excitation in frogs, followed later by a central paralysis, which occurs 2 hours before peripheral paralysis appears.

4. *Summary.* Curare, which affects the electrical activity of the brain has also a distinct effect on the temperature in the brain in combination with pentobarbital narcosis. These temperature changes in the brain indicating changes of oxidation processes are increased by curare under light pentobarbital narcosis, and decreased under deep pentobarbital narcosis. This latter central action may be involved in the synergistic narcotic effect.

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Growth of Chicks on Purified and Synthetic Diets Containing Amino Acids.*

T. D. LUCKEY, P. R. MOORE, C. A. ELVEHJEM, AND E. B. HART.

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Although amino acids have been used in recent investigations[1-3] as the principal nitrogen source in chick diets in order to determine the amino acid requirements of the chick, such diets have various other applications in nutritional investigations. We have fed amino acid diets in an attempt to determine (1) if the amino acids present in casein can be replaced by an equivalent amount of free amino acids; (2) if chicks will grow as well on a mixture of essential amino acids as when additional nonessential amino acids are added; (3) if chicks require strepogenin;[4,5] (4) if chicks will survive and develop normally on a purely synthetic diet (a chemically-defined diet consisting of C.P. chemicals); and (5) if a chemically-defined diet can be used effectively in the elucidation of unknown factors such as the monkey antianemia factor of Cooperman *et al.*[6]

Experimental. Day-old White Leghorn chicks weighing 40-42 g were divided into groups of 3 chicks each and maintained in electrically-heated cages with raised screen bottoms. In Series 1 and 2 (Table II) the chicks were fed a protein and amino acid-free diet for 3 to 4 days (to deplete them of any possible nitrogen stores carried in the yolk sac) prior to feeding the experimental diets. Since this preliminary depletion period appeared to inhibit later growth responses it was discontinued and the animals were placed directly on the experimental diets in subsequent experiments.

The composition of the diets used is given in Table I. Diet I is a typical experimental diet with 18% casein supplemented with arginine, glycine, and cystine and adequate amounts of the vitamins known to be involved in chick nutrition. Diet II is similar to Diet I but contains free amino acids equivalent to the amount present in casein with arginine, glycine, and cystine additions as in Diet I. Diet III contains the amino acids essential to the chick in amounts adequate to equal the nitrogen contents of Diets I and II. Diets IV, V and VI represent

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TABLE I.
Diet Composition in Percent.

Constituent	I	II	III	IV	V	VI	VII
Dextrin*	61.0	61.0	61.0	33.0	33.0	33.0	
Cerelose				33.0	33.0	33.0	
Celluflour†				3.0	3.0	3.0	
Glucose C.P.							71.0
Soybean oil	5.0	5.0	5.0	5.0	5.0	5.0	
Salts V‡	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Haliver oil§	.04	.04	.04	.04	.04	.04	
B-vitamins¶							
Calcium gluconate				0.4	0.4	0.4	0.5
Casein (SMA)	18.0				8.0	14.0	
d,l-Alanine		1.23		0.2	0.1		0.2
l(+)Arginine HCl	0.6	1.59	2.4	1.2	0.6	0.6	1.8
d,l-Aspartic acid		1.38		0.2	0.1		0.2
l(+)Cysteine HCl		0.09					
l(—)Cystine	0.3	0.39		0.4	0.2	0.4	0.3
l(+)Glutamic acid		5.00		1.5	0.75		1.5
Glycine	2.0	2.11	2.0	1.0	0.5	1.0	1.8
d,l-Histidine HCl • H_2O		0.68	1.2	0.6	0.3		0.2
d,l-Homocystine		0.05					
l(—)Hydroxyproline		0.05		0.1	0.05		
d,l-Isoleucine		2.85	4.0	2.0	1.0		2.0
l(—)Leucine		2.66	3.0	1.5	0.75		1.5
l(+)Lysine HCl		1.88	2.5	1.2	0.6		1.8
d,l-Methionine		0.77	2.0	1.0	0.5		1.5
d,l-Norleucine		0.05					
d,l-Phenylalanine		1.13	3.0	1.0	0.5		2.0
d,l-Proline		1.80		0.2	0.1		0.2
d,l-Serine		0.11		0.2	0.1		0.15
d,l-Threonine		1.72	4.0	1.4	0.7		2.0
l(—)Tryptophane		0.26	2.0	0.5	0.25		0.5
l(—)Tyrosine		1.40		0.5	0.25		1.0
d,l-Valine		2.94	4.0	2.0	1.0		2.0
Total Nitrogen**	3.38	3.38	3.36	1.87	2.20	2.59	2.36

* Corn starch moistened with water and autoclaved at 15 lb for 2 hr, dried, and ground.

† A commercial cellulose product.

‡ Diets IV, V, and VI contained 0.5% Na_3PO_4 and diet VII contained $NaHCO_3$ 1.0%, Na_2HPO_4 0.5%, $Al_2(SO_4)_3$ 0.1%, $Na_2B_4O_7 \cdot 10H_2O$ 0.1%, $VaCl_2$ 0.05%, $NiSO_4$ 0.03%, $Co(NO_3)_2$ 0.03%, and K_2SiO_4 0.1% in addition to salts V.

§ Haliver oil fortified with vitamin D_3 to supply .006 mg/100 g ration.

¶ Diets I, II, and III contained α-tocopherol 0.6 mg, 2-me-1,4-naphthoquinone 0.1, thiamine Cl 0.5, riboflavin 1.2, nicotinic acid 10.0, calcium pantothenate 4.0, pyridoxine HCl 0.8, choline Cl 200.0, inositol 100.0, biotin 0.04, and folic acid 0.5 mg per 100 g of ration.

Diets IV, V, and VI contained double the above amounts of α-tocopherol, thiamine Cl, nicotinic acid, riboflavin, calcium pantothenate, pyridoxine HCl and biotin and 2-me-1,4-naphthoquinone 0.1, ascorbic acid 100 mg, *p*-aminobenzoic acid 5.0, folic acid 0.05, and *i*-inositol 100 mg per 100 g of ration and 0.2 mg cholic acid per 100 g of ration. Diet IV contained 200 and diets V and VI 300 mg respectively of choline Cl per 100 g of ration.

Diet VII contained carotene 0.6, ascorbic acid 100, vitamin D_3 .005, α-tocopherol 33, 2-me-1,4-naphthoquinone 2.0, thiamine Cl 0.5, riboflavin 1.0, nicotinamide 10.0, Ca pantothenate 2.0, pyridoxine Cl 1.0, pyridoxal Cl 0.1, β-pyracin Cl 0.5, choline Cl, 200.0, *i*-inositol 100.0, biotin 0.05, folic acid 0.05, and *p*-aminobenzoic acid 10.0 mg per 100 g of ration. In addition Diet VII contained ethyl linolate 0.1, tripalmitin 1.0, ribose 0.03, and arabinose 0.5 g and cholic acid .2 mg per 100 g of ration.

** Non-utilized forms of amino acids are omitted in this sum.

modifications of amino acid mixtures with and without protein supplementation. Diet VII is a chemically-defined diet that contains additional growth factors and trace elements. All differences in total weights of the nitrogen sources were compensated for by modifying the level of carbohydrate.

Results. A direct comparison of individual groups within a given series is difficult, but a comparison of similar lots in the first 3 series demonstrates that casein diets (Groups 1, 7 and 12, Table II) produce better growth

TABLE II.
Amino Acid Diets in Chick Growth.

Series No.	Group No.	Diet No. and description	% nitrogen	Days on experiment	G gain per day
1	1	I—casein, arginine, glycine, and cystine	3.38	18	6.7 (3)*
1	2	II—22 amino acids	3.38	18	2.9 (3)
1	3	III—11 essential amino acids	3.36	14	1.0 (3)
2	4	IV—19 amino acids	1.87	10	1.5 (2)
2	5	V—19 amino acids + 8% casein	2.20	10	2.0 (3)
2	6	III—11 essential amino acids	3.36	10	0.6 (3)
2	7	VI—casein, arginine, glycine, and cystine	2.59	10	4.7 (3)
2	8	VI—casein,† arginine, cystine, and glycine	1.30	10	1.8 (3)
3	9	IV—19 amino acids	1.87	8	3.9 (2)
3	10	V—19 amino acids + 8% casein	2.20	8	3.0 (2)
3	11	III—11 essential amino acids	3.36	8	1.6 (2)
3	12	VI—casein, arginine, glycine, and cystine	2.59	8	4.1 (2)
3	13	VI—casein, arginine, glycine, and cystine	1.30	8	2.6 (2)
4	14	VII—chemical diet—no protein	0.00	16	—0.5 (3)
4	15	VII—18 amino acids	2.36	16	0.7 (3)
4	16	VII—16% amino acids + 4% liver powder‡	1.82§	16	1.5 (2)
4	17	VII—16% amino acids + 4% casein	2.45	16	3.0 (3)
4	18	VI—casein, arginine, glycine, and cystine	2.59	16	5.8 (3)
4	19	VI—casein, arginine, glycine, and cystine	1.30	16	1.0 (2)
4	20	VII—12% amino acids + 4% liver powder + 8% casein	2.67	16	2.5 (3)

* Figures in parentheses refer to number of survivors at the conclusion of the experiment.
† Casein, arginine, glycine, and cystine mixture reduced by one-half and replaced by dextrin.
‡ Whole liver powder.
§ Nitrogen content of the whole liver powder is not included in this sum.

responses in chicks than diets containing the 11 essential amino acids plus nonessential amino acids (Groups 2, 4 and 9) and that the latter diets are in turn superior to diets containing the 11 essential amino acids alone (Groups 3, 6 and 11). Growth differences between similar groups in the different series are not significant since different depletion technics and different experimental times were involved. Furthermore, although uniform chicks were used in any given series, dietary differences in the diets of the hens used as the source of eggs could affect the stores of the chicks which would alter their growth responses on similar diets in series run at different times.

Substitution of one-half of the weight of the amino acids in Diet IV with an equivalent weight of casein (8%) (Groups 5 and 10) produced no better growth responses than the amino acid diets (Groups 4 and 9) or a casein diet in which the nitrogen level was reduced 50% by feeding one-half of the casein and supplementary amino acids of Diet VI (Groups 8 and 13).

Chicks fed an amino acid-free synthetic diet (Group 14) lived for a remarkably long period and displayed no macroscopic deficiency symptoms other than loss of weight and lethargy. Chicks fed the complete synthetic Diet VII (Group 15) showed poor growth responses. Addition of 4% whole liver powder at the expense of the amino acids doubled the growth response (Group 16). The use of casein in place of whole liver powder gave even better growth responses in the chicks (Group 17) while the addition of both liver powder and casein (Group 20) appeared to produce no better growth response than 4% casein alone.

Discussion. The maximum growth responses on our amino acid diets (3.9 and 3 g per day) compare favorably with those of Hegsted (2.7 and 3.5 g per day).[2] We recognize that an absolute comparison cannot be made since different sources of animals and different experimental periods were involved.

The general failure of the amino acid diets to produce growth responses equal to diets containing casein supplemented with arginine, glycine and cystine can be explained only partially by the lack of a growth factor since whole liver substance apparently failed to supplement casein (source of strepogenin) on our synthetic diet. Our results do not, however, eliminate an extremely labile growth factor absent from the whole liver powder used. It is doubtful that the growth differences are due to missing trace elements since the whole liver powder would probably carry adequate amounts of any unidentified essential minerals.

Growth inhibition due to toxicity of some of the *d*-isomers is debatable. Replacement of part of the amino acids in the diet containing the 19 amino acids by casein failed to produce a significant growth response in chicks over a reduced casein diet (Groups 5, 8, 10 and 13) which would be expected if toxic *d*-isomers were involved. This occurred in spite of the fact that some of the amino acids were borderline in the reduced casein diet. Alternatively, the poor growth responses obtained on the diet containing the 11 amino acids (3.36% nitrogen) when compared to the diets containing the 19 and 22 amino acids (2.20 and 3.38% nitrogen respectively) could be explained by the higher percentage of the *d*-isomers in the diet containing the 11 amino acids, or by the possibility that some of the so-called nonessential amino acids are essential on such highly purified diets. It is possible that the problem is physiological rather than nutritional and involves amino acid imbalances other than toxicity of the *d*-isomers, disturbed osmotic pressure relationships, interference with buffer mechanisms in the intestine, poor retention in the tract, or impaired absorption. Inhibition of enzymes or beneficial bacteria in the intestinal tract are other possible effects.

The role of strepogenin in the nutrition of the chick remains obscure. The addition of casein or whole liver powder to our synthetic diet (Diet VII) produced a positive strepogenin-like response on chick growth (Groups 14, 16, 17, and 20) in contrast to the results with the purified diet (Diet IV) where replacement of one-half of the amino acids with casein failed to give increased growth in the chicks. The slightly higher nitrogen content of the supplemented synthetic diets could have been a factor involved. It is entirely possible that factors other than strepogenin were lacking and it is questionable if uniform growth responses to strepogenin would occur on such a deficient diet. There is also the possibility that the chick carries adequate stores of strepogenin and a true strepogenin response could not be expected on such short term experiments necessitated by the high cost of amino acids.

Summary. Chicks fed a completely synthetic (chemically-defined) diet in which amino acids are the sole source of nitrogen show a definite subnormal growth response.

Casein is superior to its component amino acids as a source of nitrogen for the chick.

Diets containing some of the nonessential amino acids are superior to diets containing the 11 amino acids now recognized as essential for the chick.

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