NITROGEN RETENTION IN PREGNANCY

BY

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Summary

Nitrogen balance studies were performed in 21 primigravida who were eating their usual diets in the third trimester. The mean apparent nitrogen retention was 1.17 g. per day. This figure tends to contradict the belief that large amounts of protein are laid down as stores by the mother in pregnancy.

There is still some disagreement about the amount of nitrogen stored by the pregnant woman. The common belief that pregnancy leads to a maternal storage of protein is based on the results of several nitrogen balance studies and the observation that maternal weight gain is usually greater than the weight gained by reproductive structures. Hoffstrom (1910), who studied a woman from 17 weeks gestation to term, found that the amount of nitrogen apparently retained exceeded that attributable to growth of the products of conception and enlargement of the reproductive organs, and termed the remainder “rest nitrogen”. Since then many studies have supported his findings. A comprehensive bibliography of work done before 1934 was published by Macy and Hunschner (1934). From 954 published daily balances they estimated that the amount of nitrogen stored was about 370 g. Hunschner et al. (1936) found a maternal storage of 333 g. of nitrogen during the last 145 days of pregnancy in a single patient studied continuously. Lister and Keith (1960) found a nitrogen retention which varied from 1 to 8 g. per day in three patients studied for one day before elective Caesarean section, while Lister (1961) found a range of mean values between 2 and 5 g. nitrogen retained per day in five antenatal cases. Lower values were obtained by Venning et al. (1959), whose patients had a low calorie intake and all lost weight during the study, and by Hummel et al. (1937) and Freyburg et al. (1938). The most recent estimate of nitrogen storage for the whole pregnancy has been 150 g. in excess of known requirements for fetus, placenta, uterine and breast hypertrophy (Zuspan and Goodrich, 1968).

On the other hand, Hytten and Leitch (1971) have argued that much of the weight gain previously unaccounted for is due to an increase in extracellular water, and that the remainder is likely to be due to storage of fat, not of protein. This was supported by the serial measurements of skinfold thickness during pregnancy made by Taggart et al. (1967). Hytten and Leitch (1971) pointed out that there is no satisfactory explanation of where the excess nitrogen is stored, and were doubtful whether storage of nitrogen on this large scale could occur in the human,
particularly as albumin and amino acid levels are lowered in pregnancy. Any errors in nitrogen balance experiments tend to increase the apparent retention by systematically overestimating intake and underestimating losses, and it is possible that small accumulated errors may be responsible for results which otherwise seem unlikely.

In Aberdeen over 40 nitrogen balance studies have been performed on primigravidae between 30 and 34 weeks pregnant who were invited to participate after the studies had been explained to them. The cases were chosen to contrast women with normal, high or low weight gain before 28 weeks gestation. The results from the group who satisfied the requirements for "normal" pregnancy are presented.

**PATIENTS AND METHOD**

All subjects were in their first pregnancy and were studied between 30 and 34 weeks gestation. The criteria required for selection of normal cases was as follows: none had a past significant illness, all were at or above average height for Aberdeen women (1.575 m.), and aged between 17 and 25. The weight-for-height fell in the interquartile range on the Kemsley scale (Kemsley *et al.*, 1962) and the weekly weight gains from 20 to 28 weeks were between 340 and 567 g. per week. The date of the last menstrual period was known with certainty, and no complication of pregnancy developed. Delivery of healthy babies occurred after the 38th completed week of pregnancy, and the birthweights were all greater than the 10th percentile on the scale of Thomson *et al.* (1968).

Twenty-two patients fulfilled these criteria, but one patient who was in negative nitrogen balance is not included. The results are, therefore, from 21 patients.

Before hospital admission a dietary history was taken from the patient by a dietitian, and a diet was arranged which corresponded as closely as possible to the subjects' usual intake. Each investigation lasted 12 days, the balance period itself starting on the fifth day and lasting six days. At the beginning of each study enough food was collected for the 12-day period to minimize variation, and exactly the same amount was eaten every day, the food being prepared and weighed in a diet kitchen attached to the Research Unit. All meals were eaten, and no patient refused food or vomited during the study. The patients used a flexible spatula to clean the plates, and invisible nitrogen returns, which were estimated for each patient on one or two days, never exceeded 60 mg. per day, the mean figure being 30 mg. per day. All food was sampled, cooked food being sampled at the time of weighing before the patient was served, as it was found that nitrogen loss occurred during cooking. Urine specimens were immediately transferred to stoppered bottles and refrigerated, and faecal specimens were kept deep frozen before they were homogenized prior to analysis. Carmine red was used as a marker. Skin losses were not measured. For analysis of nitrogen a macro-Kjeldahl method with mercury as catalyst was used. This method was essentially that of the Association of Official Agricultural Chemists (1966) and in this laboratory proved very accurate in recovery experiments with different amino acids. The overall mean difference between duplicate results during the study was less than 0.5 per cent.

**RESULTS**

These are summarized in Table I. The mean daily nitrogen intake was 12.49 g. or 78 g. protein, and the mean calorie intake, calculated from a food table, was 2409 k. cals. per day, with a standard deviation of 211 k. cals. per day. The mean nitrogen loss as "invisible returns" was 0.03 g. Losses in the urine and faeces were 10.24±1.15 g. per day and 1.04±0.40 g. per day respectively. The mean weight gain during the balance period was 464 g. per week. The mean weight gain between 28 and 36 weeks gestation was 432 g. per week. All patients were in positive nitrogen balance and the daily balance was +1.17±0.64 g. per day.

**DISCUSSION**

In this study care was taken to ensure that each patient had a food intake during the study which accurately represented her usual home intake. The mean weight gain during the balance period (464 g. per week) was similar to the mean 28–36 week gain (432 g. per week), suggesting
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### Results for 21 normal primigravidae

<table>
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<tr>
<th>Age</th>
<th>Nitrogen intake (g./day)</th>
<th>Energy intake (Cals/day)</th>
<th>Faecal nitrogen (g./day)</th>
<th>Urinary nitrogen (g./day)</th>
<th>Plate waste (g./day)</th>
<th>Apparent nitrogen retention (g./day)</th>
<th>Birthweight of fetus (g.)</th>
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Mean: 21.7 12.49 2409 1.04 10.24 0.03 1.17 3324
S.D.: 2.3 1.26 211 0.40 1.15 0.016 0.64 424

that there was no systematic alteration in the pattern of weight gain. Furthermore, the intake values were close to the expected values of the means found by Thomson (1959) in his detailed survey of the diets of 489 Aberdeen primigravidae. For this total population he found a mean protein intake of 75 g. and a mean calorie intake of 2449 calories per day, which are similar to the means of 78 g. and 2409 calories respectively in this study.

The mean faecal nitrogen loss was 1.03 g. per day. This figure is rather low compared with several previous studies. Coons and Blunt (1930) found a mean faecal loss of 1.86 g. per day and Hunschner et al. (1935) found a loss of 1.96 g. per day, while Venning et al. (1959) assumed average values of 1.3 g. lost per day. It is also slightly low compared with the averages found in large numbers of male and non-pregnant female cases. For example, an average of 1.28 g. per day faecal nitrogen loss was found by Reifenstein et al. (1945) and 1.12 g. per day by Dempsey et al. (1958). However, Hummel et al. (1936) found an identical mean of 1.03 g. per day in their study of a single patient who was followed for the last 65 days of her pregnancy.

The mean apparent nitrogen retention in our patients was certainly lower than previous workers have found, and an attempt is made to set this against known requirements. The known components of nitrogen storage in pregnancy are the fetus, placenta, liquor, uterus, breasts and blood. Hytten and Leitch (1964) calculated the requirements for these sites, and when their conversion figures from protein to nitrogen are used the total requirements from 20 to 40 weeks gestation would be approximately 110 g. nitrogen. This would involve a daily retention of about 0.8 g. per day, assuming that nitrogen is retained to meet these requirements at a steady rate during this time. In the present study the apparent nitrogen retention was calculated as the difference between the nitrogen intake and the sum of the values for urinary and faecal nitrogen. However, this is not a true value as there are several unmeasured pathways of nitrogen loss.
Some of these such as colonic flatus (Kirk, 1959) and ammonia in respiration (Jacquez et al., 1959) may prove to be negligible, but integumental losses are certainly not. Estimates of epithelial nitrogen losses obtained by direct measurement in men and non-pregnant women have been 333 mg. per day (Voit, 1930), 338 mg. per day (Cuthbertson and Guthrie, 1934), 277 mg. per day (Freyburgh and Grant, 1937), 360 mg. per day (Mitchell and Hamilton, 1949), 254 mg. per day (Darke, 1960) and 143 mg. per day (Sirbu et al., 1967). However, there have been no published studies of epithelial nitrogen loss in human pregnancy, and any difference from the non-pregnant state is difficult to predict. On the one hand Sirbu et al. (1967) have shown that blood urea levels and dermal excretion of nitrogen are directly correlated, and as blood urea concentration falls in pregnancy it could be argued that epithelial nitrogen losses would also be reduced. On the other hand it is possible that nitrogen losses may be greater than in the non-pregnant state because of increased fingernail growth and hypertrichosis (Hillman, 1960) and increased sweating (Burton et al., 1970). In the absence of reliable figures for pregnancy the mean of the reported studies in men and non-pregnant women—284 mg.—will be assumed. The mean apparent nitrogen retention for this study would then be roughly 0·9 g. per day. With a predicted nitrogen requirement of approximately 0·8 g. per day, this suggests that there is little or no storage of nitrogen outside known sites in normal patients taking their usual diets at this period of gestation. Certainly there is no evidence for maternal storage of the very large amounts of surplus nitrogen suggested by previous authors.

Macy and Hunschner (1934) for example, found that the mean apparent nitrogen retention in the second half of pregnancy was 2·83 g. per day. However, many of the studies summarized were done on outpatients, and it is likely that this biased the results, for errors in the balance technique usually tend to increase the apparent retention. When metabolic ward technique was used, as for example, in the studies by Hummel et al. (1937) and Zuspan and Goodrich (1968) the apparent retention was lower. The latter authors, in a careful long-term study found an apparent mean retention of 1·3 g. nitrogen per day for five cases in late pregnancy, but calculated that this would involve a maternal storage in excess of known requirements of 150 g. during pregnancy. However, they not only ignored skin loss of nitrogen, which is likely to account for about half of this amount, but they assumed that their figure for nitrogen retention, obtained in late pregnancy, would be the same in early pregnancy, and this is not necessarily so.

In conclusion, during pregnancy nitrogen is retained to meet the requirement of the product of conception and the growth of the reproductive organs. There may also be a retention of nitrogen for other maternal organs such as the alimentary tract and liver. However, the amount involved is likely to be small and previous very high estimations of "protein stores" are probably the result of the technical errors tending to occur in nitrogen balance studies.

Acknowledgements

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References