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NUTRITIONAL STUDIES IN PREGNANCY

Part II

EFFECT OF VARIOUS LEVELS OF DIETARY PROTEIN DURING GESTATION PERIOD ON THE NUTRITION OF RATS AND THEIR OFFSPRINGS

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ABSTRACT

Rats of Long Evans strain were fed on 22%, 16%, 12%, 6% casein and protein free diet respectively throughout the gestation period. All mother rats and weaning pups were uniformly fed on 22% casein diet during lactation and postweaning period. The effect of the different dietary protein levels during gestation on the nutrition of the mother and offspring has been investigated. The dietary protein would be mobilized to become the protein of pregnant products rather than mother tissue protein when the mother rats were subjected to protein deficiency stress such as the 6% dietary protein feeding. From the point of view of birth and weaning body weight of the offspring, body weight change of the mother and nitrogen balance pictures during gestation period, 12% of dietary protein level during pregnancy could achieve adequate reproduction performance and 22% level would cause too heavy a burden for breakdown of the extra dietary protein and urinary excretion of a large amount of waste nitrogenous products. Thus we suggest that 12% of the dietary protein could get a good result for rat reproduction and that the percentage should not exceed 16%.

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Concerning the utilization of dietary protein in pregnancy, the authors (1) found that not only was there an increase in the food intake after conception but there was concomittantly a greater protein retention. They also found that in the initial stage of gestation a smaller part of the retained protein would become a constituent of reproduction products while the greater part would become mother's tissue protein. Though the food intake markedly decreased in the last 3 days of gestation, the development of pregnant products would seem to be greater in this period.

So far as protein requirements for reproduction of rats are concerned, Russell (2) reported levels of 17 to 20% were adequate for diet including protein of good quality. Nelson and Evans (3) reported that 5% of protein as unsupplemented casein was the minimal level which would allow reproduction to occur, while optimal performance occurred at 15 to 20%. Gander and Schultze (4) found 15 to 16% levels could achieve good results for reproduction, and Goettsch (5) determined 16.7% to be adequate for diet.

In order to understand the role played by the dietary protein of the mother in the development of reproduction product during the gestation period, the pregnant rats were fed on protein free diet right after conception, and the nutritional state of pregnancy under this extreme dietary condition was observed. For comparative purpose the same study was also carried out with different levels of protein in the diet. The present paper reports the results of such a series of experiments.

MATERIALS AND METHODS

One hundred and twenty female and 30 male rats of Long Evans strain were selected after weaning and were fed on standard casein diet for 6 months until they reached maximum body weight. Then 45 female and 15 male adult rats were reselected as test animals by the resemblance of their growth curves. The body weight of the adult female and male rats were 252 ± 10 gm and 384 ± 13 gm respectively. As soon as the positive vaginal test had been observed after mating, the pregnant rats were transferred to individual metabolic cages. Five groups of such rats, each containing 6 individuals were fed respectively on 22, 16, 12, 6% casein diet and protein free diet for the whole gestation period. Body weight and nitrogen balance were measured according to Mitchell's method (6) in 4-day intervals for the first 20 days of the gestation period, and nitrogen balance of the last 2 days were also measured.

The changes in the mother body weight at the time of conception, after delivery and weaning were observed besides the birth and weaning weights of the offsprings. And thus the food efficiency or the rate of yield during pregnancy and lactation performance were calculated on the basis of the food consumption throughout these periods. For lactation performance study, each group of 6 dams were fed on 22% casein diet for 21 days soon after delivery and the number of suckling litter mates was fixed at 8.

The weaning pups born to the mothers which fed on various levels of protein diet were uniformly fed on 22% casein diet for 8 weeks. The weight gain of both male and female offsprings were recorded weekly, and the food efficiency as the ratio of body weight gain to total amount of food consumption during 8 weeks was measured.

The composition of the diet was as follows:

TABLE I

The composition of the diet

Protein level	22%	16%	12%	6%	0%
1, Casein (purity 93.6%)	23.5%	17.1%	12.8%	6.4%	—
2, Corn starch	64.5%	70.9%	75.2%	81.6%	88.0%
3, Vegetable oil	5.0%	5.0%	5.0%	5.0%	5.0%
4, Hegested's salt	4.0%	4.0%	4.0%	4.0%	4.0%
5, Vitamin premix	1.0%	1.0%	1.0%	1.0%	1.0%
6, Fiber	2.0%	2.0%	2.0%	2.0%	2.0%

RESULTS AND DISCUSSION

I. *Food utility during gestation period*

When the rats were fed on an extreme dietary condition of protein free ration the birth of offsprings with ordinary litter size was still observed. However, the birth weight of each pup was evidently smaller than that of the offsprings of the rats fed on protein containing diets. Most of the offspring died soon after birth. Only 3 in a litter out of 60 pups in 6 litters survived to the adult.

TABLE II shows that when the pregnant rats were fed on 22, 16, 12 and 6% casein diet respectively, the birth weight of the young revealed practically no difference among the groups receiving 22, 16 and 12% casein diet, but significantly ($0.02<P<0.05$) smaller birth weight was observed in the group receiving 6% casein diet. Concerning the food efficiency, the ratio of litter birth weight or mother body weight gain to the total amount of food consumption throughout the gestation period, there were no statistically significant differences among the groups fed on 22, 16 and 12% casein diet, except that the group fed on 6% casein diet showed a smaller efficiency rate than the other 3 groups ($P=0.07$).

The results of these experiments suggested that the dietary protein would be mobilized to become the protein of the pregnant product rather than the mother tissue protein when the mothers were subjected to protein deficiency stress such as the 6% protein feeding.

II. *Food utility during lactation period*

TABLE II summarizes the lactation performance of the pregnant rats and their pups during the preweaning period, such as the mean weight gain of male and female pups and body weight loss during the lactation period fed on various levels of the dietary protein during pregnancy. The result showed that there was no significant difference (male, $0.3<P<0.4$; female, $0.1<P<0.2$) in the lactation performance among groups fed on 22, 16 and 12% casein diet, while the group fed on 6% casein diet showed an inferior performance. This fact suggests that the lactation potentiality was the same for mothers fed on 22, 16 and 12% casein diet, but it was smaller for the mother fed on 6% casein diet during gestation period.

TABLE II

Effect of various levels of dietary protein during gestation on performance of reproduction and lactation of the 5 groups of rats

Protein level		22%	16%	12%	6%	0%
Litter size		10.2	10.5	10.4	10.2	10.0
Lit birth W in gm		57	54	56	49	33
Mean birth W in gm		5.5	5.5	5.4	4.8	3.4
$\frac{\text{Lit birth W}}{\text{Food consumption during gestation}} \times 100(1)$		18	18	19	15	
$\frac{\text{Mother body W gain}}{\text{Food consumption during gestation}} \times 100(2)$		+12	+11	+10	+3	
Prot efficiency ratio during gestation		13.5	18.7	23.7	29.1	
Mean wean W in gm	♂	49.2±3.5	49.1±3.6	48.5±3.9	40.3±8.5	
	♀	47.8±1.2	47.6±1.5	47.1±1.6	39.9±9.8	
$\frac{\text{Wean W—birth W}}{\text{Food consumption during laction}} \times 100(3)$		53	53	53	55	
$\frac{\text{Motoer body W loss}}{\text{Food consumption during lactation}} \times 100(4)$		−6	−6	−6	−6	
Prot efficiency ratio during lactation		21.2	29.3	39.5	81.6	
Mean body W gain in gm after 8 weeks feeding	♂	346±7	338±19	328±26	282±47	
	♀	232±11	231±13	231±13	228±17	
Food efficiency	♂	31	32	33	32	
	♀	25	26	26	25	

All figures denote means in each group of dams.
The suckling pups in each litter were fixed at 8.
One litter pups born to the mother fed on 6% casein diet, and 5 litter pups whose mothers were fed on protein free diet died after birth.
W : Weight
Lit : Litter
Prot : Protein
Wean : Weaning

$$\text{Protein efficiency ratio during gestation} = \frac{(1)+(2)}{\text{Protein consumption during gestation}}$$

$$\text{Protein efficiency ratio during lactation} = \frac{(3)+(4)}{\text{Protein consumption during lactation}}$$

III. *Weight gain and food utility of weaning pups after 8 weeks of standard feeding*

The effect of the different dietary condition of the mother during gestation on the nutritional fate of the offspring might be observed as the body weight gain of the pups and food efficiency during postweaning period. As TABLE II shows that the mean body weight gain of the offsprings born to the mother re-

ceiving 6% casein diet during the gestation period was consistently below those groups whose mothers received 22, 16 and 12% casein diet during gestation. However, there was no statistically significant difference of weight gain of pups among the groups receiving 22, 16 and 12% casein diet (male, $0.1 < P < 0.2$; female, $P > 0.9$).

For a comparison of the food utility of the pups, the food efficiency as the ratio of body weight gain to the total amount of food consumption during 8 weeks was measured, and the differences in the food efficiency among the 4 groups showed statistically non-significant ($P = 0.3$).

IV. *Nitrogen balance of rats fed on protein free diet during gestation period*

In order to study the dynamic state of mother body protein in the development of reproduction product during the gestation period, the mother rats were fed on protein free diet right after conception and the nitrogen excretion throughout the gestation period was observed. Unmated rats served as the controls. The gestation period was divided into the first and second 8-day periods and the last 6-day period. The average daily food intake and nitrogen balance are presented in TABLE III.

The average daily food intake of the pregnant and cotrol unmated rats was almost the same for the first 8 days, but a significant decrease was observed from the second 8-day. The decrease was very obvious in the last 6-day period of the gestation.

The average daily fecal nitrogen excretion was almost the same in the first 8-day period for the pregnant and unmated control rats, but in the second 8-day and last 6-day periods the fecal nitrogen excretion of pregnant rats showed a decrease accompanied by a decrease in the food intake.

The average daily urinary nitrogen excretion of the pregnant rats was smaller than that of the unmated rats in the first 8-day period inspite of the same food intake for both lots of rats. This result suggested that the increased utilization of the ingredients of the decomposed mother protein occurred for the synthesis of pregnant products. And in the second 8-day period the urinary nitrogen excretion of the pregnant rats was the same as the control unmated rats although the former showed decreased

TABLE III

Nitrogen balance of rats fed on protein free diet

Gestation period	Pregnant Rats					Unmated Rats				
	FI Mean± SD(cal)	FI/BM	FN Mean± SD(mg)	UN Mean± SD(mg)	NB Mean± SD(mg)	FI Mean± SD(cal)	FI/BM	FN Mean± SD(mg)	UN Mean± SD(mg)	NB Mean± SD(mg)
I	296±15	1.36	127±10	397±24	−524±30	298±16	1.39	137±12	442±26	−579±34
II	202±11	1.17	81±7	310±18	−391±22	248±13	1.24	111±9	284±16	−395±19
III	78±7	0.66	34±4	192±14	−226±14	136±7	1.13	58±5	125±11	−183±12
Total	576±23		242±15	899±41	−1141±54	682±26		306±20	851±42	−1157±53

FI : Food intake
BM : Basal metabolism
FN : Fecal nitrogen
UN : Urinary nitrogen
NB : Nitrogen balance
6 rats in each group
I =First 8 days
II =Second 8 days
III =Last 6 days

food intake in this period. In the last 6-day period the urinary nitrogen excretion of the pregnant rats was significantly greater than that of the unmated rats. In this period the food intake of the pregnant rats had decreased to the level of about two-thirds of basal metabolism or about half of the energy requirement for body weight maintenance, therefore body protein decomposition would unavoidably occur in the mother not only for the development of reproduction products but also for the caloric need, (Basal metabolism, K, cal,=70 $W^{3/4}$, where W expresses body weight in kg) (7). On the other hand, the decrease in food intake of the unmated rats in this period was smaller than that of the pregnant rats, and these data correspond to 1.15 times basal metabolism, or approximately corresponding to the energy requirement for body weight maintenance, therefore, the urinary

TABLE IV

Nitrogen balance of rats fed on various levels of protein diet during gestation period.

Protein level \ Gestation period		The first 4–day	The second 4–day	The third 4–day	The fourth 4–day	The fifth 4–day	Total
22%	FI cal	172	198	208	217	236	1031
	NI mg	1547	1780	1870	1934	2107	9239
	FI/BM	1.6	1.8	1.8	1.9	1.8	
	FN mg	126	155	150	165	195	791
	UN mg	1273	1035	1534	1522	1165	6529
	NB mg	148	189	160	247	427	1171
16%	FI cal	168	178	204	226	246	1022
	NI mg	1162	1228	1408	1561	1689	7048
	FI/BM	1.7	1.6	1.8	1.9	1.9	
	FN mg	140	125	138	155	170	734
	UN mg	850	920	1090	1178	1050	5088
	NB mg	172	183	180	228	463	1226
12%	FI cal	178	183	214	223	250	1048
	NI mg	912	935	1089	1140	1277	5353
	FI/BM	1.6	1.5	1.9	1.9	2.1	
	FN mg	113	120	132	135	152	652
	UN mg	635	707	764	768	695	3569
	NB mg	164	107	193	236	431	1131
6%	FI cal	113	201	196	219	228	1017
	NI mg	455	526	515	575	598	2669
	FI/BM	1.6	1.8	1.7	1.9	1.9	
	FN mg	78	86	88	90	102	444
	UN mg	384	408	325	346	335	1798
	NB mg	−7	32	102	139	161	427

FI : Food intake
BM : Basal metabolism
UN : Urinary nitrogen
NI : Nitrogen intake
FN : Fecal nitrogen
NB : Nitrogen balance

nitrogen excretion would be minimized toward the endogenous origin. However, the decrease in the food intake became greater the longer the duration of the test feeding for both groups of the pregnant and the unmated rats. This fact might be due to the unavoidable general physical emaciation caused by the extreme dietary condition such as protein free diet feeding and especially in the case of the pregnant rats which bore a heavy burden in the development of reproduction products.

Though the urinary nitrogen excretion of pregnant rats during the last 6 day period of gestation was greater than that of unmated rats, the total amount of nitrogen excretion throughout the whole gestation period for the two groups was almost the same.

TABLE V

Dietary protein utility of rats during gestation period

Dietary protein level	22%	16%	12%	6%
Apparent biological value	138	195	241	192
Biological value	266	392	460	643
Protein efficiency ratio	135	187	237	291

Apparent biological value : $\frac{NB}{NI - FN}$

Biological value : $\frac{NB+MN+EN}{NI-(FN-MN)}$

Protein efficiency ratio : $\frac{\text{Litter birth weight+mother body weight gain}}{\text{Protein intake during gestation period}}$

NI : Nitrogen intake UB : Nitrogen balance
FN : Fecal nitrogen UN : Urinary nitrogen
MN : Metabolic fecal nitrogen EN : Endogenous urinary nitrogen
Mitehell's method (6) was used for the calculation

V. *Nitrogen balance of rats fed on various levels of protein diet during gestation period*

TABLE IV shows that the average daily food intake of the pregnant rats fed *ad libitum* on 22, 16, 12 and 6% casein diet was almost the same throughout the whole gestation period.

The average daily fecal urinary nitrogen excretion of each 4-day period was as great as the increase of nitrogen intake, and the total amount of urinary nitrogen excretion throughout the whole gestation period increased with the increase in the total amount of protein intake.

The nitrogen balance of each 4-day period showed almost the same when the pregnant rats were fed on 22, 16 and 12% casein diet but when they were fed on 6% casein diet the nitrogen balance was significantly smaller than the above 3 groups. This result coincides with the fact that the pregnant rats fed on 22, 16 and 12% casein diet showed the same litter birth weight, but when fed on 6% casein diet a smaller litter birth weight was observed.

The above data and TABLE V suggested that adequate performance for reproduction occurred at 12% protein feeding during the gestation period accompanied by less urinary

nitrogen excretion which showed the best utility of the dietary protein during the gestation period among the 3 groups. On the basis of the birth and weaning body weight of the offesprings, the body weight change of the mother and the nitrogen balance pictures during the gestation period, 12% protein diet seemed to be adequate for pregnant rats. And from the standpoint of protein nutrition, 22% protein diet is inadequate during the gestation period owing to the heavy burden of the mother for breakdown of the extra dietary protein and urinary excretion of a large amount of waste nitrogenous products. Therefore, we suggest that during pregnancy of rat, 12% of the dietary protein level could produce favorable result for reproduction and the dietary protein should not exceed 16%.

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LITERATURE CITED

1. WANG, Y. T., C. T. HSU, C. P. SHIEH, L. Y. YANG, H. S. CHIANG, C. T. HHEU and T. H. YANG. 1965. Nutritional studies in pregnancy. Part. 1. The influence of pregnancy upon the dietary protein utilization of rats. *Formosan Sc.* **19:** 67-76.
2. RUSSELL, F. C. 1948. Diet in releation to reproduction and viability of the young. Part 1. Rats and other laboratory animals. *Commonwealth Bull. Australia Tech. Comm. No. 16.*
3. NELSON, M. M. and H. M. EVANS. 1953. Relation of dietary protein levels to reproduction in the rats. *J. Nutr.* **51:** 71-84
4. GANDER, J. E. and M. O. SCHULTZE. 1955. Concerning the alleged occurence of an "Animal protein factor" required for the survival of of young rats. *J. Nutr.* **55:** 543-557.
5. GOETTSCH, M. 1962. Minimal protein requirement for growth in the rat. *Natl. Acad. Sc., Natl. Res. Council Public. No* **990** pp 51-80.
6. MITCHELL, H. H., T. S. HAMILTON and J. R. BEADLES. 1945. A method of determining the biological value of protein. *J. Nutr.* **29:** 13-25.
7. MAYNARD, L. A., and J. K. LOOSLI. 1956. *Animal Nutrition* 4th Ed., McGraw-Hill, N.Y. p 315.