We have found that sulfanilamide, which produces essentially the same micromelia as insulin,[8] does not alter the blood sugar (unpublished). However its micromelic effects are also reversed by nicotinamide (unpublished). There is a possibility that the low concentration of reducing sugars in the insulin-treated embryos may result in a higher nicotinamide requirement, while the sulfonamide may affect the nicotinamide content in another fashion. Levy and Young[9] have shown that virtually all of the nicotinamide (or nicotinic acid) in the chick embryo is bound in the diphosphopyridine nucleotide (coenzyme I). They also indicate (as has been found in other animal tissues[10]) that excess nicotinic acid (or amide) does not result in an increase in the coenzyme beyond its maximum levels. If these same conditions obtain under the abnormal circumstances induced by insulin (and sulfanilamide), then it seems likely that these substances may interfere, in some manner, with the synthesis of the coenzyme.

The hypoglycemia-alleviating effect of nicotinamide demonstrated here is not without precedent. Burke and McIntyre[11] have shown that this substance increases the tolerance of rats to insulin; a decrease in the duration of hypoglycemia was noted following the injection of a standard dose of insulin into nicotinamide-treated animals. These are essentially the same results which we have obtained with chick embryos.

That α-ketoglutaric acid also prevents insulin abnormalities as well as decreasing the duration of the hypoglycemia may indicate that pyruvate metabolism is, in some way, inhibited (possibly via coenzyme lack) and that this alternate pathway may be utilized as an energy source. At any rate these data indicate that carbohydrate metabolism is of considerable importance for normal limb formation in chick embryos.

Summary. The duration of insulin-induced hypoglycemia is reduced following the administration of nicotinamide and α-ketoglutaric acid to insulin treated chick embryos. Nicotinamide, which is the more effective in preventing insulin-induced abnormalities, is also more effective in decreasing the duration of the hypoglycemia.

[8] Ancel, P., *Ann. d'Endocrinol.*, 1945, **6**, 1.

[9] Levy, M., and Young, N. F., *J. Biol. Chem.*, 1948, **176**, 185.

[10] Schlenk, F., A Symposium on Respiratory Enzymes, Univ. of Wis. Press, 1942.

[11] Burke, J. C., and McIntyre, A. R., *J. Pharm. and Exp. Therap.*, 1939, **67**, 142.

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17273. Influence of Methionine and Thiouracil on Nitrogen Balance Index and Organ Weights of Adult Rat.*

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Allison, Anderson, and Seeley[1] have demonstrated that small quantities of methionine conserve body nitrogen when added to the diet of the dog. Earle, Small, and Victor[2] and Reisen, *et al.*[3] have found, however, that larger amounts of the amino acid will cause a marked loss in body weight in rats. This is in apparent contradiction to Harrison and Long[4] who reported that a diet containing 2% of methionine would increase the liver nitrogen of rats. Lea-

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[1] Allison, J. B., Anderson, J. A., and Seeley, R. D., *J. Nutrition*, 1946, **33**, 361.

[2] Earle, D. J., Jr., Small, K., and Victor, J., *J. Exp. Med.*, 1942, **76**, 317.

[3] Reisen, W. H., Schweigert, B. S., and Elvehjem, C. A., *Arch. Biochem.*, 1946, **10**, 307.

[4] Harrison, H. C., and Long, C. N. H., *J. Biol. Chem.*, 1945, **161**, 547.

them[5] has found the same apparent contradiction in rats fed anti-thyroid drugs. The animals in his experiments lost weight, probably due to a restriction in food intake, but the protein content of the liver was remarkably increased. Leathem and Seeley[6] have also demonstrated that the plasma proteins of rats fed the anti-thyroid drugs were increased with the largest increase occurring in plasma globulins.

In an attempt to resolve these apparent contradictions, the following experiments were designed to determine the nitrogen balance at various levels of methionine and thiouracil in the diet and to determine the nitrogen content of the plasma and tissues.

Methods. Nitrogen balance indexes‡ were determined on adult rats of the Long-Evans Strain using the diets and technics previously described for the dog.[7] The diet contained 12% casein on a dry weight basis and the amino acid and thiouracil were added at various levels. Rats were pair fed with those receiving methionine (4.8%) where the food intake was markedly restricted. Experiments were also performed on animals fed *ad libitum* as a secondary control. The experiments were continued for 20 days with urine and fecal collections throughout the period. At the end of the experiment the animals were bled by heart puncture and autopsied. The plasma was analyzed by the method of Howe[8] as modified by Robinson, Price, and Hogdem.[9] The livers and kidneys were dried to constant weight at 95°C and were then analyzed for nitrogen by the micro-Kjeldahl procedure.

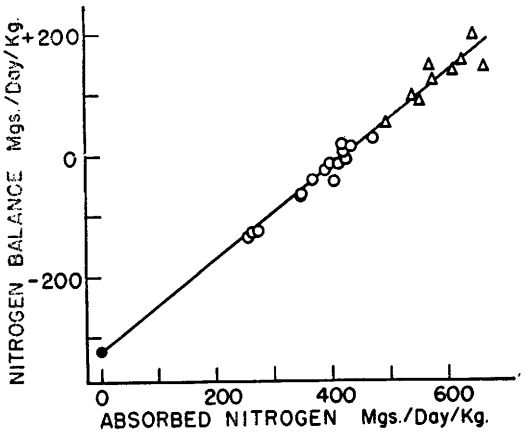

FIG. 1.
The relationship between nitrogen balance and absorbed nitrogen in rats fed a 12% casein ration *ad libitum* as indicated by the triangles, a 12% casein ration on a restricted food intake as indicated by the open circles, and a protein-free diet as indicated by the closed circle.

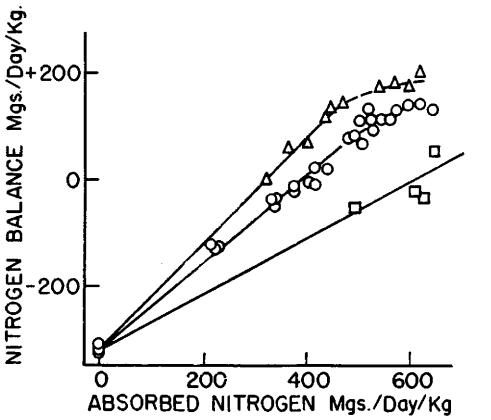

FIG. 2.
The relationship between nitrogen balance and absorbed nitrogen in rats fed a diet of 12% casein as indicated by the open circles, 12% casein to which was added 0.67% methionine (△), and 12% casein to which 0.6% thiouracil (□) had been added.

The urine and feces were also analyzed by the micro-Kjeldahl procedure. The Folin method[10] was used for the analysis of creatine and creatinine.

Results. The points plotted in Fig. 1 illustrate data obtained on rats fed a 12% casein ration (dry weight basis) *ad libitum* as shown by the triangles, and rats fed the 12% casein

5 Leathem, J. H., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 203.

6 Leathem, J. H., and Seeley, R. D., *Am. J. Physiology*, 1947, **149**, 561.

‡ The nitrogen balance index may be defined by the equation

$$NB = K(AN) - NE^{\circ}$$

Where NB is the nitrogen balance, AN the absorbed nitrogen, and NE° is the excretion of nitrogen on a protein free diet. Under these conditions, K may be defined as the nitrogen balance index.

7 Allison, J. B., and Anderson, J. A., *J. Nutrition*, 1945, **29**, 413.

8 Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 93.

9 Robinson, H. W., Price, J. W., and Hogdem, C. G., *J. Biol. Chem.*, 1937, **120**, 491.

10 Folin, O. S., *J. Biol. Chem.*, 1914, **17**, 469.

TABLE I.

Excretion of Creatine in Rats Fed a Basal 12% Casein Diet to Which Was Added Varying Amounts of Thiouracil and Methionine. Experiments on a restricted food intake are followed by R and those in which the animals are fed *ad libitum* are indicated by A. The nitrogen balance index and the weight change over a 20-day period are included.

Dietary regime	No. of animals	Avg. wt. change 20-day period, g	N. balance index	Creatine, mg/day/kg
Control (R)	18	—17	.77	11.8 ± .3*
" (A)	14	50	.78	9.6 ± .3
Thiouracil				
.12 (R)	10	0	.85	8.7 ± .2
.67 (R)	8	—20	.58	5.6 ± .2
.67 (A)	12	+40	.58	3.0 ± .2
Methionine				
.67 (A)	10	56	.96	8.1 ± .1
.67 (R)	12	5	.96	9.0 ± .1
2.4 (R)	8	—40	.60	7.9 ± .1
4.8 (R)	14	—56	.60	9.2 ± .2

* $E = \sqrt{\frac{\Sigma d^2}{N(N-1)}}$ Note: This equation for the standard error applies to all figures in the tables for which such data are given.

ration on a restricted caloric intake as shown by the open circles. The relationship between nitrogen balance and absorbed nitrogen is linear throughout the region of negative and of low positive nitrogen balance with an average nitrogen balance index of 0.77.[11] The recent work of Bricker and Mitchell[12] has confirmed this relationship in the rat.

The nitrogen balance index of the casein may be altered by adding certain compounds to the diet. The data in Table I demonstrate that the addition of 0.67% methionine (dry weight basis) to a casein diet increased the nitrogen balance index of casein in the rat from 0.77 to 0.96. The addition of larger amounts of methionine, however, decreased the nitrogen balance index markedly. The larger doses of methionine caused a definite restriction of food intake and necessitated the paired feeding of other groups to the methionine group. This restriction in food intake is paralleled by the weight change shown in Table I.

Thiouracil also had a marked effect on the nitrogen balance index of the casein. The addition of 0.6% thiouracil to the casein diet gave a nitrogen balance index of 0.58. When .12% thiouracil was fed to rats on a restricted caloric intake, the nitrogen balance index was 0.85, and a value somewhat above that obtained with casein alone. Very high levels of thiouracil were not fed because Astwood[13] has reported that rats refuse diets containing high levels of the drug.

Despite the variation in nitrogen balance index produced by the addition of thiouracil or methionine to the casein diet, the straight line relationship between nitrogen balance and absorbed nitrogen is still valid. The data plotted in Fig. 2 demonstrate that the addition of methionine (0.6%) which increased the nitrogen balance index, and of thiouracil (0.6%) which decreased the nitrogen balance index did not alter the linearity of the relationship. The bending of the line at high nitrogen balance values is to be expected and has been reported by Allison and Anderson.[7]

The data in Table I demonstrate also that thiouracil decreases the excretion of creatine. The decrease in creatine excretion of the thyroidectomized animal has been reported by Allison and Leonard[14] and Wilkins and

[11] Brown, J. H., and Allison, J. B., Abstract 112th Meeting of Am. Chem. Soc., 1947, 51c.

[12] Bricker, H., and Mitchell, H. H., *J. Nutrition*, 1947, **34**, 491.

[13] Astwood, E. B., *J. Pharm. and Exp. Therap.*, 1943, **78**, 79.

[14] Allison, J. B., and Leonard, S. L., *Am. J. Physiol.*, 1941, **132**, 185.

TABLE II.
Data Obtained on Autopsy of Rats Fed a 12% Casein Diet *ad libitum* (A) and a 12% Casein Diet on a Restricted Food Intake (R) to Which Was Added Varying Amounts of Methionine Thiouracil.

Dietary regime	Thyroid, mg/100 g., B.W.	Liver, mg/100 g., B.W.	Kidney, mg/100 g., B.W.
Control (R)	6.7 ± 0.9	3230 ± 48	684 ± 24
" (A)	6.2 ± 0.5	3352 ± 52	691 ± 36
Thiouracil			
.12 (R)	9.5 ± 0.8	3450 ± 142	673 ± 32
.67 (R)	12.8 ± 1.2	3892 ± 210	684 ± 37
.67 (A)	16.2 ± 1.3	4221 ± 178	684 ± 31
Methionine			
.67 (A)	6.8 ± 0.7	2931 ± 96	811 ± 82
.67 (R)	6.4 ± 0.6	3193 ± 103	705 ± 40
2.4 (R)	7.2 ± 0.9	2961 ± 80	850 ± 32
4.8 (R)	8.7 ± 0.9	3290 ± 92	925 ± 46

TABLE III.
Influence of Various Concentrations of Methionine and Thiouracil on Liver Nitrogen of Rats Receiving a 12% Casein Basal Ration.

Dietary regime, sulfur	No. of animals	Liver N, %	Total Liver N, g/100 g., B.W.	H_2O, %
Casein (A)	18	9.2 ± .6	.098 ± .004	69.8 ± 1.2
" (R)	14	9.6 ± .4	.099 ± .003	71.2 ± 0.8
Methionine				
.67 (A)	10	9.7 ± .5	.107 ± .004	72.0 ± 1.3
.67 (R)	12	10.4 ± .4	.103 ± .003	72.7 ± 1.1
2.4 (R)	8	10.8 ± .1	.104 ± .003	71.1 ± 0.9
4.8 (R)	14	11.2 ± .6	.109 ± .004	70.3 ± 0.8
Thiouracil				
.12 (A)	10	8.6 ± .3	.091 ± .004	67.4 ± 1.0
.67 (A)	8	9.4 ± .3	.130 ± .010	69.5 ± 1.1
.67 (R)	12	9.3 ± .4	.106 ± .008	69.2 ± 1.2

Fleischman[15] have demonstrated that the creatine excretion of the thyrotoxic patient can be decreased by the administration of thiouracil. The data presented here demonstrate that the excretion of creatine is decreased in normal rats rendered hypothyroid by the administration of thiouracil. Methionine also demonstrated some tendency to decrease the excretion of creatine.

Thiouracil increases the size of the thyroid in rats fed the compound, (Table II). These data demonstrate that thiouracil not only increases the size of thyroid but also markedly increases the size of the liver. The increased size of livers in rats fed large doses of thiouracil has been previously reported by Leathem,[5] but his data was not correlated with a change in the nitrogen balance index.

Methionine, on the other hand, had no effect on the size of the liver but brought about a definite hypertrophy of the kidneys of rats fed high concentrations of this amino acid. Methionine in high level dosage also enlarged the thyroid. The enlargement of the thyroid upon the addition of methionine to the diet may be due to the high concentration of sulphur in the diet or to an antithyroid effect from the methionine. The changes in organ weights with both compounds are directly proportional to the dosage.

The nitrogen content of certain tissues of the body was altered by the addition of either

[15] Wilkins, L., and Fleischman, W., *J. Clin. Invest.*, 1946, **25**, 360.

TABLE IV.
Influence of Various Concentrations of Methionine and Thiouracil on the Plasma Proteins of the Rat Fed a Basal Ration Containing 12% Casein.

Dietary regime	No. of animals	Albumin G. %	Globulin G. %	A/G	Hematocrit, %
Control (A)	18	3.10 ± .14	2.89 ± .14	1.10	48
" (R)	14	2.96 ± .22	2.86 ± .16	1.14	48
Thiouracil					
.12 (R)	10	3.20 ± .18	3.73 ± .18	0.86	46
.67 (A)	8	2.97 ± .16	3.74 ± .19	0.68	43
.67 (R)	12	3.00 ± .14	3.48 ± .17	0.67	43
Methionine					
.67 (R)	10	2.86 ± .19	3.69 ± .21	0.78	44
.67 (A)	12	2.81 ± .16	3.77 ± .19	0.74	45
2.4 (R)	8	2.89 ± .22	3.69 ± .18	0.78	47
4.8 (R)	14	3.02 ± .23	3.65 ± .19	0.82	46

thiouracil or methionine to the casein diet. The data presented in Table III demonstrate that methionine increased the nitrogen per gram of tissue of the liver and produced an over-all increase in liver-nitrogen. Thiouracil also increased the total liver nitrogen markedly, but the increase was due to the increased size of the organ rather than to the increased nitrogen per gram of tissue. Both methionine and thiouracil increased the globulin fraction of plasma with little change in albumin and decreased the A/G ratio (Table IV).

Summary. The addition of methionine and of thiouracil to the diet of rats altered the nitrogen balance index in proportion to the level of the compound in the diet. Although both methionine in high concentration and thiouracil caused a decrease in body weight and a reduction in nitrogen balance index, the compounds increased the total liver nitrogen; however, methionine increased the nitrogen per gram of tissue without affecting the size of the organ while thiouracil increased the size of the organ without affecting the nitrogen per gram of tissue. Both methionine and thiouracil increased the size of the thyroid, while methionine alone increased the size of the kidney. Both compounds increased the total plasma proteins by increasing the plasma globulin. Both methionine and thiouracil decreased creatine excretion; however, the effect of thiouracil is marked in comparison to methionine. These data indicate that the result of feeding methionine or thiouracil is very similar in end result but that the mechanism may not be identical because the results are reached through different pathways.

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17274. Replacement of a Portion of the Common Bile Duct with a Segment of Uterine Horn.

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Attempts at repair are comparatively unsuccessful when the common bile duct is accidentally so injured that an end-to-end anastomosis is not feasible. The present studies were undertaken to ascertain whether a grafted tubular structure with independent blood supply would maintain its viability and carry the bile from the common bile duct to the duodenum. Dogs were selected for the experiments and the uterine horn was used for