EFFECT OF PROTEIN DEPLETION DURING PREGNANCY AND PROTEIN REPLETION AFTER PARTURITION ON CELLULAR GROWTH IN RATS

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The effect of protein-free diet on cellular growth of the liver and muscle, in terms of cell size (protein/DNA) and cell number (total DNA), in pregnant and nonpregnant rats was investigated. Slight but significant decreases in the size and number of liver and muscle cells were observed in both groups of rats when fed a protein-free diet ad libitum for three weeks. When rats received only 5 g of the protein-free diet a day for three weeks, the total DNA in their liver and muscles decreased greatly. When these depleted animals were refed, the size and number of liver and muscle cells increased to normal levels within 10 to 14 days. Similar changes in cellular response to the nutritional impacts were observed in pregnant and nonpregnant animals.

It is known that protein-calorie malnutrition in young animals causes decreased synthesis of nucleic acids and protein in different organs resulting in reduced cellular growth. In adult animals the effect of protein-calorie malnutrition on the cell size (protein/DNA) and cell number (total DNA) of various organs has been investigated by many workers, but results on the cell number are conflicting. CAMPBELL and KOSTERLITZ (1), and THOMSON et al. (2) stated that neither the total DNA in the liver nor the DNA per nucleus of hepatocytes was affected by administration of a protein-free diet for one to two weeks. On the contrary, ELY and Ross (3) found that prolonged dietary deficiency of protein affected the DNA content of the liver of young adult rats. Moreover, examination of the data of several other groups (4–7) indicates a slight but significant decrease in hepatic DNA content of adult rats receiving low protein or protein-free diets for prolonged periods. A previous report from our laboratory indicated a decrease in the DNA contents of the liver and muscles of malnourished pregnant animals receiving diets devoid of one essential amino acid
with ovarian steroids (8). In this work we examined the effect of protein deprivation during pregnancy and repletion after parturition on the size and number of liver and muscle cells.

MATERIALS AND METHODS

Female Sprague-Dawley rats, weighing about 160 g, were obtained commercially and fed on 20% casein diet until they weighed about 190 g. Then they were mated and the day of insemination was confirmed by a vaginal smear test and designated as day 1 of pregnancy. These rats were fed ad libitum on a protein-free diet throughout the gestation period and were given a daily subcutaneous injection of 0.5 μg of estrone and 4 mg of progesterone from day 3 to day 22 to maintain pregnancy. On day 23 of pregnancy, one group of 7 rats was killed while the other group was subjected to Caesarean section and then fed on 20% casein diet ad libitum for 14 days. Nonpregnant rats were used as controls and fed ad libitum on the protein-free diet, and then on 20% casein diet during a 10 day recovery period.

Animals were killed on days 1 and 23 of pregnancy and at the end of the recovery period (days 15 and 11 in multiparous and control animals, respectively). The liver and gastrocnemius muscles, and reproductive organs in pregnant females at term, were removed and stored in a deep freezer for determination of nucleic acids and protein. The carcass was dried to constant weight at 105°C and then finely ground to determine its lipid and nitrogen contents.

RNA and DNA in the liver and muscles were measured spectrophotometrically (9). Protein was determined by the method of LOWRY et al. on the 0.3 N KOH hydrolyzate obtained during nucleic acid determination (10). The lipid content of the dried carcass was determined gravimetrically after extraction with ethyl ether in a Goldfisch apparatus. A semi-micro Kjeldahl method was used for determination of carcass nitrogen.

The body weight and food consumption of the animals were measured daily. Each group contained six to seven rats.

In a preliminary experiment on the influence of more severe dietary restriction on the nucleic acid contents of tissues, pregnant and nonpregnant animals received only 5 g of the protein-free diet a day for three weeks.

RESULTS

Food intake and change in body weight

Figure 1 and Table 1 show the changes in body weight and food intake of the animals. Pregnant rats consumed a total of 163 g (7.4 g/day on an average) of protein-free diet during pregnancy, showing a gradual decrease in daily intake. Nonpregnant rats ate significantly more food than pregnant
The body weight of rats receiving protein-free diet decreased steadily during the first two weeks, regardless of whether pregnancy was maintained or not. During the last week, the body weight of pregnant rats increased, while that of nonpregnant females continued to decrease.

Data on rats killed at the end of the depletion period are not shown, but were essentially similar to those in Table 1. In the recovery period, the total food intakes of multiparous and control animals were 193 g/14 days and 119 g/10 days, respectively, with temporary peak intakes. As a result of adequate nourishment, the body weights of both groups increased to the initial levels within 10 to 14 days.

Reproductive performance

Table 2 shows the reproductive performances on day 23 of pregnancy in rats. Significantly less growth of conception products was observed in rats fed protein-free diet than in normal rats. Furthermore, in rats fed protein-free diet, whose intake was restricted to 5 g per day, the conception products were lighter than when food was taken ad libitum. Maternal protein-calorie deficiency affected protein and DNA contents in fetal whole body. Total DNA (cell number) of a fetus of the protein deprived mothers was half when compared with that in normal control, while protein/DNA ratio (cell size) was appreciably not reduced.
Table 1. Food intakes and changes in body weight during protein depletion and repletion.

Depletion period

|                        | Intake of PFD | Body weight |
|------------------------|---------------|-------------|
|                        | Total         | Daily       | Day 1  | Day 14 | Day 23 (or 22) |
| Pregnant (6)           | 163±25\(^d\) | 7.4         | 188±5  | 158±9  | 171±10         |
| Nonpregnant (7)        | 236±30        | 11.2        | 193±10 | 165±6  | 155±5          |

Repletion period

|                        | Intake of 20\% casein diet | Body weight in recovery period |
|------------------------|-----------------------------|-------------------------------|
|                        | Total          | Daily         | Day 1\(^f\) | Day 6 | Day 11 | Day 15 |
| Multiparous            | 193±13         | 13.8          | 129±7  | 144±7 | 179±6  | 194±7  |
| Nonpregnant            | 119±7          | 11.9          | 155±5  | 184±6 | 191±9  |        |

\(^a\) The periods of protein depletion in pregnant and control groups were 22 days and 21 days, respectively.
\(^b\) Protein-free diet.
\(^c\) Figures in parentheses represent numbers of rats.
\(^d\) Values (in grams) are means ±SD.
\(^e\) The periods of protein repletion in pregnant and nonpregnant animals were 14 days and 10 days, respectively.
\(^f\) Fetuses and placentas were removed by Caesarean section from pregnant females on day 23 of pregnancy, and thereafter rats were fed on 20\% casein diet. Day 1 in the repletion period corresponds to day 23 in the depletion period.

Gross carcass composition

Data on the composition of the carcass are shown in Table 3. On day 1 of pregnancy (average body weight; 185 g) the average nitrogen content of the carcass (without the liver) was 5,355 mg. After protein depletion for 3 weeks, the carcasses of pregnant and nonpregnant animals contained 3,660 mg and 4,450 mg of nitrogen, respectively. The difference between the latter two values (790 mg) was partly due to nitrogen mobilization from the maternal body to the conception products (about 480 mg of nitrogen) and probably also to increased nitrogen excretion caused by less food intake. The lower food intake of pregnant females also affected the fat content of their body, as seen in Table 3. The nitrogen and fat contents of the carcasses of these rats suggest that catabolism is greater in maternal body of pregnant animals than in control animals.

When malnourished rats were fed on adequate diets, the nitrogen and fat contents of their body returned to the initial levels within 10 to 14 days.

Nucleic acid and protein contents of the liver and muscle

Tables 4 and 5 show the nucleic acid and protein contents of the liver and gastrocnemius muscle of the animals, respectively. The liver weight, total RNA and total protein in pregnant and nonpregnant rats decreased on protein depletion for 3 weeks. There were also significant decreases in the size (protein/DNA) and
Table 2. Effect of protein depletion during pregnancy on reproductive performance and cellular growth of fetuses.\(^a\)

| Diets          | Weight of conception products\(^b\) (g) | Placenta wt (g) | Fetus |                    |                    |                    |                    | Protein/DNA        |
|----------------|----------------------------------------|-----------------|-------|--------------------|--------------------|--------------------|--------------------|--------------------|
|                | wt (g)                                 | Total protein (mg) | Total DNA (mg) |                    |                    |                    |                    |                    |
| 20\% casein\(^e\) (13)\(^d\) | 76±17                                  | 4.68±1.13       | 5.35±0.25 | 560±68             | 25.92±2.64         | 22.0               |
| PFD,\(^e\) ad libitum (12)    | 40±6\(^c\)                             | 2.85±0.73\(^c\) | 2.64±0.37\(^c\) | 263±65\(^c\)      | 14.87±2.33\(^c\)  | 18.0               |
| PFD,\(^e\) 5 g/day (3)        | 28±4\(^c,\(^γ\) | 1.90±0.09\(^c\) | 1.86±0.30\(^c,\(^γ\) | —                  | —                  | —                  |

\(^a\) Values (except protein/DNA) represent means ±SD.

\(^b\) Conception products include uterus, placentas, fetuses and amniotic fluid.

\(^c\) Cited from the data in our laboratory.

\(^d\) Figures in parentheses show the numbers of rats.

\(^e\) Protein-free diet.

\(^f\) Significantly different from values for 20\% casein group at the level of 0.1%.

\(^g\) Significantly different from values for PFD ad libitum group at the level of 1%.
Table 3. Composition of the carcass of protein depleted and repleted rats.\(^a\)

| Group\(^b\) | Body wt \((g)\) | Carcass wt \((g)\) | Nitrogen | Water | Fat |
|-------------|----------------|----------------|----------|-------|-----|
|             | \(\) | \(\) | Total \((mg)\) | Per cent \((\%)\) | Total \((g)\) | Per cent \((\%)\) | Total \((g)\) | Per cent \((\%)\) |
| **P\(1\) (7)\(^d\)** & 185±5 & 166±3 & 5,355±158 & 3.23±0.10(3.69)\(^o\) & 104±5 & 63.0±2.2(71.9)\(^o\) & 20.5±4.3 & 12.4±2.7 |
| After protein-depletion for 3 weeks |
| **P\(22\) (7)\(^d\)** & 169±7 & 122±7 & 3,660±316 & 2.99±0.27(3.20)\(^o\) & 84±6 & 68.9±1.9(73.4)\(^o\) & 7.6±1.7 & 6.2±1.3 |
| **NP\(21\) (7)\(^d\)** & 153±9 & 144±10 & 4,450±230 & 3.11±0.26(3.59)\(^o\) & 89±5 & 61.6±1.8(71.3)\(^o\) & 19.7±6.8 & 13.5±3.7 |
| After protein-repletion for 10 or 14 days |
| **MP-R\(14\) (6)\(^d\)** & 194±7 & 175±7 & 5,100±305 & 2.91±0.09(3.40)\(^o\) & 108±6 & 61.9±1.4(72.4)\(^o\) & 25.4±2.0 & 14.5±1.2 |
| **NP-R\(18\) (6)\(^d\)** & 191±9 & 176±8 & 5,370±260 & 3.05±0.07(3.61)\(^o\) & 107±6 & 61.0±2.1(71.9)\(^o\) & 26.8±3.8 & 15.3±2.2 |

\(^a\) Values represent means ±SD.

\(^b\) The abbreviations used in this column are: \(P\(_1\)\), pregnant rats on day 1; \(P\(_{22}\)\), pregnant rats protein-depleted for 22 days; \(NP\(_{21}\)\), non-pregnant rats protein-depleted for 21 days; \(MP-R\(_{14}\)\), multiparous rats repleted for 14 days after feeding protein-free diet during pregnancy and delivering the fetuses by Caesarean section at term; \(NP-R\(_{18}\)\), nonpregnant rats repleted for 10 days after feeding protein-free diet for 21 days.

\(^c\) The carcass includes the whole body other than the liver, gastrocnemius muscles and conception organs.

\(^d\) Figures in parentheses show the numbers of rats.

\(^e\) Figures represent values as percentages of the total carcass weight, while those in parentheses are values as percentages of the fat-free carcass weight.
Table 4. Total nucleic acid and protein contents of the liver.\textsuperscript{a}

| Group\textsuperscript{b} | Liver wt (g) | RNA (mg) | DNA (mg) | Protein (mg) | RNA/DNA | Protein/DNA |
|-------------------------|--------------|----------|----------|--------------|---------|------------|
| P\textsubscript{1}       | 7.86±0.55    | 84.5±6.2 | 36.6±2.8 | 1,504±98     | 2.31±0.15 | 41.2±2.4   |
|                         | After protein-depletion for 3 weeks |
| P\textsubscript{23}      | 4.80±0.63\textsuperscript{a} | 76.7±7.9 | 31.2±4.7\textsuperscript{d} | 1,010±179\textsuperscript{e} | 2.49±0.16 | 32.5±2.3\textsuperscript{e} |
| NP\textsubscript{31}     | 5.49±0.63\textsuperscript{a} | 53.5±6.3\textsuperscript{e} | 32.0±3.2\textsuperscript{d} | 1,180±176\textsuperscript{e} | 1.67±0.14\textsuperscript{e} | 37.0±5.2 |
| After protein-repletion for 10 or 14 days |
| MP-R\textsubscript{14}   | 8.37±1.15    | 94.8±17.3| 38.8±4.2 | 1,986±273\textsuperscript{e} | 2.43±0.28 | 51.1±4.6\textsuperscript{e} |
| NP-R\textsubscript{10}   | 7.31±0.83    | 78.7±7.4 | 38.3±4.2 | 1,550±273    | 2.06±0.15\textsuperscript{d} | 40.5±5.6 |

\textsuperscript{a} Values represent means ± SD.
\textsuperscript{b} The abbreviations used are shown in Table 3.
\textsuperscript{c, d, e} Significantly different from values in group P\textsubscript{1} at levels of 0.1\%, 5\% and 1\%, respectively.
Table 5. Total nucleic acid and protein contents of the gastrocnemius muscle.a

| Group | Muscle wt (g) | RNA (mg) | DNA (mg) | Protein (mg) | RNA/DNA | Protein/DNA |
|-------|--------------|----------|----------|--------------|---------|-------------|
| P     | 1.03±0.06    | 1.69±0.14| 2.18±0.27| 243±20       | 0.78±0.08| 113±18      |
| P22   | 0.82±0.04c   | 0.80±0.16b| 1.73±0.31d| 161±20c      | 0.46±0.07c| 94±13b      |
| NP21  | 0.88±0.05c   | 0.98±0.20b| 1.77±0.41d| 187±26b      | 0.56±0.10e| 111±30b     |

After protein-depletion for 3 weeks

|          |              |          |          |              |         |             |
|          |              |          |          |              |         |             |
| MP–R14  | 1.13±0.08d   | 2.10±0.51| 2.22±0.18| 249±18       | 0.98±0.33| 113±9b      |
| NP–R10  | 1.20±0.13e   | 1.69±0.15| 2.32±0.25| 261±32       | 0.72±0.02| 113±14b     |

a Values represent means ± SD.
b The abbreviations used are shown in Table 3.
c, d, e Significantly different from values in group P1 at levels of 0.1%, 5% and 1%, respectively.

Table 6. Effect of severe protein-calorie malnutrition during pregnancy on the size and number of liver and muscle cells.a, b

|          | Weight (g) | Total DNA (mg) | Protein/DNA |
|----------|------------|----------------|-------------|
| Liver    |            |                |             |
| Pregnant (3)c | 3.25±0.57  | 27.87±2.67     | 25.4±6.5    |
| Nonpregnant (6) | 3.14±0.59 | 27.60±3.91     | 25.4±3.5    |
| Muscle   |            |                |             |
| Pregnant | 0.76±0.08  | 1.39±0.16      | 105±14      |
| Nonpregnant | 0.69±0.03 | 1.42±0.13      | 111±18      |

a Dietary intake of pregnant and nonpregnant rats in this table was restricted to only 5 g per day.
b Values represent means ± SD.
c Figures in parentheses show numbers of rats.
d Gastrocnemius muscle.

number (total DNA) of liver cells. The decreases in total DNA in pregnant and nonpregnant animals which received 5 g of protein-free diet a day for 3 weeks were greater than those of rats which received the same diet ad libitum (Table 6). When the protein-deficient animals were given adequate diets, their liver weight, total RNA, total protein and the size and number of their liver cells all increased significantly. The changes in cell number induced by protein-calorie malnutrition and repletion in pregnant females were comparable with those in nonpregnant control animals.

In both groups the weight of the gastrocnemius muscle decreased significantly when the animals were given protein-free diets. Decreases in total RNA, DNA and protein and in cell size were observed in both groups. Administration of an adequate diet to these animals resulted in increases in the weight, total RNA, DNA, protein and cell size of the muscle.
The effect of malnutrition on the cells of adult animals has been studied by many workers, but results, particularly on the cell number, have not been in good agreement. CAMPBELL et al. (1) and THOMSON et al. (2) reported that the total DNA in the liver of adult animals consuming a protein-free diet for one to two weeks was comparable with that of control animals. WINICK et al. (11) investigated cellular growth in energy deficiency and found no changes in the total DNA of any organs other than the thymus and spleen. On the other hand, significant decreases in the cell number can be recognized by calculating the total DNA content of the liver from the data of MUNTWYLER et al. (4), SEIFTER et al. (5), LEATHEM et al. (6), and HAIDER et al. (7) on the effect of protein-free diet for three to five weeks on adult rats. A significant decrease in the total DNA of the liver was also observed in pregnant rats fed on 50%-restricted diet (12). We obtained similar results in pregnant animals fed on diets devoid of one essential amino acid (8). In the present study, a consistent decrease in the total DNA of the liver was observed in both pregnant and nonpregnant rats on prolonged administration of a protein-free diet.

From the results of the maternal carcass composition, it was predicted that catabolism of nitrogen was greater in pregnant animals than in the nonpregnant control. Notwithstanding reduction of tissue total DNA, due to the maternal malnutrition, in both groups was about the same regardless of whether pregnancy was maintained. Specific effect of pregnancy on cellular responses has to be studied further.

The reduction in the total hepatic DNA observed in this study was not so marked as in rats fed on diets deficient in an essential amino acid. This may be because the rats in the present experiments ingested more food than rats on diets deficient in an essential amino acid which only ate about 100 g of the deficient diets in 3 weeks. In a preliminary experiment to test this possibility, pregnant and nonpregnant animals were given only 5 g of protein-free diet a day for three weeks. The total DNA and protein/DNA ratio of the liver of both pregnant and nonpregnant rats decreased much more than in rats on unrestricted protein-free diet, as shown in Table 6 and the total DNA contents of these animals were comparable with those of pregnant rats on diets deficient in an essential amino acid reported previously (8). These results strongly suggest that protein-calorie deficiency may affect the size and number of liver cells in adult rats.

In connection with the above observation, the question of whether decrease in the total DNA in the liver is due to a change in ploidy of hepatocytes arises. If this were so, a decrease in total DNA would not indicate a decrease in cell number. However, ELY and Ross (3) reported that the ploidy of liver cells in rats receiving a protein-free diet ad libitum did not differ from that in control rats.
In muscle, the total DNA in protein-energy deficient animals decreased significantly, as indicated in Tables 5 and 6. This finding is in accord with that of Leathem and Koishi (6).

These results and considerations indicate that dietary deficiencies probably result in decrease in the cell size and cell number of most organs in adult rats and that these cellular responses due to dietary deficiency are reversed within a relatively short period on administration of adequate food as seen in Tables 3, 4 and 5. On the basis of mitotic activity, Leblond and Walker (13) divided the cell populations of the adult organ into three groups, that is, static, expanding and renewing cell populations. According to their classification, striated muscles as well as nerve cells are included in the static cell population where cells, if lost, are not replaced. Leblond et al. also include the hepatocytes in the expanding cell population, in which slow tissue expansion is observed throughout life, because only the minimal mitotic activity (not exceeding 0.1% per day) has occurred. As stated above, however, rapid increases in numbers of muscle and liver cells were observed during the recovery period in our study, differing from Leblond's view. To confirm the finding obtained in the present work, we are investigating the rate of DNA synthesis of liver and muscles in protein-depleted and protein-repleted rats by using ³H-thymidine.

REFERENCES

1) Campbell, R. M. and Kosterlitz, H. W., J. Endocrinol., 6, 308 (1950).
2) Thomson, R. Y., Heagy, F. C., Hutchinson, W. C., and Davidson, J. N., Biochem. J., 53, 460 (1953).
3) Ely, J. O. and Ross, M. H., Science, 114, 70 (1951).
4) Muntwyler, E., Seifter, S., and Harkness, D. M., J. Biol. Chem., 184, 181 (1950).
5) Seifter, S., Muntwyler, E., and Harkness, D. M., Proc. Soc. Exp. Biol. Med., 75, 46 (1950).
6) Leathem, J. H. and Koishi, H., Amer. J. Anat., 135, 572 (1972).
7) Haider, M. and Tarver, H., J. Nutr., 99, 433 (1969).
8) Niiyama, Y., Kishi, K., Endo, S., and Inoue, G., J. Nutr., 103, 207 (1973).
9) Wannemacher, R. W., Jr., Banks, W. L., Jr., and Wunner, W. H., Anal. Biochem., 11, 320 (1965).
10) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem., 193, 265 (1951).
11) Winick, M. and Noble, A., J. Nutr., 89, 300 (1966).
12) Srivastava, U., Vu, M-L., Bhargava, S., and Goswami, T., Can. J. Physiol. Pharmacol., 50, 832 (1972).
13) Leblond, C. P. and Walker, B. E., Physiol. Rev., 36, 255 (1956).