The biphasic nature of protein metabolism during pregnancy in the rat

By D. J. NAISMITH AND B. L. G. MORGAN

Department of Nutrition, Queen Elizabeth College, London W8 7AH

1. Additional protein given to malnourished rats early in pregnancy was found to increase substantially the weight and cellularity of their foetuses and placentas at term.

2. This observation confirms the hypothesis that protein is stored in early pregnancy for use during the period of rapid foetal growth.

It is now well established that undernutrition of the mother can impair the growth and development of the conceptus. A relationship between the level of protein intake and the weight and protein content of the foetus and placenta was first reported, in the rat, by Naismith (1966). Shortly thereafter it was demonstrated that maternal protein restriction retards cellular development, as measured by DNA content, in all foetal tissues including, notably, the brain (Winick, 1968, 1970; Zamenhoff, Van Marthens & Margolis, 1968; Zeman & Stanbrough, 1969). Varying the protein intake over a very wide range, however, induces comparatively small changes in the birth weight of the pups. In the experiments of Nelson & Evans (1953), for example, a reduction in the concentration of protein in the maternal diet from 20 % to 5 % brought about a fall of no more than 20 % in the average weight of the young. Such results strongly suggest that a mechanism exists whereby the foetus is protected from the harmful effects of poor maternal nutrition.

Analyses of the carcasses of rats at parturition have shown that there is no net gain of protein resulting from pregnancy (Boyne, Chalmers & Cuthbertson, 1953; Naismith, 1966). By day 14 of pregnancy, however, a substantial retention of nitrogen has occurred, representing an increase of 8·5 % in lean tissue, and equal to half the amount of N found in the foetuses and placentas at term (Naismith, 1969). The metabolism of protein during pregnancy thus appears to follow a biphasic course, as illustrated in Fig. 1.

During the first two weeks of pregnancy, the anabolic phase, the protein increments of the foetuses and their supporting tissues are insignificant, and the dam builds up a reserve of protein. During the last week, when rapid growth of the foetuses takes place, the protein reserve is withdrawn. This catabolic phase occurs irrespective of the protein intake of the mother, indicating that it is under hormonal rather than dietary control.

The utilization of protein stored in early pregnancy to subsidize the rising cost of protein synthesis in late pregnancy could play a major role in moderating the influence of severe protein restriction on foetal growth. To test this hypothesis, the effects of supplementing a diet low in protein for a short period in early pregnancy have been investigated.
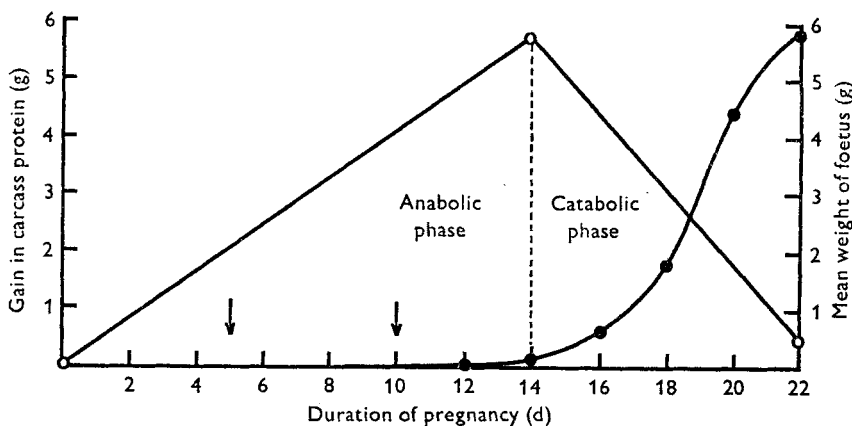
Fig. 1. Schematic representation of gain in carcass protein (g) due to pregnancy (o–o), as reported by Naismith (1969), and mean weight of individual foetuses at different stages of pregnancy (●–●), calculated from the data of Beaton, Beare, Ryu & McHenry (1954). ↑↑, Protein supplement given in the experiment.

METHODS

Eleven litter-mate pairs of rats of the Sprague–Dawley strain, weighing approximately 210 g, were mated, pregnancy being dated from the appearance of a mating plug. One of each pair was fed throughout pregnancy on a diet containing only 50 g protein (casein)/kg, but adequate in all other respects. The second animal received the same diet, but from days 6 to 10 inclusive, the casein content was raised to 250 g/kg at the expense of maize starch, and 2·5 g DL-methionine/kg was added. Thus the protein supplement was withdrawn before rapid growth of the foetuses began (Fig. 1). On the last day of gestation (day 22) the litters were removed by caesarian section, the foetuses and placentas were weighed, and their contents of protein and DNA were determined. The brains of the pups were dissected for separate analyses. N was estimated by the kjeldahl procedure. DNA was extracted from the tissues by the method of Klemperer (1963) and estimated by the diphenylamine reaction described by Burton (1956). For the statistical evaluation of the results, Student's 't-test for related samples' was used.

RESULTS AND DISCUSSION

The offspring of rats deprived of protein during pregnancy show diminished hyperplasia in all tissues examined. Furthermore, the impaired rate of cellular growth persists even when good nutrition is instituted at birth, by fostering the pups on to well-nourished mothers (Allen & Zeman, 1971). The effects of malnutrition in utero are therefore both absolute and permanent.

In the present study, total food intakes of the rats given the low-protein diet and the supplemented diet were 305 and 321 g respectively, the small difference (5 %) being accounted for by an upsurge in appetite that occurred when the high-protein diet was given. Total protein intake was doubled by the protein supplement (from 13·4 to 29·3 g).

Table 1. *Analyses for protein and DNA in individual placentas and foetuses from rats fed a low-protein diet† throughout pregnancy, or given a protein supplement in early pregnancy*

(Mean values with their standard errors for 11 matched pairs of dams)

Placenta			Foetal carcass		
Weight (mg)	Protein (mg)	DNA (mg)	Weight (g)	Protein (mg)	DNA (mg)
Control					
406 ± 6	$37 \cdot 6 \pm 1 \cdot 8$	$0 \cdot 67 \pm 0 \cdot 034$	$3 \cdot 29 \pm 0 \cdot 18$	203 ± 10	$13 \cdot 0 \pm 0 \cdot 17$
Supplemented					
$448^{**} \pm 9$	$47 \cdot 6^{**} \pm 2 \cdot 3$	$0 \cdot 95^{**} \pm 0 \cdot 036$	$4 \cdot 74^{**} \pm 0 \cdot 28$	$296^{**} \pm 16$	$16 \cdot 9^{**} \pm 0 \cdot 64$

Foetal brain		
Weight (mg)	Protein (mg)	DNA (mg)
Control		
$183 \pm 9 \cdot 1$	$9 \cdot 37 \pm 0 \cdot 42$	$0 \cdot 42 \pm 0 \cdot 01$
Supplemented		
$216^{**} \pm 5 \cdot 3$	$10 \cdot 57^{*} \pm 0 \cdot 36$	$0 \cdot 62^{**} \pm 0 \cdot 04$

* $P < 0 \cdot 05$.   ** $P < 0 \cdot 01$.
† For details see page 564.

The results of the analyses are summarized in Table 1. No differences were found in litter size between the rats fed the low-protein diet and those that received the supplement. Feeding extra protein in early pregnancy, however, raised the mean birth-weight of the foetuses by 44 % and the protein content of their carcasses by 46 %. A similar difference in carcass DNA was found ($+30$ %) indicating that a marked improvement in the rate of cell multiplication had occurred.

The influence of early malnutrition on the growth and development of the brain has attracted much attention during the last decade, particularly since it has been suggested that a critical period of brain growth may exist during which malnutrition, even for a short period, may produce irreversible retardation. The brains of the foetuses from dams that had received the protein supplement were significantly heavier, and contained more protein and DNA than the brains of their controls. In the rat, 94–97 % of the adult complement of neurons is present in the brain of the new-born (McIlwain, 1966), the period of most active neuronal replication occurring during the last week of pregnancy (Dobbing & Sands, 1973). Thus the striking effect that the dietary supplement had on the DNA content ($+48$ %), and hence on the number of neurons in the foetal brain, could not have been exerted during the time of its administration. As a corollary to our findings, demonstrated earlier by Zamenhoff, Van Marthens & Grauel (1971), a protein-free diet fed throughout the first 10 d of pregnancy was found to retard foetal brain development. These authors concluded that the effects were unlikely to be due to a deficiency of amino acids *per se*, as required for embryonal protein synthesis. The period of protein deprivation imposed, however, coincided

with the anabolic phase of protein metabolism, and would therefore have precluded the normal build-up of the protein reserve.

The rate of growth of the foetuses is determined not only by the availability of amino acids and other nutrients in the maternal circulation, but also by the rate of transfer of nutrients across the placenta. Since the transport of nutrients is a surface phenomenon, and, in some instances, is dependent on specific carrier proteins in the placenta, it seems probable that the larger placentas, containing substantially more protein, that were found in the group that received the protein supplement would function more efficiently in this respect. Winick & Noble (1966) have shown that the amount of DNA in the placenta begins to increase rapidly around the twelfth day of gestation. Extra protein ingested by the mother between days 6 and 10 of pregnancy could not, therefore, have affected cell multiplication in the placenta at that time. To what extent the active transport of nutrients is regulated by the size of the placenta, by its protein content, and by its cellularity, has yet to be determined, but it is likely that the beneficial effect of an early protein supplement on foetal growth was mediated, in part, through its influence on placental growth.

The findings of this experiment confirm the hypothesis that protein stored during the anabolic phase of pregnancy is used to sustain the growth and development of the foetuses in late pregnancy, and so to limit the influence of protein deprivation.

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