Sulfur Amino Acid Requirement for Lactation in the Rat.*† (24478)

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The classic studies of Rose(2) on amino acid requirements for post-weaning growth and nitrogen balance in rats have not been extended to the study of amino acid requirements for either pregnancy or for lactation. Numerous reports have been made, however, on the beneficial effects of cystine or methionine supplementation for lactation in rats maintained on diets containing protein sources known to be low or lacking in sulfur amino acids, *e.g.* casein(3,4), alfalfa proteins(5,6), peanut meal(7), and yeast protein(8). In fact, cystine and methionine have been proposed as specific dietary lactogogues(3). In the present study purified diets varying in casein level and in cystine supplementation have been used to determine an approximate value of total sulfur amino acid requirement for optimal lactation in the Long-Evans rat.

Methods. The short-term lactation test of 3 weeks is the same as used in previous studies(1,9,10,11). Normal female rats of Long-Evans strain, together with their litters, were placed on experimental diets at parturition. All groups were balanced in regard to average age and body weight of mother at parturition. The litters were limited to 6 young, preferably 3 males and 3 females. Mother and young were weighed every 5 days and the young were weaned on 21st day following parturition. Dietary casein levels of 12, 18, 24, 30, 36 and 48% were used, with sucrose and casein being interchanged isocalorically in the basal purified diet.‡ L-cystine (0.5%) was added to the 12, 18, 24, and 30% casein diets. In addition, 0.2% L-cystine and 0.2% DL-methionine were tested separately with the 24% casein diet. Ten to 12 litters were used for each experimental group and all groups receiving the standard level of 24% casein were repeated. Control litters were maintained on stock diet of natural foodstuffs which contained approximately 24.3% protein (N x 6.25) or the protein equivalent to that furnished by 28% casein purified diet. All diets were given *ad lib.* and food intake was measured every 5 days. Criteria used for lactation performance in this short-term lactation test were those used previously by Nelson & Evans(9): (1) average weaning weight of young, and (2) average weight change of lactating mother during the 21-day period.

Results. Table I demonstrates improvement in lactation performance resulting from cystine supplementation of casein-containing purified diets. Addition of 0.5% L-cystine to 12, 18 and 24% casein diets resulted in significantly heavier weaning weights of young and significant improvement in maternal weight change. Cystine supplementation of the 30% casein diet improved weaning weights of young significantly with less beneficial effects on maternal weight. As judged by

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‡ The basal purified diet contained 24% alcohol-extracted casein, 64% sucrose, 8% hydrogenated vegetable oil and 4% salts No. 4(12). Crystalline vitamins/kilo diet were: 300 μg *d*-biotin, 5.5 mg pteroylglutamic acid, 5 mg 2-methyl-1,4 naphthoquinone, 5 mg thiamine HCl, 5 mg pyridoxine HCl, 10 mg riboflavin, 10 mg p-aminobenzoic acid, 20 mg niacin or niacinamide, 50 mg *d*-calcium pantothenate, 400 mg inositol and 1 g choline chloride. One cc of fat-soluble vitamin mixture containing 6 mg *dl*-alpha-tocopherol, 115 chick units vit. D, 800 U.S.P. units vit. A and 650 mg corn oil (Mazola) was given weekly to each litter. Vit. B_{12} was not included in the vitamin mixture for this study. However, succeeding studies have shown that addition of 25-50 μg crystalline vit. B_{12}/kilo of the 24% casein diet did not improve lactation performance either in presence or absence of cystine supplementation.

TABLE I. Effect of Supplementary Cystine on Lactation in the Long-Evans Rat. Three-week test—litters limited to 6 young.

Dietary casein level, %	Cystine suppl., %	No. of litters	No. of young	Avg wt young day 21,* g	Maternal wt change during lactation, g	Avg daily food intake, g	Dietary sulfur amino acids Methionine + cystine = total SAA†
			Casein-containing purified diets				
12	.0	11	66	31 ± 2	−43 ± 8	23 ± 1	.33 + .04 = .37
	.5	10	60	41 ± 2	−14 ± 3	29 ± 1	.33 + .54 = .87
18	.0	13	78	41 ± 1	−24 ± 2	27 ± 1	.50 + .06 = .56
	.5	12	72	54 ± 1	0 ± 3	34 ± 1	.50 + .56 = 1.06
24	.0	34	204	49 ± 1	− 1 ± 2	31 ± 1	.66 + .08 = .74
	.2‡	23	138	54 ± 1	+ 2 ± 3	33 ± 1	.66 + .28 = .94 .86 + .08 = .94
	.5	36	216	54 ± 1	+17 ± 2	35 ± 1	.66 + .58 = 1.24
30	.0	12	72	50 ± 1	− 2 ± 3	32 ± 1	.83 + .10 = .93
	.5	12	72	54 ± 1	+ 9 ± 4	34 ± 1	.83 + .60 = 1.43
36	.0	12	72	52 ± 1	+19 ± 4	33 ± 1	.99 + .12 = 1.11
48	.0	12	72	51 ± 1	+18 ± 5	34 ± 1	1.32 + .16 = 1.48
			Stock Diet I + lettuce *ad lib.*				
28§		102	612	49 ± 1	+14 ± 2	35 ± 1	

* This column gives avg weaning wt of young as calculated from litter avg weights, each litter corrected to the 3:3 sex ratio by means of mid-sex correction factor (CF). All columns give the mean together with stand. error of mean.

† Calculated from standard tables(13) and expressed in proportion to 16% nitrogen.

‡ 0.2% L-cystine was used for 12 litters and 0.2% DL-methionine for 11 litters. The data have been combined as there was no significant difference between the 2 groups.

§ The protein content of stock diet is equivalent to that furnished by a 28% casein-containing purified diet.

both young and mother, optimal casein level in the presence of additional cystine was between 18% and 24%, whereas in the absence of cystine supplementation optimal casein level was probably close to 36%.

The data suggest that slightly more than 1% of total dietary sulfur amino acids (SAA) was necessary for optimal lactation of Long-Evans rats, *i.e.* 1.11 to 1.24% SAA. This SAA requirement for optimal lactation is considerably higher than that needed for post-weaning growth of rats as determined by Wretlind and Rose(14) *i.e.* 0.5-0.6% SAA furnished by 0.3-0.4% methionine and 0.2% cystine. There are several possible explanations for this difference, *e.g.* (1) higher SAA requirements for lactation than for growth, (2) strain differences in SAA requirement and (3) loss or non-availability of methionine or cystine resulting from process of alcohol-extraction.§

The purified diets containing 18-30% casein and supplemented with cystine resulted in young with weaning weights superior to those produced previously by similar diets supplemented with 8 B vitamins and a liver eluate powder(10). Average weaning weights of 54 g on these cystine-supplemented diets closely approached the optimal weaning weights of the Long-Evans rat reported by Dunn *et al.*(15) *i.e.* 54 g for females and 56 g for males.

Summary. A short-term lactation test has been used to study the effects of cystine supplementation for purified diets containing casein as sole protein source. Supplementa-

§ Microbiological assays for methionine with *L. fermenti*[36] showed an apparent loss of methionine in all alcohol-extracted caseins tested. Methionine content for casein used was only 2.65% in comparison with the value of 3.3% given in standard tables(13) and with that of 3.2% for casein prepared by isoelectric precipitation and analyzed by the same microbiological assay at the same time as the alcohol-extracted caseins. These assays were carried out by Dr. S. Shankman of Shankman Laboratories, Los Angeles, Calif. Alcohol-extraction had no apparent effect on cystine content of casein preparations when cystine was assayed with *L. mesenteroides*.

tion of 12, 18, and 24% casein diets with 0.5% L-cystine markedly improved lactation performance as judged by weaning weights of young and mother. Cystine supplementation of 30% casein diet improved weaning weights of young significantly with less beneficial effects on maternal weight. The data indicate that total sulfur amino acid requirement for optimal lactation in the Long-Evans rat may be placed at slightly more than 1% of the diet and that cystine may furnish at least half of this requirement.

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Relationship between Pituitary Growth Hormone Content and Age in Rats.* (24479)

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The question of whether or not the amount of pituitary growth hormone varies with age has been asked by several laboratories using beef(1), swine(2), and fetal and infant rat pituitaries(3) in the tibia test assay. To date, there is no consistent opinion as to the relationship between growth rate and amount of growth hormone in the pituitary. To determine whether growth hormone potency of the female rat pituitary varies as the animal ages from 10 to 630 days of age, the following methods were employed:

Methods. Female rats descended from the Sprague-Dawley and Wistar strains were weighed and sacrificed with ether at 10, 42, 56, 70, 168, 308, 376, and 630 days of age. At necropsy, the pituitaries were removed, weighed, and placed in absolute acetone, dried and stored in a vacuum desiccator for use in the tibia test assay for growth hormone. The growth hormone potency of the pituitaries was determined according to the procedure of Evans *et al.*(4) using the parallel line method. For each assay, the whole dried pituitaries were weighed, triturated with saline and injected at 2 dose levels; and 3 groups of hypophysectomized rats received graded doses of a purified beef growth hormone preparation (Armour and Co.). Pituitaries from 2 age groups (42 and 70-day-old females) were assayed twice to test the accuracy of the procedure.

Results. Table I shows epiphyseal widths and statistical evaluation of growth hormone potency of the donor pituitaries. Table II is a compilation of data, using body and pituitary weights of the donor rats, and assayed growth hormone content of the pituitaries, expressed in terms of μg of growth hormone/mg pituitary, or per g rat.

Within the limits of the assay procedure,

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