titrating the potency of new skin test antigen lots. A second experiment was performed to compare the degree of sensitization obtained by the footpad method using non-infectious material to that obtained by injecting the guinea pigs with live *Histoplasma capsulatum*. There was essentially no difference in the degree of sensitivity obtained in the animals using the two methods.

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Influence of Nonessential L-Amino Acids on Growth of Rats Fed High Levels of Essential L-Amino Acids. (31178)

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There has been renewed interest in the role of nonessential amino acids (NEAA) in diets containing mixtures of crystalline amino acids as the sole source of dietary nitrogen for young rats. The work in this field has been comprehensively reviewed(1,2). However, there have been few reports(3-7) of studies using only the natural (L) isomers of both the essential and nonessential amino acids.

Breuer and coworkers(7) recently reported that the diet of Rechcigl *et al*(8), containing a high level (15.68%) of essential amino acids (EAA) and no nonessential amino acids (except cystine and tyrosine), supported significantly better growth than did diets containing lower levels of EAA with NEAA to provide additional nitrogen. However, these workers did not study the effect of addition of NEAA to the high level EAA mixture. A casein diet was superior to any of the amino acid diets they tested. They later(9) reported that proline, asparagine, and glutamic acid were necessary for maximal growth of the rat using other amino acid mixtures.

Recently we reported* that weanling rats fed the L-amino acid mixture of Rechcigl *et al*(8) supplemented with NEAA grew at a significantly faster rate than rats fed only a mixture of high levels of EAA. The studies reported here were designed to investigate further the effects of addition of NEAA to this mixture on growth of weanling rats. In addition, observations were made on the effect of NEAA on liver composition and the plasma amino nitrogen levels.

Materials and methods. Weanling male Holtzman rats having initial weights of 45-50 g were housed in individual cages in a room maintained at 22 ± 1°C. Food intakes and body weights were recorded twice weekly. Rats were fed a complete diet, with 14% vitamin-free casein (General Biochemicals, Inc.) for a 10-day adaptation period except in Experiments III and IV. The rats were then provided with their assigned experimental diets and tap water *ad libitum* for 21 days.

The composition of the diets is given in

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* Adkins, J. S., McCombs, J. S., Hove, E. L., Fed. Proc., 1965, v24, 168.

TABLE I. Percentage Composition of Diets.

		Diet	
Ingredient	A	B*	C
Casein	14.00	—	—
L-arginine • HCl	—	2.12	2.12
L-histidine • HCl • H_2O	—	.79	.79
L-isoleucine (allo-free)	—	2.08	2.08
L-leucine	—	1.93	1.93
L-lysine • HCl	—	2.84	2.84
L-methionine	—	.50	.50
DL-methionine	.18	—	—
L-cystine	—	.45	.45
L-phenylalanine	—	1.09	1.09
L-tyrosine	—	.86	.86
L-threonine	—	1.16	1.16
L-tryptophan	—	.23	.23
L-valine	—	1.63	1.63
L-alanine	—	—	.33
L-aspartic acid	—	—	.65
L-glutamic acid	—	—	1.14
Glycine	—	—	.33
L-proline	—	—	.39
L-serine	—	—	.78
Constant ingredients†	39.71	39.71	39.71
Dextrin, corn	46.11	44.61	40.99
Total	100.00	100.00	100.00
Protein or equivalent (N × 6.25)	12.5	13.2	15.9

* Patterned after mixture 2-L, Rechcigl *et al*(8).

† The diet had a constant percentage composition of the following ingredients: 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline, 0.0125 (Santoquin, Monsanto Chemical Co., St. Louis, Mo.); glucose, 15.0 (Cerelose, Corn Products Co., Argo, Ill.); cellulose, 2.0 (Alphacel, Nutritional Biochemicals Corp., Cleveland, Ohio); hydrogenated vegetable oil, 14.0 (Crisco, Procter & Gamble, Cincinnati, Ohio); vitamin supplement, 2.2 (Vitamin Diet Fortification Mixture in Dextrose, Nutritional Biochemicals Corp., Cleveland, Ohio); mineral supplement, 4.0 (Jones & Foster(17)). Purchased from General Biochemicals, Inc., Chagrin Falls, Ohio; $NaHCO_3$, 2.5.

Table I. The basal amino acid diet (diet B) was patterned after Rechcigl *et al*(8) with all optically active amino acids supplied as the National Research Council grade L-isomers (General Biochemicals, Inc.). Diet C was the basal amino acid diet supplemented with a mixture of 6 NEAA. The amino acids were finely ground and well mixed to insure uniformity of the mixture.

At the end of the 3-week experimental period, all rats were anesthetized lightly with ether and a blood sample was drawn from each heart by a heparinized syringe. The sample was transferred to a heparinized centrifuge tube and the plasma separated by centrifugation at 2500 rpm and stored at —15°C for analysis. Blood plasma was analyzed for plasma amino acid nitrogen(10). The rats were sacrificed and the livers excised, blotted, and weighed. Moisture was determined on a sample obtained from each lobe by difference after drying the liver sample *in vacuo* (100 mm Hg) at 100°C for 5 hours.

The remainder of the organ was held at —15°C until analyzed. Total liver lipids were determined gravimetrically after extraction with chloroform-ethanol 1:2 and ethyl ether. Nitrogen was determined by a conventional micro-Kjeldahl procedure on the fat-free dried livers and the protein equivalents of the diets were calculated (N × 6.25). Data were analyzed statistically by the "t" test(11).

Results. Body weight gain, food efficiency, and liver composition data are summarized in Table II. Daily weight gain was significantly greater ($P<0.001$) with diet A (casein) or diet C than with diet B. The growth rate of rats fed amino acid diet C was essentially the same as that of rats fed the casein diet. Further, addition of the NEAA mixture resulted in a significant ($P<0.01$) improvement in food efficiency over diet B.

Rats fed the casein diet had greater liver weights expressed as % body weight ($P<0.01$) than did rats fed either amino acid diet. Kidney and spleen weights and the percentage of liver moisture did not differ significantly between groups (data not presented). Rats fed amino acid diet C had significantly more liver fat ($P<0.001$) expressed as % of wet weight and liver nitrogen ($P<0.05$) on a fat-free dry basis than rats fed the casein diet. On diet C, rats had significantly more ($P<0.01$) liver fat than on diet B, but the liver nitrogen was the same for both groups. Livers of all rats were normal in gross appearance. Rats on diet B had a significantly higher ($P<0.01$) plasma amino nitrogen (PAN) value than rats fed diet C. For rats fed the casein diet, PAN values were significantly lower than for rats fed either diet B ($P<0.01$) or diet C ($P<0.05$).

Next, the six NEAA were added individually to diet B. It can be seen (Table III) that the growth rate of rats fed diet C, with or without serine, was essentially the same

TABLE II. Effect of the Nonessential Amino Acid (NEAA) Mixture on Growth, Plasma Amino Nitrogen and Liver Composition of Rats Fed Amino Acid and Casein Diets for 3 Weeks.

Diet	No. of rats	Avg wt gain, g/day		Food efficiency, g gain/g food		Liver wt, % body wt		Liver fat, % wet		Liver N, % fat-free dry		Plasma amino nitrogen, mg/100 ml	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
A (casein)	8	5.6	.2	.38	.01	4.60	.17	4.77	.29	12.48	.40	8.8	.2
B (EAA)	16	3.8	.2	.33	.02	4.11	.05	5.35	.18	13.10	.14	12.6	.5
C (EAA + NEAA)	16	5.2	.2	.41	.01	4.03	.06	6.24	.14	13.24	.17	10.6	.4

as that of control rats fed the casein diet. Of the 6 individual NEAA and urea tested, only proline enhanced weight gain and food efficiency significantly ($P<0.01$) over diet B. The significance of proline in the diet was further investigated by omitting this amino acid from diet C. Growth was significantly less ($P<0.05$) when proline was omitted and the food efficiency was also significantly lower ($P<0.01$).

Discussion. The present data indicate that the basal amino acid diet supplemented with so-called "nonessential" amino acids is superior to the diet lacking these compounds and is more nearly equivalent to the intact casein protein diet. This probably indicates that under the conditions of this experiment, one or more of the six NEAA were not synthesized fast enough to support maximal growth and food utilization. These results are of interest especially since the basal amino acid mixture contained cystine and tyrosine and supplied a high level of "nonspecific-N" from excess L-isomers of the essential amino acids(8).

In a preliminary study,* and in the study presented here, it should be noted that the growth rate exceeded 5 g/day only when the diet contained proline.† This response to proline is in agreement with the early work of Womack and Rose(12) and the recent finding of Breuer *et al*(9) who showed that proline was necessary in the diet for optimal weight gains of weanling rats. The need for proline and other NEAA(6,9) may be related in some way to the levels of other amino acids in the diet. The levels of dietary arginine and glutamic acid may be important factors relating to the need for proline because of the known interconversion of these amino acids with proline(12).

Harper(13) has shown that the development of fatty livers in rats may depend upon the amino acid balance of the diet. In the present studies, the higher liver fat values observed for rats fed the NEAA mixture as compared to diet B may be due to improper balance of amino acids in the diet. However, addition of the NEAA mixture clearly im-

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† Proline is a naturally occurring component of casein.

TABLE III. Effect of Individual Nonessential Amino Acids (NEAA) on the Growth of Rats Fed Amino Acid and Casein Diets for 3 Weeks.

Exp No.	Source of dietary N	Protein equivalence (N × 6.25), (% diet)	No. of rats	Avg wt gain, g/day		Food efficiency, g gain/g food	
				Mean	S.E.	Mean	S.E.
II	Casein, 14% (diet A)	12.5	6	5.9	.3	.38	.01
III	*idem*, 17%	15.2	10	6.3	.4	.52	.01
II	EAA + NEAA, (diet C)	15.9	6	5.5	.2	.42	.04
IV	*idem*	15.9	9	5.4	.1	.49	.01
III	" , minus serine	15.2	12	6.1	.1	.54	.01
IV	" , minus proline	15.6	9	4.8	.2	.41	.01
II	EAA (diet B)	13.2	7	4.2	.2	.34	.01
II	*idem* + proline, 0.39%	13.5	5	5.1	.3	.41	.02
II	" + glycine, 0.33%	13.6	7	4.7	.2	.35	.02
II	" + glutamic acid, 1.14%	13.9	5	4.6	.4	.36	.02
II	" + glutamic acid, 3.62%	15.4	7	4.5	.5	.32	.02
II	" + aspartic acid, 0.65%	13.6	6	4.5	.3	.36	.04
II	" + alanine, 0.33%	13.5	8	4.2	.4	.35	.02
II	" + serine, 0.78%	13.8	5	4.2	.2	.33	.02
II	" + urea, 0.93%	15.9	7	4.0	.3	.34	.03

proved the growth promoting potential of diet B. The addition of NEAA to diet B significantly reduced the PAN level. It may be hypothesized that an improper balance of amino acids in the diet slowed protein synthesis and thereby permitted the total concentration of amino nitrogen to build up in the plasma. Our growth and PAN data seem to support this hypothesis.

In these studies, sodium bicarbonate was added to all diets at the 2.5% level. Many workers(8,9,15) have added $NaHCO_3$ to amino acid diets to neutralize or "buffer" the hydrochloride present. This level of $NaHCO_3$ was added since the diets were patterned after Rechcigl *et al*(8). Breuer *et al*(9) showed that growth rate and feed consumption were significantly increased when an amino acid diet containing hydrochloride forms of arginine, histidine and lysine and the salt mixture of Jones and Foster(17) was supplemented with 2.58 or 4.58% $NaHCO_3$. Addition of 2.5% $NaHCO_3$ to the diet resulted in a total Na content of 0.9% (calculated).

Addition of $NaHCO_3$ to the casein diet was not detrimental as growth was essentially the same with or without $NaHCO_3$ as established in preliminary studies. The $NaHCO_3$ was included in these studies so that the basal mixture of ingredients could be used for both the casein and amino acid diets. The pH of the various diets measured in solution (1:1 w/v) was 7.3, 7.1 and 6.7 for diets A, B and C respectively. The pH of a casein diet similar to diet A but without $NaHCO_3$ was 6.6.

When all experiments are considered, our findings are in accord with the recent work of Rogers and Harper(14) and other investigators(5,6,15), which indicates that diets containing crystalline amino acids as the sole source of nitrogen can promote weight gain equal to that obtained with an intact protein diet. In our studies, this rapid gain was obtained without the inclusion of dietary asparagine(9), agar(14), or water(14,16) used by other workers. Proline, however, was needed in diets to give this excellent rate of growth.

Summary. Weanling rats were fed purified L-amino acid diets containing high levels of essential L-amino acids (EAA) to determine the effect of nonessential amino acids (NEAA) on growth. Observations were made also on the liver composition and plasma amino nitrogen (PAN) levels. A diet containing high levels of only the EAA resulted in poor growth (3.8 g/day), as compared with a casein diet (5.6 g/day). However, when six NEAA were added to this amino acid diet, growth rate (5.2 g/day) and food efficiency were significantly improved. Proline added singly caused a significant growth improvement; on the other hand, glycine, glutamic acid, aspartic acid, alanine, serine, or urea did not significantly improve the growth rate.

Addition of NEAA to the EAA diet resulted in significantly lower PAN levels but elevated liver fats.

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γ G Antibodies Appearing Early in the Primary Response of the Mouse.* (31179)

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The sequence of appearance of molecular species of antibody following immunization with particulate and soluble antigens has been studied by ultracentrifugation of antisera and localization of antibody activity within the globulin fractions. Gel filtration has also been used to fractionate serum proteins according to molecular weight and sedimenting characteristics, and antibody activity can be localized in the globulins of the 7S or 19S species. Using the passive hemagglutination test of Boyden(1) to measure antibody activity, various investigators(2,3) have concluded that the primary immune response in various animal species to bovine serum albumin (BSA) consists of 19S macroglobulin formation followed by the 7S variety. In the course of studying the primary immune response to BSA in the inbred mouse with and without the adjuvant effect of endotoxin, the presence of an early appearing 7S antibody response was detected. This antibody response was easily demonstrable by the Farr antigen binding capacity (ABC) technique(4), but poorly evident using passive hemagglutination. Rosenquist and Guilden(5) previously reported finding early 7S antibody activity to BSA in chickens, and since our studies were undertaken, reports of similar findings in the chicken and rabbit by Benedict and coworkers(6,7) and by Wei and Stavitsky(8) in the rabbit have been published. We wish to report the evidence in yet a third species and discuss the significance of these findings in relation to the general immune response to BSA.

Materials and methods. Antigen. BSA (Calbiochem, lot 43648) dissolved in saline and endotoxin (lot SMTCA AA-1) prepared from *Serratia marcescens* by Dr. A. Nowotny was injected intraperitoneally into mice 7-10 weeks old. Dosage was 3 mg BSA plus 10 μg endotoxin in a volume of 0.5 ml. The mice were maintained on Purina Mouse Checkers

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