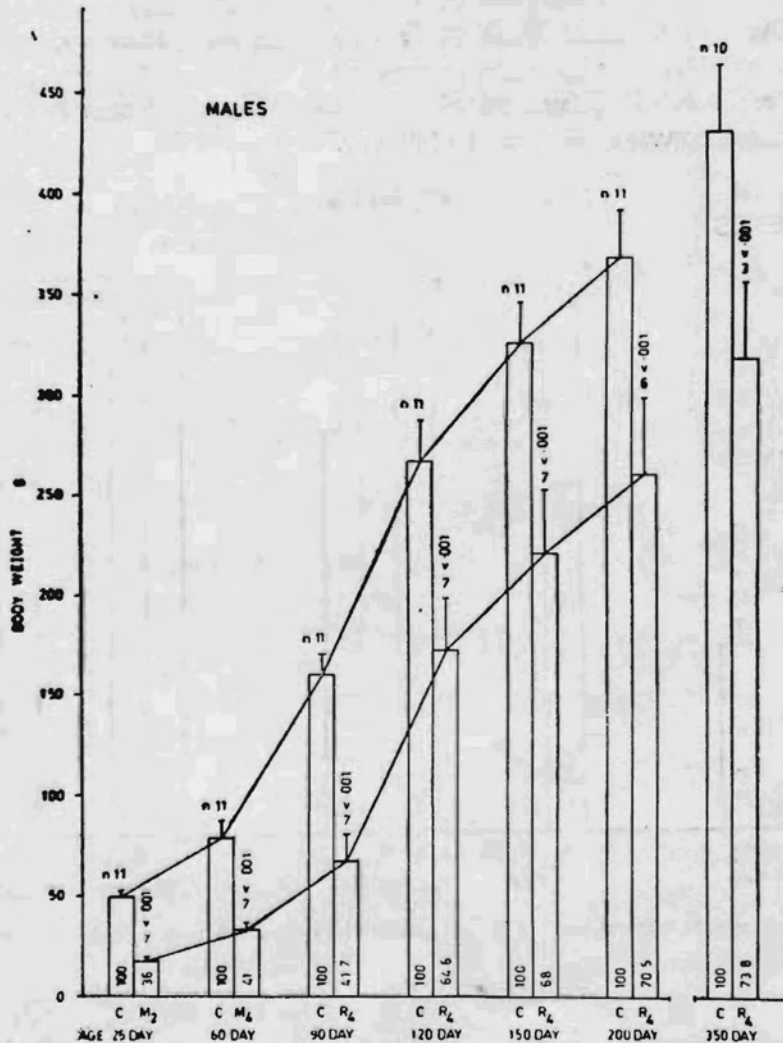


Fig. 5 : Mean body weight histograms of rehabilitated male rats (R_4) treated as in Fig. 4, and of normal category of] rats similarly protein-malnourished between 41st and 60th day and then put back to normal nutrition (C).

rition than the males, the residual unrecoverable deficit being about 66 g versus 108 g respectively for the sex-related growth rate differences. Further, the R_4 category carried much more residual permanent deficits than the less severely undernourished category of R_2 (Fig. 3, 4).

Brain weights : At weaning, the M_2 category which had a body weight deficit of about 65% showed a brain weight deficit of about 375 mg or about 21% of control. Continuing the undernutrition till 425 days when the body weight deficit was about 55% (both sexes average), the brain weight deficit was about 16% (Fig. 6). On the other hand, rehabilitation of the pups from 21st day till 425 days of age still left a permanent brain weight deficit of about 115 mg or about 6%. However, this means that the recovery possibilities for the brain growth (weight) is much better than for the body weight. The recovery of R_4 was slightly less than R_2 as the former had an extra dose of undernutrition between 40th and 60th day.

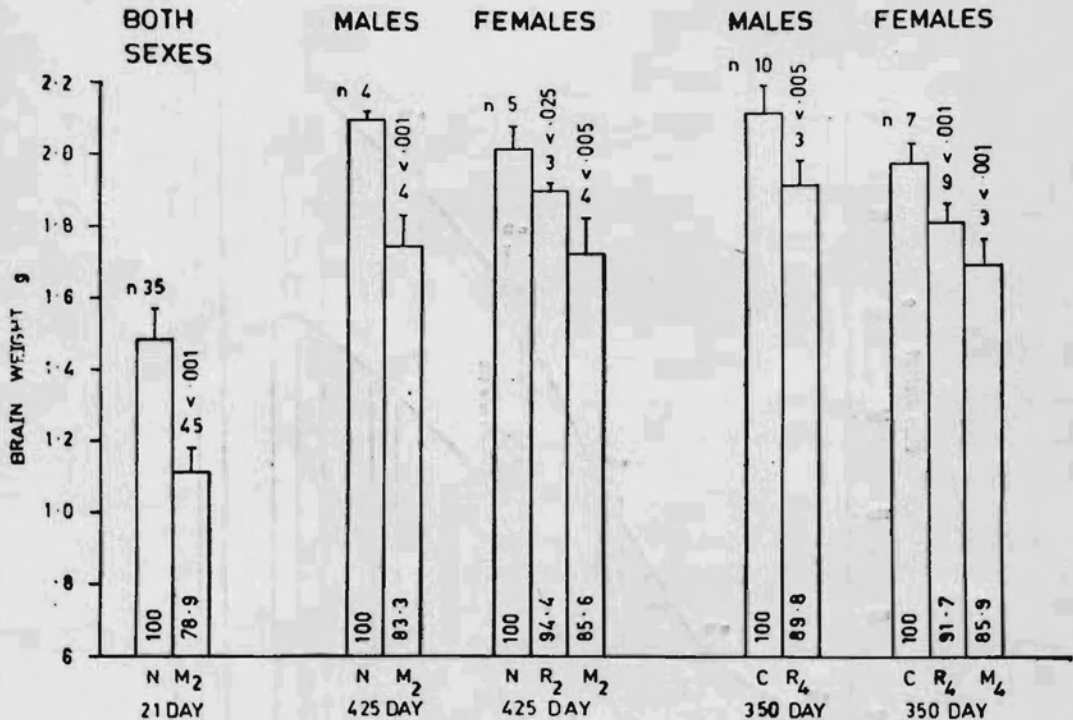


Fig. 6 : Brain weight histograms of different ages of the malnourished and rehabilitated groups.

DISCUSSION

The present data adds to the point that different degrees of undernutrition imposed in gestational and/or lactational phases can lead to different degrees of severity of deficits

in body weights and brain weights (Fig. 1 and Table I). When the deficits in the body weights in 21 days old groups of pups were 28%, 64% and 77%, the correlated brain weight deficits were only 8%, 21% and 30% respectively. On the contrary, on day 1, when the body weights were deficient by only 13%, 15% and 20%, the correlated brain weights were deficient by 14%, 16% and 19% respectively. Thus, the data (Table II) confirms that the brain attains its growth better than the body when energy supplies are low and also probably because the growth of the former is programmed to be completed much earlier.

It was observed that even while the undernutrition was continuing in the post-weaning age, the body weights could undergo stabilization at certain levels by about 150 days (M_2 in Figs. 2, 3) and be maintained thereafter. This observation indicates that even under continued malnutrition, the adaptation for a homeostatic regulation of body weight of the organism can be achieved in spite of actualization of only a part of the genetic potentialities of body growth. The physiological reorganization of this adaptation of optimizing body growth to the deficient nutritional situation has to be understood further in the future. This seems to be the natural way of coping up to the imposed restriction. The slightly better recovery of the females during the continued post-weaning undernutrition could be due to their growth rates being slower than the males, and also due probably to their getting a relatively more diet than the males, because the 8 g diet per day would work out as a slightly higher ration to a female than the male.

Rehabilitation experiments showed that the amounts of deficits in body growth established early by the undernutrition remain permanent in spite of better nutrition provided in later age. Pups having suffered a mean deficit of about 21 g (Fig. 3- R_2) by 21st day of age could rehabilitate by attaining upto only 91% of control weight, i.e., the deficit of about 21 g of body weight continued to remain under the new growth occurring with age after 21st day. The statistics could not clearly bring out this small but consistent value of permanent deficit in the body weight because this small figure was hidden or diluted in a much larger value having a relatively larger variation. When the deficiency in body weight was increased to about 60% by increasing the severity of undernutrition, the amount of the underlying permanent deficit became larger, being about 108 g and 65 g for the males and females respectively by 90th day of age onwards. These deficits are reflected in the expressions of overall body weight deficits of 29% in males and 22% in females at the age of 200 days (Figs. 4 and 5). On the contrary, with the nutritional supplementation rehabilitation, the proportion of the early deficits of brain weights seemed to be recovering considerably better (from 375 mg to about 115 mg) than the deficits in body weights (Figs. 3-6).

The data of this study affirms that early deficits in body or brain weight imposed by undernutrition during the developmental ages will be permanent and not recoverable

later on by subsequently provided better nutrition. The nutritional rehabilitation can bring about only an amount of growth appropriate to the age available after the introduction of the rehabilitation, i.e., what has been lost earlier cannot be recovered later. The degree of the permanent deficits or retardations, and the occurrence of growth will essentially depend on three critical factors: (i) severity of the restriction on the nutrition imposed; (ii) age span of development during which the undernutrition is imposed; and (iii) the age span of growth left available after the introduction of the rehabilitatory nutritional support.

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