by causing a prolonged and greatly increased sensitivity toward thiouracil.

A basic difficulty in attempting any interpretation of the mixed treatment is that while the effects of antithyroid treatment are well known, the actual mechanism by which the effects are produced is not known with certainty for any antithyroid substance. Further investigation of thiouracil-iodide mixtures may help illuminate such mechanisms, and also provide interesting results in growth and maturation studies.

Summary. Young rats grew normally on diets containing either thiouracil or iodide at a level of 3 μmoles/g of feed, but growth was severely repressed on diets containing this concentration of both substances. After several weeks' treatment, growth repression was quickly overcome by adding thyroid powder to the thiouracil-iodide diet, or by removing thiouracil. Conversely, after being established, the repression was completely maintained by thiouracil alone at the stated concentration. It is concluded that the primary cause of the growth repression was a thyroxine deficiency induced by a synergistic action of thiouracil and iodide on the thyroid gland.

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Effect of Feeding Different Amino Acid Diets on Growth Rate and Nitrogen Retention of Weanling Rats.* (30079)

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The essential amino acid requirements of weanling rats fed amino acid diets containing 10% protein (N $\times$ 6.25) have been reported from this laboratory†(1-3). The investigations had been undertaken as part of a study to develop a method of protein evaluation by relating the essential amino acid content of the protein to the essential amino acid requirements(4) of the animal or man rather than to whole egg protein(5,6).

The growth rate of weanling rats fed the best amino acid diets was 4-5 g/day. Recently, Rogers and Harper‡ have reported that weanling rats fed an amino acid diet containing 15.2% protein and high in arginine and containing asparagine, agar and 50% water, showed a rate of growth of 45-50 g/week, equivalent to that obtained with a diet containing 18% whole egg protein. Since it is necessary that amino acid requirements be known accurately before a correlation can be established between the amino acid content of the protein and its biological

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TABLE I. Percentage Composition of the Diets.

	Experiment I					Experiment II					Experiment III					
	AI[1]	BI[1,2]	CI[1]	DI[3]	EI	AII[4]	BII[5]	CII[6]	DII[7]	EII[8]	AIII[9]	BIII[10]	CIII[10]	DIII[10]	EIII[10]	FIII[8]
Essential amino acids																
L-arginine·HCl	.254		.254	1.355	—		.891	.386	1.355			.700	.500	.350	.254	
L-histidine·HCl·H_2O	.338		.338	.446	—		.338	.514	.446							
L-isoleucine	.550		.550	.820	—		.550	.836	.820							
L-leucine	.700		.700	1.110	—		.730	1.064	1.110							
L-lysine·HCl	1.125		1.125	1.800	—		1.184	1.710	1.800							
L-methionine	.500		.500	.820	—		.539	.760	.820							
L-phenylalanine	.700		.700	1.160	—		.763	1.064	1.160							
L-threonine	.500		.500	.530	—		.500	.760	.530							
L-tryptophane	.150		.150	.150	—		.150	.228	.150							
L-valine	.550		.550	.820	—		.550	.836	.820							
Nonessential amino acids																
DL-alanine	.467		.402	.350	—		.230	.611	.095							
L-aspartic	1.006		.865	.350	—		.230	1.314	.095							
L-glutamic	3.294		2.833	3.500	—		2.199	4.306	.955			2.731	3.290	3.709	3.977	
Glycine	.300		.258	2.330	—		1.394	.393	.635							
L-proline	1.877		1.644	.350	—		.230	2.453	.095							
L-cystine	—		—	.350	—		.230	—	.095							
DL-serine	.974		.837	.350	—		.230	1.273	.095							
L-tyrosine	—		—	.350	—		.230	—	.095							
L-asparagine	—		.600	.600	—		.395	.912	.164							
Casein	—		—	—	18.0		—	—	—							
Agar	—		3.0	3.0	—		—	—	—							
Vitaminized sucrose[11]	15.0		15.0	15.0	15.0		15.0	15.0	15.0							
Salts 446[12]	4.0		4.0	4.0	4.0		4.0	4.0	4.0							
Cellulose	2.0		2.0	2.0	2.0		2.0	2.0	2.0							
Sodium chloride	1.0		1.0	1.0	1.0		1.0	1.0	1.0							
Corn oil[13]	4.0		4.0	4.0	4.0		4.0	4.0	4.0							
Dextrin	60.715		58.224	53.459	56.0		62.437	54.580	62.665			62.096	61.737	61.468	61.296	
Total	100.0		100.0	100.0	100.0		100.0	100.0	100.0			100.0	100.0	100.0	100.0	
Protein (N × 6.25):																
calculated	10.0	10.0	10.0	15.2	—	15.2	10.0	15.2	10.0	—	10.0	10.0	10.0	10.0	10.0	—
analyzed	10.2	10.1	9.9	15.5	16.0	15.3	10.1	15.3	10.1	16.0	9.8	9.8	9.9	9.8	9.9	16.0

NOTES TO TABLE I.

[1] Essential amino acids based on determined requirements(3). Nonessential amino acids as in casein(8).
[2] Same as A I, plus 3% agar added at expense of dextrin.
[3] Amino acid mixture (composition presented at 48th Annual Fed Proc Meeting, Chicago) of Rogers and Harper (see footnote ‡).
[4] Same as D I, except that nonessential amino acids were substituted with isonitrogenous amounts (12.016 g) of glutamic acid.
[5] Amino acids of diet D I proportionally decreased to correspond to 10% instead of 15.2% protein. Wherever the level of essential amino acids fell below that of diets A I-C I, the latter values were retained.
[6] Modified diet C I to contain 15.2% protein.
[7] Nonessential amino acids of diet D I proportionally decreased to contain 10% protein.
[8] Same as diet E I.
[9] Same as diet B II.
[10] Except for differences shown, identical with diet A III (or B II).
[11] Thiamine•HCl and riboflavin, 10 mg each; Ca pantothenate, 50 mg; pyridoxine•HCl, 5 mg; nicotinic acid, 20 mg; folic acid, 2 mg; p-aminobenzoic acid, 50 mg; menadione and biotin, 0.1 mg each; vit B_{12} in mannitol, 1 g; inositol, 330 mg; choline chloride, 660 mg; vit A palmitate, 11 mg (250,000 IU/g); α-tocopherol acid succinate, 10 mg; and sucrose to 100 g.
[12] Spector(9).
[13] 4 g corn oil contained 200 IU of vit D.

value, using the requirement figures as the "reference" protein(4) further studies were undertaken. These involved formulation of an amino acid mixture capable of supporting maximum growth and nitrogen retention in rats fed diets containing the previously determined 10% protein requirement(1,2) in contrast to a 15.2% protein diet.

Materials and methods. In the 3 experiments to be described, groups of 8-10 weanling rats (Sprague-Dawley strain), averaging about 50 g initially, were used. They were housed individually in wire screen cages and fed *ad libitum* the pure amino acid and casein diets for 14 days. The diets as offered to the rats contained 50% water added in advance and were stored in the cold room (4°) in a manner to prevent any loss of moisture. Addition of water to the diets did not result in diet separation or settling out of the solids in the absence of agar. Daily food intake and alternate day weight gain records were kept. At the end of the experimental period, all rats were sacrificed, their intestinal contents washed out and the carcasses frozen. The frozen carcasses were weighed individually, digested in HCl in an autoclave for 30 minutes at 18 lb pressure, thoroughly homogenized in a Waring blendor, and suitable aliquots in duplicate then taken for nitrogen analysis by the Kjeldahl method. A group of 8-10 rats killed at the beginning of each experiment was treated in the same manner to determine initial body nitrogen.

The composition of the diets used is given in Table I. The calculated nitrogen contents of the diets were confirmed by the Kjeldahl method. The data were subjected to analysis of variance and Duncan's(7) multiple range test.

Results and discussion. The results are summarized in Table II.

Experiment I. Rats fed diet B I, containing agar as a diet thickener, did not show significant ($P > .01$) increase in growth rate over those fed diet A I devoid of agar, but otherwise identical in composition. Growth rate and nitrogen retention were not significantly improved in rats fed the diet containing agar plus asparagine (diet C I). An improvement in amino acid diets due to asparagine has been reported‡(10). Protein efficiency ratios (PER), indicating the efficiency of protein utilization by the rats, were about equal for the diets A I to C I. The diet containing the amino acid mixture of Rogers and Harper‡ (diet D I) supported a high rate of growth, 6.8 g/day, equal to that obtained with an 18% casein diet and significantly higher ($P < .01$) than that obtained on diet C I. Rogers and Harper‡ reported similar results using 18% whole egg diet. Sauberlich (11) reported a growth rate of 40 g/week in weanling rats fed pure amino acid and 20% casein diets. Assuming the effect of nonspecific nitrogen to be constant, data obtained with diets C I and D I appeared to indicate that essential amino acids added at levels higher than recommended†(3) resulted in significant ($P < .01$) improvement in the growth rate of the rats.

Experiment II. This experiment was conducted to study some modifications of the amino acid mixtures of Exp. I, as they influenced the performance of the growing rats. Substituting "nonessential amino acids" nitrogen of diet D I with glutamic acid as in

TABLE II. Effect of Feeding Pure Amino Acid Diets on Growth Rate and Nitrogen Retention of Weanling Rats (14 days).

							Gain in body nitrogen	
Diets	Description	No. rats	Protein* (N × 6.25) (% diet)	Avg food* intake, g/day	Avg wt gain, g/day	PER†	g/2 weeks	mg/day/g gain in body wt
				Experiment I				
AI	No agar	9	10.0	10.5	4.4 ± .16‡	4.2 ± .05‡	1.53 ± .12‡	24.8
BI	AI with agar	9	10.0	11.0	4.5 ± .20	4.1 ± .05	1.46 ± .09	23.2
CI	Agar plus asparagine	9	10.0	11.2	4.8 ± .10	4.3 ± .07	1.69 ± .09	25.1
DI	Amino acids according to Rogers and Harper	9	15.2	12.8	6.8 ± .20	3.5 ± .05	2.42 ± .15	25.4
EI	18% casein	10	16.0	13.2	6.9 ± .25	3.3 ± .03	2.72 ± .18	28.1
				Experiment II				
AII	Glutamic replacing non-essential amino acids in DI	9	15.2	10.7	4.7 ± .14	2.9 ± .07	1.58 ± .06	24.0
BII	Amino acids in DI, reduced by 10/15.2	9	10.0	12.8	6.2 ± .22	4.8 ± .08	2.07 ± .08	23.8
CII	Amino acids in CI, increased by 15.2/10	9	15.2	11.6	5.7 ± .22	3.2 ± .06	1.97 ± .12	24.7
DII	Nonessential amino acids in DI, reduced to obtain 10% protein equivalent diet	9	10.0	11.7	5.3 ± .18	4.5 ± .06	1.78 ± .06	24.0
EII	18% casein	9	16.0	12.9	6.7 ± .08	3.2 ± .02	2.51 ± .07	26.8
				Experiment III				
AIII	Same as BII	8	10.0	13.2	6.2 ± .14	4.7 ± .03	2.01 ± .05	23.2
BIII	AIII with .700% arginine•HCl	8	10.0	13.9	6.8 ± .16	4.9 ± .05	2.05 ± .03	21.5
CIII	AIII with .500% arginine•HCl	8	10.0	13.3	6.7 ± .07	5.0 ± .05	1.89 ± .03	20.1
DIII	AIII with .350% arginine•HCl	8	10.0	13.2	6.2 ± .19	4.7 ± .05	1.83 ± .06	21.1
EIII	AIII with .254% arginine•HCl	8	10.0	12.5	5.7 ± .10	4.6 ± .04	1.60 ± .05	20.0
FIII	18% casein	9	16.0	13.9	7.4 ± .14	3.3 ± .03	2.38 ± .05	23.0

* Expressed on moisture-free diet basis.
† Protein efficiency ratio: g weight gain/g protein consumed.
‡ ± S.E.

diet A II, resulted in a sharp decrease in growth rate from 6.8 g to 4.7 g/day, in nitrogen retention from 2.42 g to 1.58 g/2 weeks, and in PER from 3.5 to 2.9. This tends to agree with the report of Rose *et al* (12), which indicated that a single source of "dispensable nitrogen" was inferior to a mixture of nonessential amino acids. Sauberlich(11) reported that "nonessential amino acids" appeared to be necessary in the diet, in order to obtain an optimum early growth rate. Diet D II contained all the nonessential amino acids of diet D I, but at a reduced level to contain 10% instead of 15.2% protein. This resulted in some, although nonsignificant ($P>.01$) improvement in growth rate and nitrogen retention over diet A II. By virtue of a wide difference in protein levels, the PER increased from 2.9 at the 15.2% protein level to 4.5 at the 10% protein level. Proportionally increasing diet C I amino acids to the level to correspond to 15.2% protein as in diet C II, further improved, but insignificantly ($P>.01$), the growth rate and nitrogen retention of the rats fed this diet. As in Exp. I, highest and almost identical body weight and nitrogen gains were obtained in rats fed the intact casein diet followed closely by diet B II. Diet B II had amino acids incorporated at levels lower than those in diet C II excepting arginine. Diet B II was a definite improvement over diet A I, the amino acid diet of Rama Rao *et al*† (1-3) giving a growth rate of 6.2 g/day, total nitrogen retention of 2.07 g/2 weeks, and a PER value of 4.8. The dietary protein level in each case was maintained at 10%, reported(1,2) as the minimum total protein requirement for the growing rat. Except for arginine·HCl, which was added at 0.891% level compared to 0.254%(3), all other essential amino acids in diet B II were incorporated at essentially the same levels previously reported(3) to be the requirements of the weanling rats. It thus appeared that most of the beneficial response was due to the high level of arginine. Since, following the reports of Laguna and Carpenter(13) and of Keane *et al*(14), water was added to all diets, it was not a variable, and only a small insignificant response had been obtained to asparagine in Exp. I.

Experiment III. Since the data of the previous experiments indicated that arginine was a limiting factor in the growth of rats on the Rama Rao *et al* diet, an experiment was carried out to determine the arginine level required for optimum growth and nitrogen retention. Arginine·HCl was added to the diets (A III-E III) at levels ranging from 0.254% to .891%, the two values representing, respectively, the levels recommended by Rama Rao *et al*(1-3) and that incorporated in the best amino acid diet (diet B II) of Exp. II. Increasing the level of arginine·HCl from 0.254% (diet E III) to 0.35% (diet D III) improved, but insignificantly ($P>.01$), growth rate, total nitrogen retention, and PER in the rats fed diet D III. At 0.5% dietary arginine·HCl level, rats showed a daily weight gain of 6.7 g, PER value of 5.0, and a total nitrogen retention of 1.89 g/2 weeks. Further increases in the level of dietary arginine did not support any significant improvement in the performance of the rats suggesting, therefore, that 0.5% arginine·HCl is required by the weanling rats.

From Table II it can be seen that gain in mg in body nitrogen per day per g gain in body weight, although somewhat lower in Exp. III because of the exceptionally high rate of growth obtained, was not much different for all the 13 amino acid diets (average 23.1 with a range of 20.0 to 25.4). This suggests that the nitrogen content of the growing tissues was reasonably constant, *i.e.*, each unit gain in body weight was accompanied by a uniform increase in body nitrogen, typical of the growing tissue.

Summary. True body growth of over 6 g/day approaching that obtained with an intact protein diet was obtained by feeding weanling rats a pure amino acid diet equivalent to 10% protein. This rapid rate of growth was obtained by increasing the arginine content of the diet over that previously used. 0.5% arginine·HCl appeared to be the requirement level for optimum growth.

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