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Nitrogen Balances on Rats During Pregnancy and Lactation.

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The present investigation is a continuation of previous studies carried out in this laboratory on the balances of elements in rats during the period of pregnancy and lactation.[1] It has not yet been possible to maintain rats on synthetic diets for successive generations with production of normal litters.[2] This probably indicates that these diets are not adequate in all respects. It was hoped that, by comparing the nitrogen balances obtained when the animals were fed various synthetic diets with similar data when the rats were maintained on a stock ration that has proved adequate for the colony, some information as to the required dietary factors could be obtained.

Adult female rats weighing over 200 g and having a record of one or 2 litters when maintained on the stock diet were employed in the present experiments. The animals were placed on the experimental diets for a period of 3 weeks or until they had attained weight equilibrium before the balance experiments were initiated. Throughout the experiments food and water were supplied *ad lib.* The amount of food consumed was determined by weighing. The excreta were collected in 0.5 N H_2SO_4 to prevent loss of nitrogen. Balance experiments were carried out during a fore-period of 3-8 days before mating. These were resumed subsequent to mating. The litters were reduced to 6 in all cases. They were weaned at about 21 days. The amount of nitrogen loss to the mothers as a result of littering was determined by estimating the nitrogen content of one or more of the young shortly after birth. The amount of nitrogen supplied by the mother to the litter during lactation was estimated by determining the nitrogen content of one or more of the young at the time of weaning and subtracting from this value the nitrogen content of the young at birth. It is not possible to separate the excreta of the litter from that of the mother during the lactation period. Since the amount of nitrogen gained by the litter was taken to be L_{w-b}, no error results by including the excreta of the litter with that of the mother. The nitrogen balances were followed on the mothers for 7-14 days after removal of the young.

The following diets were fed: Group I, washed casein, 27 (increased to 30 for rats in the 300 series); cane sugar, 52; salt mixture,* 4; crisco, 7; corn oil, 7%. To this was added (mg per kilo) thiamin, riboflavin, and pyridoxin, 6 each; nicotinic acid, 30; pantothenic acid, 60; inositol, 200; 2-methyl-1,4-naphthoquinone, 0.05; choline chloride, 500; *p*-aminobenzoic acid, 5. In addition the diet contained 12 cc wheat germ oil, 5,000 μ vitamin D, and 65,000 μ vitamin A per kilo. The Group II rats received the same diet as the animals in Group I except that in addition each animal was given 50 mg *dl*-methionine daily. The Group III rats were maintained on the Group I diet to which 3% liver extract (Lilly)† had been added. The Group IV animals were maintained on the stock diet the composition of which was as follows: skimmed milk powder, 3.5; corn, 6; wheat germ, 2.25; whole wheat, 8; fish meal, 4.25; alfalfa meal, 1.25; lard, 1.5; NaCl and $CaCO_3$, each 0.125 lb; and fish liver oils, 50 cc. The average per cents of nitrogen in the diets were: diet I (27% casein) 3.3 and (30%

[1] Goss, H., and Schmidt, C. L. A., *J. Biol. Chem.*, 1930, **86**, 417; Heppel, L. A., and Schmidt, C. L. A., *Univ. of Calif. Pub. Physiol.*, 1938, **8**, 189.

[2] Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED. 1940, **45**, 625. In this connection see also Vinson, L. J., and Cerecedo, L. R., *Arch. Biochem.*, 1944, **3**, 389.

* The salt mixture contained: ferric citrate, 31.9; calcium lactate, 352; $Ca(H_2PO_4)_2 \cdot H_2O$, 146; K_2HPO_4, 258; $NaH_2PO_4 \cdot H_2O$, 93.8; NaCl, 46.7; $MgSO_4 \cdot 7H_2O$, 156; $MnSO_4 \cdot 4H_2O$, 1.3; NaI, 2.0; $Ca_3(PO_4)_2$, 132; $CuCO_3$, 2.0 g.

† Kindly supplied by Eli Lilly and Company.

TABLE I.

Foreperiod			Period of pregnancy						Period of lactation						Afterperiod			
Rat No.	Days	Bal.	Days	I	L_b	O	O + L_b	Bal.	Days	I	L_{w-b}	O	O + L_{w-b}	Bal.	Days	Bal.	Days	Bal.
Group I (Synthetic diet)			Totals for period						Totals for period									
11	3	+0.06	21	11.42	0.63	10.51	11.14	+0.28	Failed to lactate									
3	3	—0.03	21	11.71	0.70	11.58	12.28	—0.57	,,	,,	,,							
6	3	+0.06	21	7.20	0.38	8.42	8.80	—1.60	,,	,,	,,							
103	3	+0.02	21	8.62	0.22	9.68	9.90	—1.28	21	9.77	3.57	9.04	12.61	—2.84				
104	3	+0.10	21	8.73	0.61	9.14	9.75	—1.02	21	10.99	2.31	11.29	13.60	—2.61				
305	8	—0.08	22	12.19	0.64	11.97	12.61	—0.42	20	16.27	4.34	12.95	17.29	—1.02	9	—0.41		
311	7	—0.04	22	12.36	0.68	12.51	13.19	—0.83	21	18.33	4.39	12.30	16.69	+1.64	7	—0.07		
Group II (Synthetic diet + methionine)			Totals for period						Totals for period									
319	7	—0.02	22	12.63	1.32	11.59	12.91	—0.28	21	16.23	2.97	15.90	18.87	—2.64	8	+0.78	7	+0.33
320	7	—0.01	22	9.47	1.04	9.42	10.46	—0.99	21	15.04	2.36	13.73	16.09	—1.05	7	+0.19	7	0.11
321	7	+0.10	22	10.15	0.77	9.46	10.23	—0.08	21	15.73	3.60	13.26	16.86	—1.13	8	+0.55	7	—0.20
325	7	+0.02	22	11.29	0.70	10.71	11.41	—0.12	Failed to lactate						7	+0.18		
Group III (Synthetic diet + liver extr.)			Totals for period						Totals for period									
18	3	+0.12	21	11.73	0.85	9.77	10.62	+1.11	22	21.48	5.34	13.00	18.34	+3.14				
10	3	+0.18	21	13.93	0.81	12.18	12.99	+0.94	23	22.30	6.02	16.62	22.64	—0.34				
102	3	+0.12	21	12.20	0.65	9.78	10.43	+1.77	22	17.48	5.72	13.38	19.10	—1.62				
312	7	—0.17	22	11.31	0.84	8.84	9.68	+1.63	21	17.23	1.72	19.51	21.23	—4.00	7	—0.07		
323	7	0	22	12.04	1.30	9.18	10.48	+1.56	20	15.81	4.38	11.28	15.66	+0.15	9	+2.76	8	—0.15
324	7	+0.10	23	11.44	1.61	8.86	10.47	+0.97	20	18.95	5.00	13.08	18.08	+0.87	8	+0.52	7	+0.09
Group IV (Stock diet)			Totals for period						Totals for period									
105	3	+0.07	21	11.76	0.62	10.44	11.06	+0.70	21	21.06	5.25	16.83	22.08	—1.02				
106	3	+0.06	21	14.36	0.84	12.42	13.26	+1.10	22	29.04	6.62	18.69	25.31	+3.73				
107	3	—0.01	21	11.69	0.81	9.18	9.99	+1.70	23	21.82	5.75	17.94	23.69	—1.87				
310	7	—0.07	22	12.32	0.67	11.52	12.19	+0.13	21	19.41	4.21	11.80	16.01	+3.40	7	—0.84		
326	7	—0.09	21	10.63	0.73	8.08	8.81	+1.82	21	19.75	4.37	14.23	18.60	+1.15	6	+0.05	7	—0.12
327	7	—0.16	22	11.43	0.78	8.57	9.35	+2.08	21	14.77	3.25	12.64	15.89	—1.12	7	+0.25	7	+0.14

I = input of nitrogen; O = output of nitrogen; L_b = nitrogen content of litter at birth; L_{w-b} = nitrogen content of litter at weaning minus that at birth; O + L = total nitrogen output; all nitrogen values are in g.

casein) 3.9; diet III, 3.8; diet IV, 3.3.

The data are summarized in Table I. Although the input and output of nitrogen were determined during weekly periods the values are not included in the table since they are quite variable and show no particular trends. The data are, however, on file.

Analysis of the data shows the following: (a) All of the Group III and Group IV rats had gained nitrogen at the end of the period of pregnancy while with one exception those of Groups I and II had lost nitrogen. During this period all animals gained weight as would be expected. (b) Most but not all of the animals in Groups III and IV gained nitrogen at the end of the lactation period while with one exception the Groups I and II rats lost nitrogen. Three animals (103, 305, and 311) in Group I, and 2 (319 and 321) in Group II lost weight during the lactation period while one in each of these 2 groups maintained its body weight. Of the Group III rats, 4 maintained their body weights, 1 (323) lost and 1 (312) gained weight. Five animals of Group IV lost weight and 1 (105) maintained its weight. There was no correlation between loss or gain in body weight and loss or retention of nitrogen during lactation in any of the groups. (c) Examination of the weekly nitrogen balances of the Group III and Group IV rats shows no uniform correlation between nitrogen retention and development of the fetus although in many cases the maximum nitrogen retention occurred in the third week of pregnancy. In Groups I and II there is no correlation between loss or retention of nitrogen and the period of pregnancy. Similar statements may be made of the lactation periods of all groups of animals. (d) Under the conditions of the experiment and of the diets studied the liver extract supplemented diet compares most favorably with the stock ration in preventing loss of nitrogen in pregnant and lactating rats. This may be indicative of the presence of one or more factors in liver extract required by rats during pregnancy and lactation; however, direct proof is lacking

Although a great many studies on nitrogen metabolism during pregnancy and lactation have been published, apparently none of them have dealt extensively with the rat.‡ For women the experiments of Macy and her coworkers[3] are representative. They found that the reproductive cycle is a period of nitrogen acquisition for the mother although at the time of delivery and in the early period of lactation the nitrogen balance may be negative. On the other hand Murlin[4] found in dogs that nitrogen loss occurs during the first half of pregnancy and particularly in the third and fourth week while nitrogen is retained in the last half of pregnancy. The adequacy of the diet used by Murlin may be questioned. Campbell[5] in this laboratory showed that rats, maintained on a diet containing 2.4% nitrogen but less adequate in the vitamins than in the present experiments, stored amounts of nitrogen that met the needs of the fetus. When maintained on a similar diet but containing only 1.2% nitrogen the amount of nitrogen retained was not adequate for the requirements of the fetus. During the lactation period the animals on the first mentioned diet were in negative nitrogen balance but were able to rear their young while those maintained on the diet of lower nitrogen content were unable to do so unless at the onset of the lactation period there was an unusually large reserve of nitrogen upon which to draw.

Summary. Nitrogen balances have been carried out on rats maintained on various diets during the period of pregnancy and lactation. The liver extract supplemented diet compared most favorably with the stock ration in preventing loss of nitrogen.

‡ In this connection see Poo, L. J., Lew, W., and Addis, T., *J. Biol. Chem.*, 1939, **128**, 69.

[3] Hunscher, H. A., Donelson, E., Nims, B., Kenyon, F., and Macy, I. G., *J. Biol. Chem.*, 1932-33, **99**, 507; Hunscher, H. A., Hummell, F. C., Erickson, B. N., and Macy, I. G., *J. Nutr.*, 1935, **10**, 579.

[4] Murlin, J. R., *Am. J. Physiol.*, 1910, **27**, 177.

[5] Campbell, W. W., *The Nitrogen Balance in the Rat During Pregnancy and Lactation*, Thesis, Univ. of Calif., 1938.