Studies on rats have shown that deficiencies in the protein composition of a diet fed to mother rats during pregnancy and lactation is associated with poor food utilization in the progeny. A preliminary study of 11-year-old children in Formosa produced results pointing to an interpretation similar to that obtained with rats. Further study is required of the role of maternal diet during pregnancy and lactation in human beings.

MATERNAL NUTRITION AND METABOLISM OF THE OFFSPRING: STUDIES IN RATS AND MAN

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Introduction

SINCE the early years of this century, it has been known from work in animals that the long-term effect of temporary malnutrition is dependent on the stage of life during which the malnutrition occurs. When Jackson and Stewart[5] reviewed this subject in 1920 it had already been shown that underfeeding of dogs and rats during the period of lactation prevents their attaining normal adult size upon later full feeding, whereas there was complete recovery in body size after refeeding rats which had been underfed for short periods after four weeks of age. In spite of the later well-known studies of Widdowson and McCance and their group[7,10] confirming the irreversible effect of neonatal undernutrition on body size, the implications of this phenomenon with respect to human development and nutrition have, until recently, been largely disregarded.

In 1964 Chow and Lee[2] demonstrated even broader and more profound effects on the development of the ad libitum fed progeny of mother rats which had been subjected to 50 per cent dietary restriction during pregnancy and lactation. Such progeny attain an ultimate body size from 20 to 30 per cent less than the progeny of ad libitum fed mothers. The growth stunting is not attributable to lower food intake since these animals consume more food per unit body weight than do the normal controls.[6] They waste nitrogen by excreting an abnormally high proportion of the nitrogen which they absorb.[6]

Growth stunting also occurs when dietary restriction is applied to the mother during gestation alone[2]; moreover, Venkatachalam and Ramanathan[9] have demonstrated significant growth stunting in the progeny of mother rats subjected to protein-free feeding in any *single week* of gestation with ad libitum feeding during the remainder of gestation and lactation. Hsueh, Agustin, and Chow[4] have recently assembled evidence suggesting that the quantity and quality of protein in the maternal diet are the critical factors in determining the size of the progeny. Thus in the rat a maternal diet in which the protein is of low nutritive value even when fed ad libitum will result in permanently stunted offspring.

This report represents another approach to the problem of assessing food

utilization and metabolism in the progeny of mothers receiving proteins of differing quality during gestation and lactation. We shall describe first a recent study involving rats which provides a useful comparison with a partially analogous study in children, the main subject of this report.

Further Observations in Rats

The earlier data on feed utilization and nitrogen metabolism in the progeny of underfed mothers were obtained under conditions of ad libitum feeding. Our more recent approach involves standardizing the dietary intake, by pair feeding, of control and experimental progeny using growth during this period as an index of feed utilization. The present experiment demonstrates the effect of both the quality and quantity of protein in the maternal diet on the feed utilization of the progeny.

Procedure

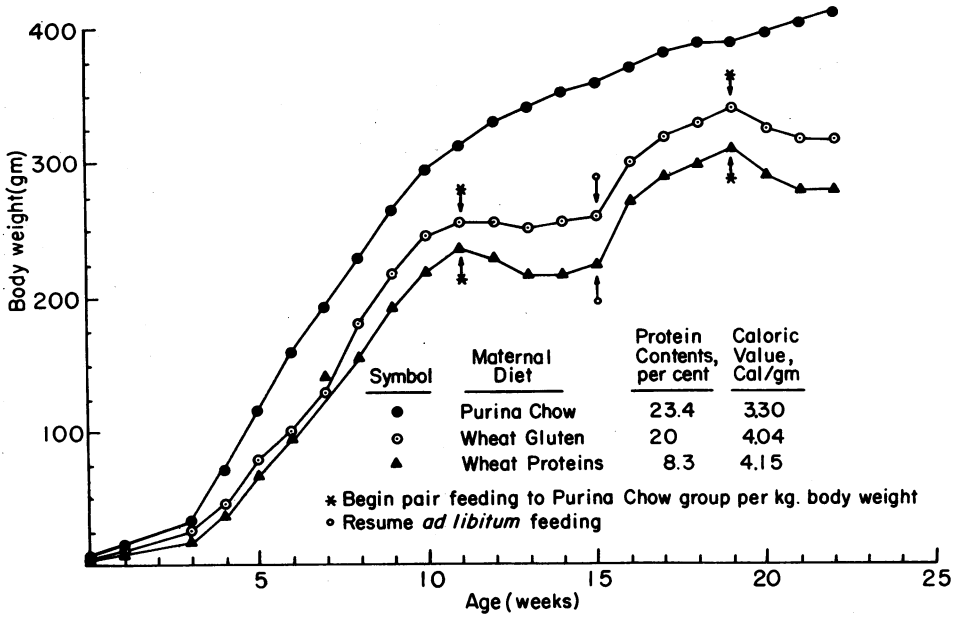
Eighteen McCollum strain female rats were mated at six months of age. These animals, from our stock colony, had been raised on a diet of Purina Laboratory Chow and all had a fully satisfactory nutritional status as judged from their body weight. Pregnancy was timed from the first appearance of sperm in a vaginal lavage after which the female animals were divided into three groups of six each, designated A, B, and C. From the first day of pregnancy to the 21st day of lactation each of the groups received a different ad libitum diet: group A mothers continued on Purina Laboratory Chow; group B mothers received a diet derived from whole wheat and containing 8.2 per cent protein; group C mothers received a formulated diet containing 20 per cent wheat gluten. Caloric intakes during pregnancy were found to be approximately equal. Because of the delayed development of the young in the two experimental groups B and C, weaning was delayed from the usual 21 days until 28 days for all groups. From the 21st day of lactation, however, all mothers were again offered ad libitum quantities of Purina Chow. No efforts were made to prevent the progeny obtaining access to the mother's feed.

After weaning, eight, six, and seven male progeny from groups A, B, and C, respectively, were fed Purina Chow ad libitum until the first period of controlled feeding at 11 weeks of age. At this time the dietary intake of the progeny from group A was measured daily; the average intake per unit body weight was calculated and the exact intake relative to body weight was fed to each of the young rats from mothers in groups B and C on the following day. This regime was continued for four weeks, i.e., until the young were 15 weeks of age. At this time Purina Chow was again offered ad libitum to the progeny from all groups for a period of four weeks, following which pair feeding was instituted again for a further period of three weeks.

Results

Of the six mothers in each group, all in groups A and C delivered young, while in group B (on the low protein diet) only three mothers carried pregnancies to term. Litter sizes in the three groups did not differ significantly. Birth weights, however, were conspicuously low in group B, for which the average was 5.42 gm for the males and 4.95 gm for the females in contrast to group A, for which the averages were 6.59 gm and 6.26 gm, respectively. The corresponding values for group C, 6.16 gm and 5.58 gm, were intermediate. The differential in body weight among the three groups at birth remained apparent at 11 weeks of age despite ad libitum intake of Purina Chow by all groups after weaning. At this time the mean weights of the animals were 312 gm, 235 gm, and 257 gm in groups A, B, and C, respectively (Figure 1). During

Figure 1—Growth rates of progeny of mothers fed ad libitum diets



the ensuing four weeks of controlled feeding, progeny of group B mothers (low protein) lost an average of 13 gm, while the controls gained 45 gm. The former animals regained 87 gm during the four-week rehabilitation period, while group A gained an additional 30 gm. Progeny from group C mothers (poor quality protein) just maintained their body weight while being fed the same relative intake as group A. Upon resumption of ad libitum feeding, food intake per unit body weight, total food intake and feed efficiency in groups B and C exceeded that of the Purina Chow group A as the experimental animals rapidly gained weight. Similar results were obtained in a second controlled feeding period of the same animals and in a repetition of this experiment.

Preliminary Observations in Children

Studies of the effect of maternal diet on the growth and development of the child require a different experimental approach from that used in the animal studies. Since dietary restriction during pregnancy appears physiologically undesirable, it is ethically unacceptable, and our planned approach to the human studies involves, essentially, a reversal of the technic employed in the animal studies. In our projected longitudinal study, suitable volunteer subjects will be chosen in a geographical area where the diet is unsatisfactory, particularly with respect to the quality and quantity of dietary protein. Varying levels of protein-rich food supplementation will be supplied to the subjects during pregnancy and lactation and their children observed and compared for growth and development. Such a study, requiring at least five years, will begin soon in Taiwan. As a preliminary to that study we have carried out short-term balance studies on school boys in Taiwan during the past two years. Some of the results of that work are the subject of this section of the present paper.

Procedure

The balance studies were conducted in an elementary school in the city of Taishi, which is located at the middle of

the western coast of Taiwan. The subjects were school boys, approximately 11 years old. Two groups, each of 18 boys, were selected for the study. The mothers of the boys in the first group were believed to have had an inadequate diet during that pregnancy and these boys will be referred to as poor diet progeny (PDP). The mothers of boys in the other group were believed to have consumed a more adequate diet during pregnancy; these boys will be referred to as adequate diet progeny (ADP). The PDP were thus drawn from economically very poor families and the ADP from families with more satisfactory incomes. From previous surveys and from the experience of public health workers in the area, diets of the poor families are known to have been extremely low in protein, particularly from animal sources and also marginal with respect to vitamins and minerals. The diets of families in the higher socioeconomic levels are quite variable, but tend to be more adequate, especially in animal protein. Economic conditions in the area a decade ago were definitely no better than those at the present; the families who are poor at the present time were also poor at that time. Approximately 25 candidates for selection were suggested by the teachers for each group; the nature of the socioeconomic condition of the family and the character of the diet was then verified through home visits by an epidemiologist and a public health nurse. From those who fulfilled the dietary criteria further selection was made with regard to the body weight rather than age. Children from both groups were chosen to fall in the same range of body weight. The mean initial body weights were 25.5 kg for the PDP group and 26.1 for the ADP group. This reasonably close matching of body weight was associated with some age difference—11.4 years in the PDP and 11.0 years in the ADP.

Two balance studies, separated by approximately five months, were made on the same subjects; the first study was for 20 days and the second for 30 days. The subjects were kept in the school continuously for the entire period of each balance study. They were fed a carefully measured diet consisting primarily of Sobee (a high-protein food supplement compounded from soybean) and rice, with smaller amounts of bread, butter, and jelly. Their total food intake was based on their body weights at the beginning of the balance study period. Their ad libitum water intake was measured to the nearest gram. Total collections of urine and stool were made and their amounts measured to the nearest gram. By means of a specially adapted balance apparatus daily body weights were estimated each morning after breakfast to the nearest gram.

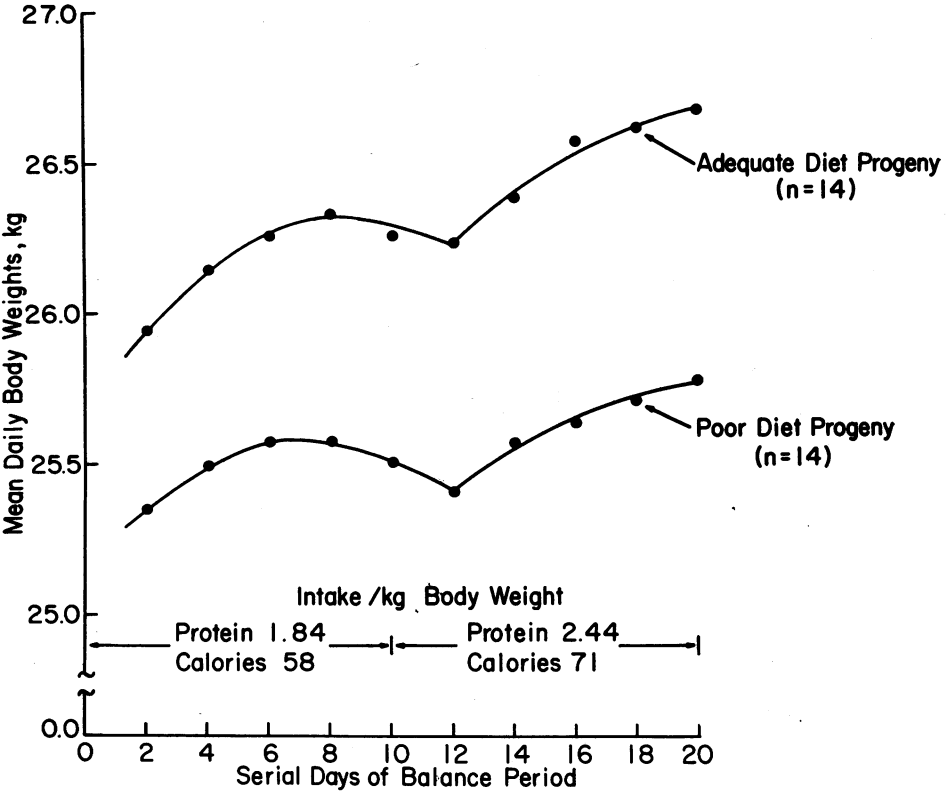
The two balance studies were carried out according to an identical protocol, except for their length. During the initial ten-day period of both studies, a total food intake of 58 calories and 1.84 grams of protein per kilogram of body weight was allowed. The intake of Sobee was then raised by 50 per cent to provide 71 calories and 2.44 grams of protein per kilogram of body weight. In the first study, the period for the higher intake was ten days, whereas in the second study it was continued for 20 days.

In the intervening five months the subjects consumed their customary diet. Body weights were obtained at intervals during that time and also following the second balance study.

For nitrogen retention estimates, the total daily urinary nitrogen was determined. Fecal nitrogen was determined only in the second portion of the second balance study.

Fourteen of the initial 18 subjects in each group completed the entire period of observation extending through the two balance study periods; results on those subjects follow.

Figure 2—Mean daily body weights during first balance study (20 days)



Results

The 14 boys in the ADP group showed a more favorable physiological response during the balance study periods than did the corresponding 14 boys in the PDP group. This is reflected both in the body weight changes (Figures 2 and 3) and in the urinary nitrogen output.

The mean daily change in body weight was consistently in favor of the ADP group in both periods of study. The mean body weight of the ADP was approximately 600 grams higher than that of the PDP at the beginning of the first balance study. This difference in means increased by 300 grams during the 20 days of the first balance study (Figure 4). At the end of the five-month interval between the two balance studies, the mean body weights had become more nearly equal. During the second 30-day study period the difference in mean body weight increased by 584 grams (Figure 5). After the boys returned home following the second study period, the PDP group again approached the body weight of the ADP group, as in the interval between the two study periods. In view of the fairly wide range of initial body weights of the children in both groups, and the small difference in the mean initial body weight of the two groups, the significance of the observed difference in weight change between the two groups has been tested by an analysis of covariance, regarding the initial body weight as the covariable. For this analysis the body weight changes in the first and second study periods have been combined. In Figure 6 these total body weight changes, on the vertical axis, have been plotted against the initial body weights,

on the horizontal axis, for each child. There is a clear dependence on the initial body weight and it appears reasonable to make the assumptions of linearity and equality of slope as required for an analysis of covariance. In essence this technic involves the fitting of regression lines to each group of data under the restraint that the slopes be equal. It is then determined whether or not the lines are separated significantly. In the present case they are significantly separated ($p<0.001$) and it would not have been possible to demonstrate anything approaching this level of significance without removing the effect of initial body weight, the covariable. Furthermore, a test that ignored this factor would be open to the criticism that the initial mean body weights were not identical in the two groups.

The data relating to nitrogen metabolism (which are to be presented in greater detail elsewhere) were consistent with those for change in body weight. The urinary nitrogen outputs for the second study are summarized in Figure 7: the mean urinary nitrogen excretion relative to body weight of the PDP group consistently exceeded that of the ADP group. Furthermore, the fecal nitrogen (which was determined only during the final portion of the second balance period) was higher in the poor dietary group. The absorbed portion of total dietary nitrogen was thus lower in the PDP group but those subjects had, nonetheless, a higher urinary output, indicating an inability to retain absorbed nitrogen, over and above the greater fecal loss.

Discussion

There is clearly a close parallel between the results obtained in rats on the one hand and in children on the

Figure 3—Mean daily body weights during second balance study (30 days)

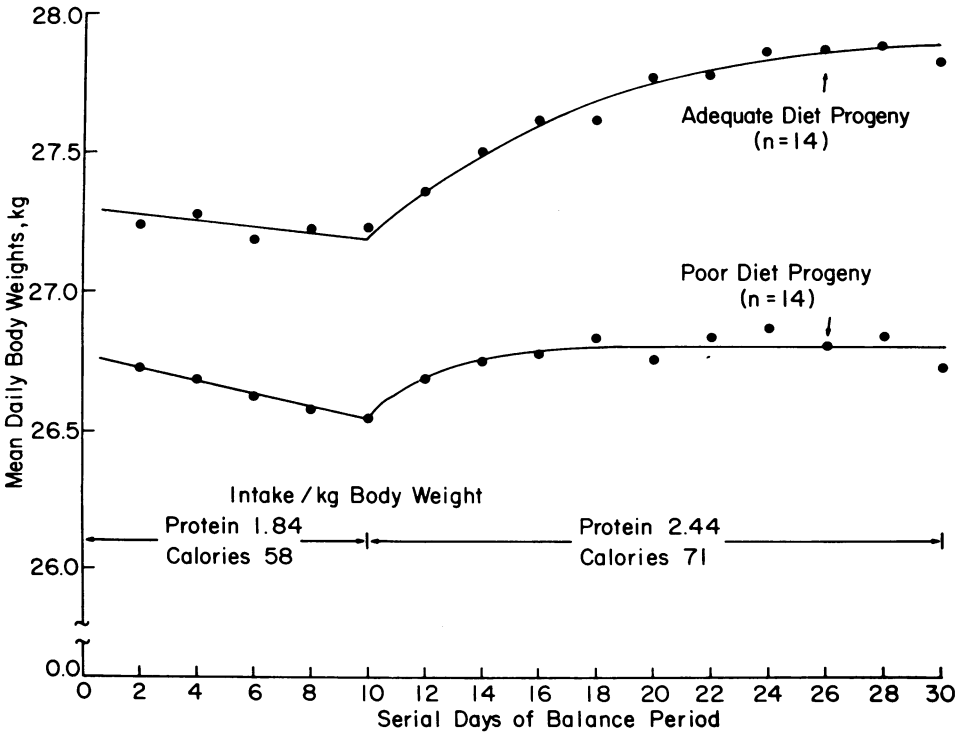


Figure 4—Differences* in mean daily body weights during first balance study (20 days) (n=14 in each group)

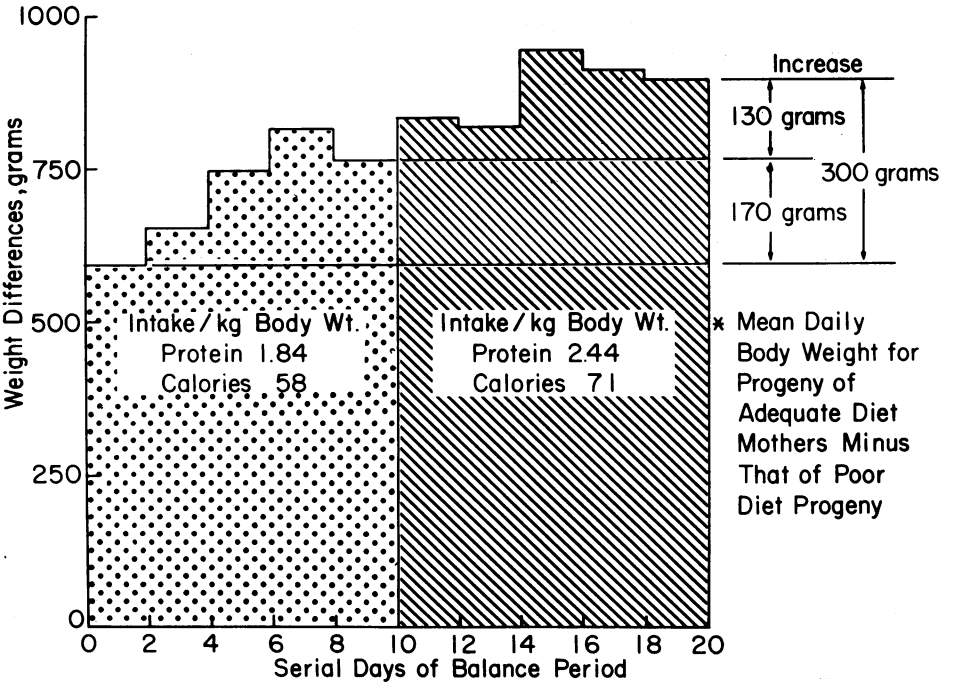
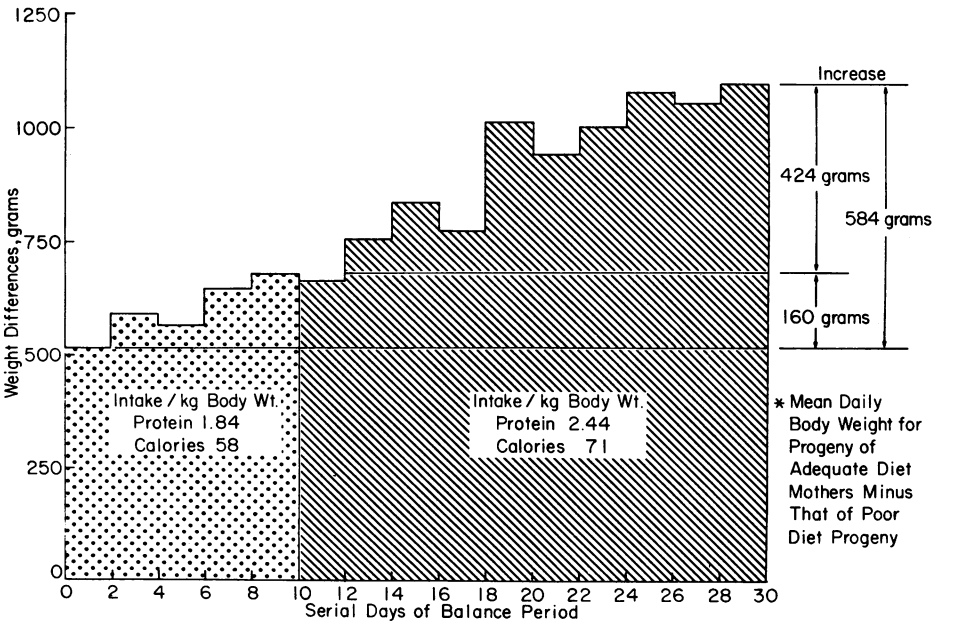


Figure 5—Differences* in mean daily body weights during second balance study (30 days) (n=14 in each group)



other. In both studies there is an association between an inadequate maternal diet and subsequent poor utilization of food by the progeny. In the case of the rats, the experiment is specific and the metabolic performance of the progeny can be directly related to the maternal diet. With the children the nature of the study precludes any such direct inference because the quality of the maternal diet was only one of several differences existing between the ADP and PDP groups. For example, the former group also had received a very much more adequate diet *after* weaning up until the time of study at about 11 years of age. Probably less important but still relevant are the social and economic differences necessarily associated with the dietary differences. However, regardless of the various factors involved, we believe that the study has demonstrated a hitherto unrecognized phenomenon of food wastage in a certain group of children, a group characterized by economic and, hence, dietary deprivation of both their mothers and themselves.

In another respect there is a dissimilarity between the observations in the rats and the children. The progeny of the poorly nourished mother rats exhibit a weight deficit of about 30 per cent in spite of ad libitum feeding with an adequate diet after weaning. On the other hand, the children of the poorly nourished mothers had achieved approximately the same body weight as their more privileged colleagues only about six months later, even though their diet had remained inadequate throughout their childhood. At the same age the PDP group showed a weight deficit of only about 6 per cent. However, from observations made on the dietary intake of the two groups of children in the five months spent at home between the

Figure 6—Body weight change during both balance periods as a function of initial body weight. Adequate diet progeny are represented by open circles and poor diet progeny by filled circles. The respective means are represented by larger circles.

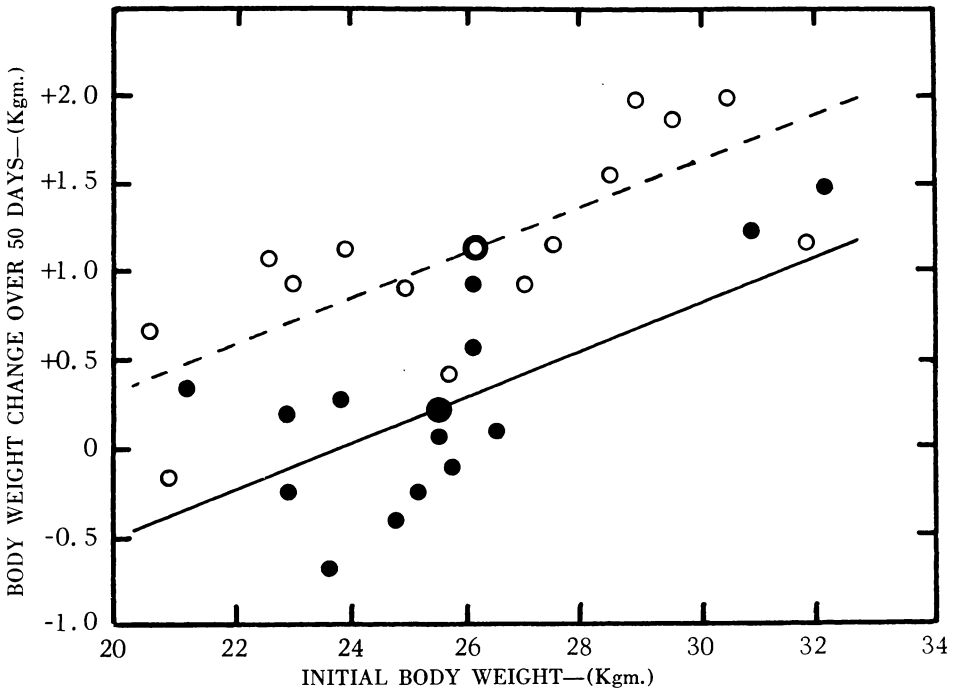
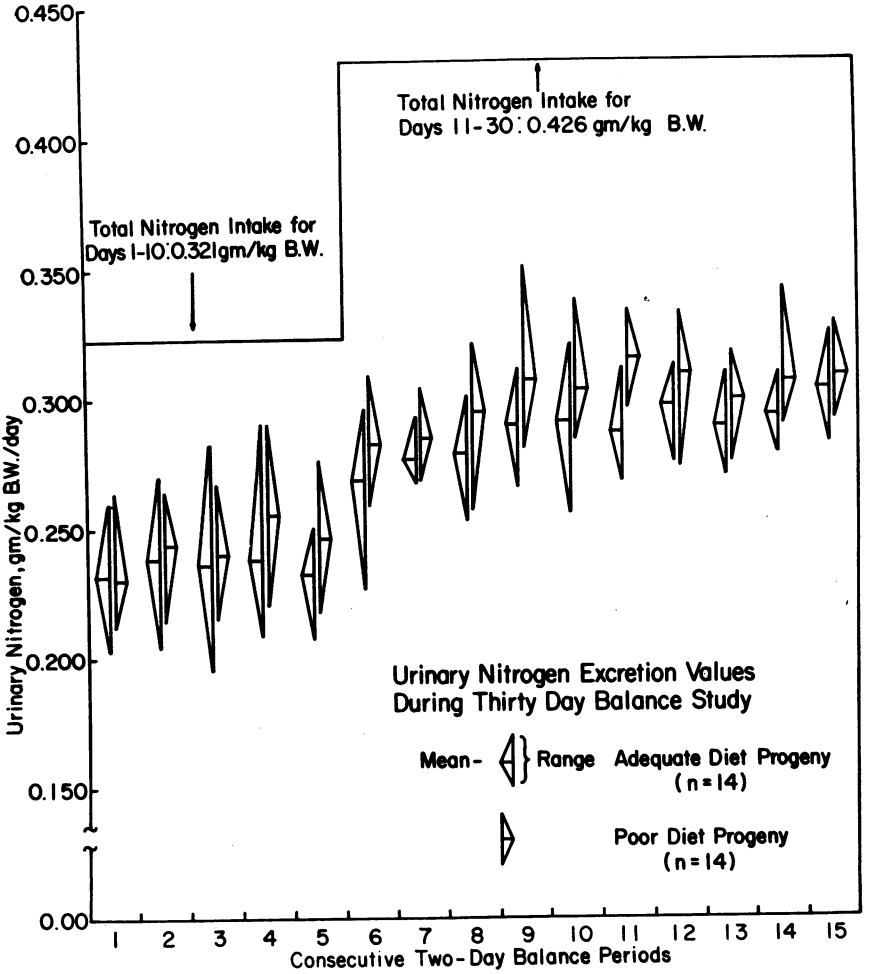


Figure 7



two balance studies, we have evidence that the caloric intake of the PDP group was much greater than that of the ADP group; this explains the fact that the PDP group more than recovered the weight deficit incurred during the first balance study, and showed a similar trend when they returned home after the second balance study. While the dietary intake of the congenitally malnourished rats is higher than that of controls, the animals do not, at any age, appear to have the ability to eat and utilize all of the additional food which would be necessary for them to achieve a normal body weight. In this regard, it is of particular interest that the children exhibit a definite metabolic abnormality in spite of a relatively normal body weight, because it emphasizes that body weight is an unsatisfactory index of past and present nutritional adequacy. This is not always recognized. It is widely believed that the human fetus develops normally in spite of quite severe quantitative and qualitative inadequacies in the maternal diet. This view appears to be based on the fact that birth weights are but little impaired under conditions of considerable maternal dietary deprivation.[1,3,8] It has been tacitly assumed that a child of normal

weight is normal in all other respects, but we are aware of no follow-up studies which adequately justify this assumption. Our present findings, for example, show that at least at one stage of growth important metabolic abnormalities can be present in a child of essentially normal body weight. We cannot be certain that these abnormalities are attributable to the maternal diet, but the parallel with our findings in rats leads us to believe they are related to it.

Summary

Qualitative and quantitative deficiency in the protein composition of diet fed to mother rats during pregnancy and lactation is associated with poor food utilization in the progeny.

In a preliminary study of 11-year-old children in Formosa, a dietary intake sufficient to permit normal weight gain in children from adequately fed families caused weight loss in children from poorly fed families.

The "poor" children showed consistently greater urinary nitrogen excretion.

This type of study does not allow us to infer that the maternal diet during pregnancy and lactation was necessarily responsible for the observed differences, but such an interpretation would be consistent with the results in rats.

Definite information about this effect in human beings can only be obtained by longitudinal study involving experimental manipulation of the maternal diet and subsequent study of the children. Such an experiment is being undertaken in Formosa.

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