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Requirement of the German Cockroach for Choline and Related Compounds.*

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Ordinarily choline is not considered a critical vitamin in insect nutrition, although its omission may result in retarded growth.[1] Recently, however, it was shown[2] that the omission of choline from the diet of newly hatched German roaches resulted in a complete cessation of growth within 10 days and the death of all of the nymphs within 30 days. The omission of each of the other vitamins singly resulted in less marked effects upon growth and survival.

In the present study the requirement of the roach *Blattella germanica* (L.) for choline was determined quantitatively with synthetic diets otherwise optimal for growth and maturation. Compounds related to choline were also studied. The 40-odd nymphs from a single egg sac were divided into groups of 5-7 and kept in wire-screened test-tube cages[3] at a temperature of 27-32°C. Each group received a different diet, based on variations of synthetic diet V (Table I). Food and water were given *ad libitum* and were replaced weekly, and the roaches were weighed under CO_2 anaesthesia at 10, 20, and 30 days of age. The age of maturation of each roach was also recorded.

Choline Requirement. Young roaches fed diet V (Table I) from which the choline was omitted failed to gain weight after the 10th day (Table II) and they all died before the 40th day. At a level of 500 γ of choline/g of diet V, the growth rate was very slow and only one-fifth of the insects reached maturity. At a level of 1000 γ of choline/g of diet the growth rate was improved but was still subnormal, although most of the insects matured. At both the levels of 2000 and 4000 γ choline/g of diet, growth was good, and the insects matured at an average age of 39 days, with practically all surviving. A further increase in the level of dietary choline to 8000 γ/g of diet resulted in suboptimal growth (Table II).

TABLE I.
Composition of Synthetic Diet V.

	Parts
Glucose monohydrate (*Cerelose*)	31
Casein ("vitamin-free")	30
*Cellu Flour**	30
Wesson's salt mixture†	4
Corn oil	3
Cholesterol	1
Vitamin mixture	0.6
	γ/g
Choline chloride	4000
Thiamine hydrochloride	12
Riboflavin	18
Nicotinic acid	100
Calcium pantothenate	40
Pyridoxine	16
Inositol	2000
Biotin	0.6
Folic acid	5

* Manufactured by Chicago Dietetic Supply House, Chicago, Ill.

† Wesson, L. G., *Science*, 1932, **75**, 339.

The finding that the cockroach requires as much as 2000 γ of choline/g of diet for optimal growth is in contrast to the experiments of Fraenkel and Blewett with other insect species[1] which were uniformly fed only 500 γ of choline/g diet. However, 2000 γ/g have been reported[4] necessary for good growth of chicks fed homocystine and a minimal level of methionine.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

1 Fraenkel, G., and Blewett, M., *Biochem. J.*, 1943, **37**, 686.

2 Noland, J. L., Lilly, J. H., and Baumann, C. A., *Ann. Ent. Soc Am.*, in press, II.

3 Noland, J. L., Lilly, J. H., and Baumann, C. A., *Ann. Ent. Soc. Am.*, in press, I.

4 McKittrick, D. S., *Arch. Biochem.*, 1948, **18**, 437.

TABLE II.
Growth, Maturation, and Survival of the Cockroach *Blattella germanica* on Synthetic Diets Containing Various Amounts of Choline.

No. of times fed	Choline Cl γ/g diet	Wt of roaches 10 days, mg	20 days, mg	30 days, mg	Avg. age at matur. days	Survival at matur.
2	0	4	3	4	dead	0/11
4	500	4	5	10	80	4/22
5	1000	6	16	33	44	26/28
7	2000	6	21	44	39	34/36
6	4000	6	21	44	39	35/35
1	8000	5	19	35	45	6/6

An effort was made to demonstrate alterations in choline requirement due to changes in the level of dietary fat, cholesterol, cystine, or niacinamide. Corn oil was included at levels of 1, 3 and 10% of the diet, and cholesterol was fed at levels of 0.3, 1 and 3% of the diet. Various combinations of these 3 levels of fat and cholesterol were fed in diets containing 500, 1000 and 4000 γ of choline/g diet with no significant differences in growth rate resulting. L-Cystine and niacinamide fed at the levels of 1% and 870 γ/g, respectively, in a diet containing 1000 γ choline/g diet, likewise failed to produce significant changes in growth or maturation. It was, therefore, concluded that the requirement of the roach for 2000-4000 γ of choline/g of diet is a reasonably constant one.

Activity of Other Compounds. DL-methionine, dimethylaminoethanol, aminoethanol, or betaine hydrochloride were added to diet V in the absence of choline or as supplements to diets containing a low level of the vitamin, 500 γ/g of diet. On the choline-free diet, methionine, dimethylaminoethanol and aminoethanol had very little growth-stimulating

TABLE III.
Response of the German Cockroach to Choline and Related Compounds Fed at Equimolar Levels in Synthetic Diet V.

Exp.*		γ/g diet	Wt of roaches 10 days, mg	20 days, mg	30 days, mg	Avg. age at matur. days	Survival at matur.
	Compounds added to choline-free diet.						
C, G	Choline Cl	1000	6	12	36	44	9/11
C, D	DL-methionine†	3210	3	6	5	dead	0/10
C, D	Dimethylamino-ethanol†‡	956	4	6	7	dead	0/10
C, D	Aminoethanol	438	4	6	7	dead	0/10
C, G	Betaine HCl	1100	6	13	36	45	10/12
D	Choline Cl	2000	5	19	46	40	5/5
D	Betaine HCl	2200	5	20	48	38	11/12
G	Choline Cl	4000	6	19	38	41	7/7
G, G	Betaine HCl	4400	6	18	33	43	13/14
	Compounds added to diet containing 500 γ of choline/g.						
A, B	None		4	6	12	78	2/10
A, B	Choline Cl	1500	6	20	40	42	8/10
A, B	DL-methionine	1610	5	10	20	72	5/10
A, B	Dimethylamino-ethanol	956	6	15	24	54	8/10
A, B	Aminoethanol	657	4	6	10	92	2/10
A, B	Betaine HCl	1650	6	22	41	40	10/10

* Dietary groups included in the same series were lettered the same.

† Added at levels equivalent to choline Cl on a methyl basis. In all other experiments the compounds were added at levels equivalent to choline Cl on a molar basis.

‡ Carbide and Carbon Chem. Corp. We are indebted to R. Grunert for this preparation.

TABLE IV.
Effect of Diet Upon the Choline Content of German Cockroaches, as Determined by the *Neurospora* Method.

Insects used		Diet		Choline content	
Sex	Age, days	Basal	Composition	Range	Avg
				γ/g live wt	
127 nymphs	3	crude	dog biscuits	900-1320	1080
2 M, 3 F	58	synth. V	choline Cl, 1000 γ/g	1520-1830	1620
3 M, 2 F	50	" III*	" Cl, 2000 γ/g	1630-1830	1730
2 M, 2 F	58	" V	" Cl, 4000 γ/g	2690-2880	2780
3 M, 3 F	58	" V	betaine HCl, 1100 γ/g	1140-1480	1290
2 M, 3 F	58	" V	" HCl, 4400 γ/g	1890-2090	2020

* Same basal diet as synth. V except vitamin K and p-aminobenzoic acid were added.

effect, since the nymphs gained only 2 or 3 mg from the 10th to the 30th days of age (Table III), and all such nymphs died before maturing. Nymphs receiving 1000 γ of choline/g diet gained 30 mg during this time. When the diets contained betaine, however, growth was equal to that of nymphs receiving an equimolar amount of choline, and the growth-promoting activity of betaine was found to equal that of choline at all the levels tested.

When the various compounds were added to a diet containing 500 γ of choline/g at levels equivalent to 1500 γ/g of choline on a molar basis, aminoethanol remained completely inactive while both methionine and dimethylaminoethanol appeared to increase growth, maturation, and survival to a considerable extent although neither compound was as active as choline itself (Table III). On the other hand the roaches fed betaine grew at least as well and matured at the same rate as those receiving choline.

Choline Content of Roaches. The successful replacement of choline by betaine in the diet of the roach in spite of the high choline requirement of this species suggested a possible interconversion of these substances *in vivo*. Accordingly, newly-hatched nymphs, and roaches which had matured on diets containing varying levels of choline or betaine were analyzed for choline with the cholineless mutant of *Neurospora crassa* 34486. The insects were starved for 48 hours, anaesthetized with chloroform and chopped with scissors into a test tube containing 10 ml of 3% H_2SO_4, and the sample was treated as described by Horowitz and Beadle,[5] except that no attempt was made to remove methionine, since the amounts of this amino acid in the roaches were too small to interfere with the determination. The mold pads were filtered from the medium, washed with distilled water, rolled into pellets and dried on a porcelain plate for 6 hours at 90°C. before weighing. The standard curve covered the range 0-20 γ choline per flask. Essentially quantitative recoveries of choline were obtained in experiments in which choline and betaine were added separately or together to aliquots of freshly chopped insects, in agreement with the finding of Horowitz and Beadle that betaine is inactive for cholineless Neurospora.[5]

The newly-hatched nymphs were found to contain an average of 1080 γ choline/g live weight (Table IV) while adults maturing on diets containing 1000, 2000 and 4000 γ choline/g diet contained an average of 1620, 1730 and 2780 γ choline/g live weight respectively. Adults which had been fed diets containing 1100 and 4400 γ of betaine hydrochloride/g of diet instead of choline contained 1290 and 2020 γ of choline /g live weight respectively. Since the newly-hatched nymphs weighed an average of 2.26 mg each, they contained only 2.4 γ choline/insect. On the other hand the insects fed no choline but 1100 and 4400 γ of betaine/g diet weighed, on the average, 68.2 and 89.0 mg, and contained 88 and 180 γ choline/insect respectively. It is therefore evident that choline had been synthesized either by these insects or by the microorganisms associated with them. Roaches fed betaine at a molar

[5] Horowitz, N. H., and Beadle, G. W., *J. Biol. Chem.*, 1943, **150**, 325.

equivalent of 1000 γ choline/g diet contained about 80% as much choline as insects fed choline itself; at a level of betaine equivalent to 4000 γ choline/g diet, they contained 73% as much body choline as those fed choline itself. The roaches fed the higher level of betaine contained 2020 γ of choline/g live weight whereas those fed 2000 γ of choline/g of diet, the optimal amount for growth and maturation, contained only 1730 γ of choline /g of live weight (Table IV). Thus, amounts of choline above those actually "needed" had accumulated in the betaine-fed group.

Discussion. The relative inactivity of aminoethanol and dimethylaminoethanol in the absence of choline suggests that in the cockroach the methylation of aminoethanol and dimethylaminoethanol by dietary methionine† must be an inefficient process if, indeed, it occurs at all. This is in contrast to the finding that aminoethanol can be methylated by methionine in the rat,[6] and that dimethylaminoethanol plus methionine supports fair growth in the chick[7] in the absence of choline.

On the diet containing a minimal level of choline the feeding of methionine, dimethylaminoethanol, aminoethanol and betaine to roaches resulted in graded growth responses which were proportional to the methyl content of the supplements, with aminoethanol being completely inactive, methionine poor, dimethylaminoethanol fair, and betaine equal to choline. Apparently, therefore some of the functions of choline can be met by methionine and dimethylaminoethanol. In this respect, the cockroach may be similar to the chick in which choline has both an "essential" and a "replaceable" role.[4] The roach is different from the chick, however, in that betaine can completely replace choline in the diet of the roach whereas it meets only the "replaceable" needs for choline by the chick.[4]

In view of the efficient synthesis of body choline by cockroaches fed betaine, and the apparent difficulty of dietary aminoethanol and dimethylaminoethanol to be methylated by methionine, it is suggested that the mechanism of choline synthesis in the cockroach may be different from that proposed[8] for the rat, *viz.*, betaine→ glycine→ ethanolamine→ choline. Rather, a direct conversion of betaine to choline is suggested.

Summary. 1. The omission of choline from an otherwise adequate synthetic diet resulted in complete failure of roaches to grow and in death shortly thereafter. For optimal growth and maturation 2000-4000 γ of choline/g of diet were found to be needed.

2. The addition of dimethylaminoethanol or aminoethanol to diets lacking choline but containing 30% of casein resulted in little or no growth. The addition of methionine, dimethylaminoethanol and betaine as supplements to a diet low in choline resulted in an increased growth response in the order named. Aminoethanol was inactive.

3. Dietary betaine replaced choline quantitatively for growth and maturation at all levels of intake, and insects fed betaine contained nearly as much choline as those fed a corresponding amount of choline.

† Synthetic diet V contains 0.9% methionine as a constituent of casein (Table I).

6 duVigneaud, V., Chandler, J. P., Cohn, M., and Brown, G. B., *J. Biol. Chem.*, 1940, **134**, 787.

7 Jukes, T. H., and Oleson, J. J., *J. Biol. Chem.*, 1945, **157**, 419.

8 Stetten, DeWitt, Jr., *J. Biol. Chem.*, 1941, **140**, 143.