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The Effect of Feeding Excess Glycine, L-Arginine, and DL-Methionine to Rats on a Casein Diet.*

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Previous reports[1-5] have demonstrated, that in rats and dogs, the addition of different levels of DL-methionine to a casein diet, affects the nitrogen balance index and may alter the physiological state of the animal. Low levels of methionine elevate the nitrogen balance index of casein, causing increased retention of nitrogen; intermediate levels do not alter the nitrogen balance index but cause weight loss and kidney hypertrophy; high levels depress the nitrogen balance index, there is a tearing down of body tissue, severe weight loss, kidney hypertrophy and an increase in creatine and creatinine excretion. It has also been shown[5] that excess L-arginine added to the high level DL-methionine diet, is effective in preventing, in large measure, kidney hypertrophy and also counteracts the lowering of the nitrogen balance index and weight loss.

The work described in this paper was designed to explore the effect of adding excess glycine alone and in combination with methionine and arginine, upon the nitrogen balance index of casein, organ and body weights, and creatine and creatinine excretion.

Methods. The methods for determining nitrogen balance indexes were the same as those described for the dog by Allison, Anderson, and Seeley[6] and for the rat by Brown.[4] These indexes (K) are functions of the rate at which the protein stores of the body are being filled by a given protein source and are calculated from the following equation: $NB = K\ (AN) - NE_O$ where NB is nitrogen balance, AN is absorbed nitrogen and NE_O is the excretion of nitrogen on a protein-free diet. Mature, male rats of Sherman strain weighing approximately 250 g were used in the experiments. The rats were divided into 6 groups, 10 in a group and fed the following nitrogen sources. Values are on a dry weight basis.

Group	Casein
I	12% (controls)
II	12% + 4.8% DL-methionine
III	12% + 4.8% glycine
IV	12% + 4.8% glycine + 4.8% DL-methionine
V	12% + 4.8% glycine + 4.8% DL-methionine + 1.7% L-arginine
VI	12% + 4.8% glycine + 1.7% L-arginine.

The diets were, in addition, adequate with respect to carbohydrates, fats, minerals and vitamins. All the rats were pair fed with those fed excess methionine (Group II) since this group restricts their food intake. The experiment was continued for 20 days; the urine and feces were discarded for the first 4 days and then collected, thereafter, in four, 4-day periods. At the end of 20 days the animals were autopsied and the livers and kidneys dried at 95°C and analyzed for nitrogen. All deviations listed are standard errors.

Results and Discussion. In Table I are recorded the nitrogen intakes, the urinary and fecal nitrogen excretions, the nitrogen balances and the nitrogen balance indexes for the 6 groups.

The values for nitrogen balance index in

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1 Miller, L. L., *J. Biol. Chem.*, 1944, **152**, 203.

2 Allison, J. B., Anderson, J. A., and Seeley, R. D., *J. Nutrition*, 1947, **33**, 361.

3 Brush, H., Willman, W., and Swanson, P., *J. Nutrition*, 1947, **33**, 389.

4 Brown, J. H., Thesis, Rutgers University Library (1948), in preparation for publication.

5 Brown, J. H., and Allison, J. B., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 196.

6 Allison, J. B., Anderson, J. A., and Seeley, R. D., *Ann. N. Y. Acad. Sci.*, 1946, **47**, 241.

TABLE I.
Nitrogen Intake and Excretion of Rats Fed Various Nitrogen Sources.
Averages of 20 values obtained on 10 rats in each group.

Group	Ingested N mg/kg/day	Urinary N mg/kg/day	Fecal N mg/kg/day	N balance g N/kg/day	N balance index
I	397.4	250.2	108.1	+.039	.87
II	537.8	422.8	82.0	+.034	.59
III	600.8	434.0	114.6	+.052	.61
IV	721.5	596.1	96.0	+.029	.55
V	836.0	623.0	95.0	+.118	.49
VI	745.0	469.6	117.2	+.159	.64

TABLE II.
Weight Changes and Creatinine and Creatine Excretions of Rats Fed Various Nitrogen Sources.
Average on 10 rats.

Group	Wt change (avg 20 days), g	Creatinine mg/kg/day	Creatine, mg/kg/day
I	7.4 ± 1.9*	25.8 ± 1.1	5.7 ± 1.0
II	-36.6 ± 6.1	28.9 ± 2.1	9.4 ± 1.6
III	$+ 1.2 \pm 3.6$	26.7 ± 1.3	7.6 ± 0.5
IV	$- 1.0 \pm 3.6$	30.5 ± 1.3	8.0 ± 0.9
V	$- 5.0 \pm 5.1$	30.2 ± 1.2	12.5 ± 1.4
VI	$- 5.1 \pm 2.1$	28.4 ± 1.1	10.3 ± 2.1

* Standard error.

Table I are calculated, relative to that of the casein fed controls (Group I). Examination of Table I shows that the addition of extra nitrogen in methionine (Group II), or glycine (Group III), or methionine plus glycine (Group IV) causes no significant change in the nitrogen balance. This may be interpreted as indicating that the nitrogen in these excess amino acids is not utilized under the conditions of this experiment. The drop in the nitrogen balance index for these groups approximately equals the index that may be calculated, assuming no utilization of the nitrogen in the excess amino acids fed. In Groups V and VI there is a significant rise in the nitrogen balance. The extra arginine fed to these 2 groups may be responsible for this rise. In these 2 groups, the nitrogen balance index calculated on the basis of no utilization of the amino acid nitrogen, is lower than that observed indicating, therefore, some usage of the excess amino acids. Previous studies indicate that excess arginine nitrogen added to casein is retained, in part, by the rat.[5] It should be pointed out that there appears to be a strain difference in the effects of excess methionine. For example, it has been found that 2.5% methionine, fed to Long-Evans strain rats, produced about the same effects in previous studies[4] as 4.8% produced in this one. Further work has shown that the addition of 7% methionine to the diet will produce negative nitrogen balance and tissue destruction in the Sherman strain rats.

The rats in all groups lost weight rapidly at first, and then more slowly during the first 6-8 days of the experiment (Table II). Thereafter, with the exception of those rats in Group II, a weight gain took place, so that at the end of 20 days the rats were nearly back to their original weights. The nitrogen balances paralleled the weight changes closely, being negative the first few days and even during the first collection period, but then positive for the last 12-14 days of the feeding. The rats receiving casein plus methionine (Group II), however, continued to lose weight throughout the 20 days, even though they were in positive nitrogen balance for the last 12 days.

In Fig. 1 is plotted the relationship between weight loss and nitrogen balance for the rats receiving casein alone (Group I). Fig. 2 illustrates the relationship between weight loss and nitrogen balance for Group II fed 12% casein plus 4.8% methionine. The loss in weight in the rats receiving excess methio-

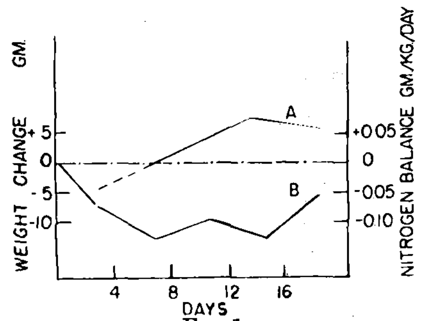

FIG. 1.
Relationship between weight loss and nitrogen balance for rats fed 12% casein.
Group I. Avg of 10 rats.
A. N balance B. Wt loss

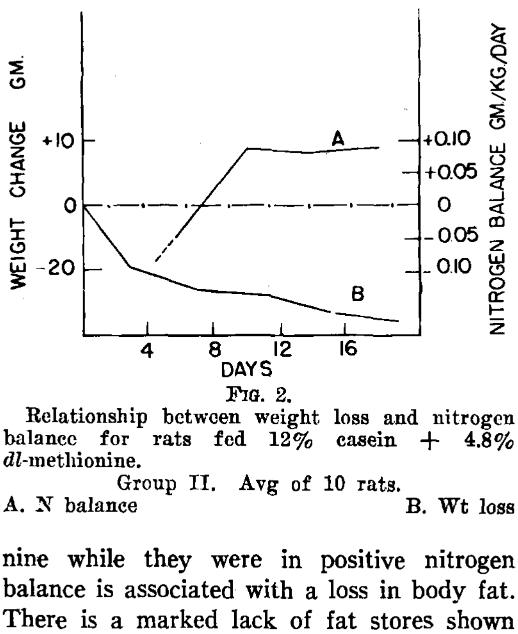

FIG. 2.
Relationship between weight loss and nitrogen balance for rats fed 12% casein + 4.8% *dl*-methionine.
Group II. Avg of 10 rats.
A. N balance B. Wt loss

nine while they were in positive nitrogen balance is associated with a loss in body fat. There is a marked lack of fat stores shown upon autopsy.[5]

The addition of glycine, or glycine plus arginine to the casein diet containing excess methionine counteracts the loss in weight caused by the excess methionine alone. Possibly glycine prevents the excessive lipotropic action of the methionine by aiding in its metabolism, or excretion. Based on previous studies,[5] larger amounts of methionine alone could cause continued loss in body nitrogen as well as fat, the animals never reaching positive nitrogen balance during the 20-day experimental period.

The excretions of creatine and creatinine are illustrated in Table II. The results are daily excretions and are averages of 20 determinations.

These data show that the addition of excess glycine to a casein diet does not significantly raise the creatinine or creatine excretion. The excretion of these materials is increased slightly by feeding excess methionine, alone, or in combination with glycine and arginine. Larger and more significant increases in the excretion of creatinine and creatine take place if more methionine is added to the diet since previous studies have demonstrated that excesses of methionine causing loss in body nitrogen will increase the excretion of creatinine.[5]

The effect of the diets on the organ weights is given in Table III.

The data in this table demonstrate that the addition of excess methionine to a casein diet causes an increase in liver and kidney weight and a slight increase in adrenal and thyroid weight; confirming previous results. The feeding of excess glycine causes an increase in liver weight but no other change. These data, together with those previously reported[5] demonstrate that both glycine and L-arginine antagonize in part the hypertrophic effect of the excess methionine.

That the increases in liver and kidney sizes were not due to increases in water content is demonstrated by the data in Table IV. The data in Table IV show that the addition of methionine, methionine plus glycine, or methionine plus arginine plus glycine, to a casein diet increases the nitrogen content of the kidneys. Glycine alone or in combination with arginine has no significant effect. Thus the hypertrophy of the kidney is associated with the presence of methionine.

Summary. In these experiments on rats, the addition of 4.8% glycine, 4.8% DL-methionine or 4.8% glycine plus 4.8% DL-methionine to a 12% casein diet did not alter the nitrogen balance as compared to controls receiving 12% casein alone but did reduce the nitrogen balance index, the excess amino nitrogen not contributing to the retention of nitrogen in the animal. The data indicates, on the other hand, that excess arginine (1.7%) contributes in part, to the retention of nitrogen. The addition of 4.8% glycine or

TABLE III.
Organ Weights of Rats Fed Various Nitrogen Sources.
Wet weights per 100 g of body weight. The results are averages obtained on 10 rats.

Group	Liver, g	Kidney, g	Adrenals, mg	Thyroid, mg	Testes, g	Seminal vesicles, g
I	2.66 ± .11	.617 ± .012	10.1	3.95 ± .26	.955	.310
II	3.15 ± .07	.856 ± .023	12.6	4.66 ± .19	1.07	.264
III	2.96 ± .05	.632 ± .014	8.8	4.01 ± .18	.912	.266
IV	2.91 ± .15	.707 ± .011	9.5	4.04 ± .07	.926	.312
V	2.79 ± .13	.788 ± .036	9.9	3.99 ± .10	.977	.228
VI	2.32 ± .11	.625 ± .016	9.1	3.88 ± .22	.960	.313

TABLE IV.
Water and Nitrogen Contents of Livers and Kidneys of Rats Fed Various Nitrogen Sources.
Averages on 10 rats.

	Water		Dry wt		Nitrogen		Total nitrogen	
Group	Liver %	Kidney %	Liver g/100 g B.W.	Kidney g/100 g B.W.	Liver %	Kidney %	Liver g/100 g B.W.	Kidney g/100 g B.W.
I	70.8	76.1	.815	.146	10.28	11.63	.084	.0169
II	70.9	76.4	.915	.201	10.23	11.61	.094	.0234
III	71.3	76.0	.846	.151	10.12	11.55	.086	.0174
IV	71.9	75.8	.794	.171	10.42	11.34	.083	.0194
V	70.3	76.9	.829	.181	10.53	11.16	.087	.0202
VI	70.7	76.0	.675	.149	10.86	11.29	.073	.0168

4.8% glycine plus 1.7% L-arginine to a casein diet containing excess methionine counteracted the weight loss and in part the kidney hypertrophy caused by the excess methionine. The slight increases in thyroid weights, associated with excess methionine, were also antagonized by glycine and arginine. Under the conditions of these experiments urinary creatinine and creatine excretion was not increased in rats by feeding excess glycine. The significance of these results is discussed.

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Therapeutic Effect of Choline Chloride in Dogs with Fat Emboli Produced by Bone Marrow Curettage.*

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Choline chloride as therapy for fat embolism was apparently used for the first time at Minneapolis General Hospital in 1948.

Moosnick, Schleicher,† and Peterson[1] observed that the fat content of human bone marrow is decreased 90% when a 1% choline chloride solution is given intravenously.

Methods. Twelve dogs were used to determine the value of choline chloride as a

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1 Moosnick, F. B., Schleicher, E. M., and Peterson, W. E., *J. Clin. Invest.*, 1945, **24**, 228.