The most significant results were obtained with pantothenic acid regenerated from its barium salt (Table II). In the presence of killed yeast, flavin-purine complex, vitamin B_6, and glutathione, growth was very slight and no larvae reached the 4th instar. The further addition of the regenerated barium salt of pantothenic acid enabled growth to proceed to the adult stage. As is evident from Table II, a concentration of 6.7 mg. of barium salt per 100 ml., corresponding to about 4 mg. of pantothenic acid per 100 ml., gave the best growth. The value of $N \times \frac{1}{T}$ was 15.8 in one experiment and 21.4 in another, and almost all of the larvae reached the

TABLE II

The Effects of Pantothenic Acid and Vitamin B_6

Concentration mg. per 100 ml. culture fluid						$N \times \frac{1}{T}$	Adults from 6 larvae		Average days to reach adult stage
Flavin complex	Riboflavin	B_6	Regenerated barium pantothenate*	Glutathione	Nicotinic amide		♀	♂	
10	0	0	6.7 (4)	0	0	1.9	0	0	
10	0	1.3	6.7 (4)	0	0	13.8	1	2	12
10	0	0	6.7 (4)	13	0	0	0	0	
10	0	1.3	6.7 (4)	13	0	15.8	4	1	14.5
10	0	1.3	6.7 (4)	13	8	17.6	2	4	14.5
0	0.04	0	6.7 (4)	0	0	0	0	0	
0	0.04	1.3	6.7 (4)	0	0	11.2	1	0	22
10	0	1.3	0	13	0	0	0	0	
10	0	1.3	13.3 (8)	13	0	9.2	1	2	17.5
10	0	1.3	6.7 (4)	13	0	21.4	2	4	13.5
10	0	1.3	3.3 (2)	13	0	9.6	1	2	18

* Number in parentheses gives the approximate concentration of pantothenic acid.

adult stage in 13 to 14 days. If glutathione was omitted, growth was always not quite as rapid as in its presence and fewer of the larvae reached the adult stage. If B_6 was omitted growth was very slow and few or none of the larvae reached the 4th instar (Table II). If riboflavin was substituted for the flavin-purine complex, growth proceeded at a much slower rate (Table II). A few of the larvae nevertheless reached the adult stage in this medium in which the liver extract was replaced entirely by known substances.

The addition to a medium of killed yeast, flavin-purine complex, vitamin B_6, pantothenic acid, and glutathione, of various other substances such as yeast nucleic acid, β-alanine, adenosine, tryptophane-betaine, nicotinic amide, inosine, etc., had no effect except in the case of nicotinic amide which in some experiments slightly accelerated growth (Table II).

Vitamin B_6, which has been recently synthesized and shown to be 2-methyl-3-hydroxy-4,5-di-(hydroxymethyl) pyridine (16), was absolutely essential for growth (Table II). Whether a more or less nearly pure preparation of pantothenic acid was used, the optimal concentration of vitamin B_6 was 1.3 mg. per 100 ml. of medium.

DISCUSSION

The major accessory factors, essential for the growth of mosquito larvae and supplied by liver extract in a medium of killed yeast in liver extract, appear to be riboflavin, pantothenic acid, and vitamin B_6, with glutathione and nicotinic amide having a lesser growth-stimulating rôle. It may be that the killed yeast, which supplies enough thiamin, also supplies almost enough glutathione and nicotinic amide. The experimental results show clearly that other essential substances supplied by liver extract remain to be discovered. These are present in the barium filtrate and more especially in the flavin-purine complex. While killed yeast, vitamin B_6, pantothenic acid, and glutathione, with flavin-purine complex, gave excellent growth, the same materials with pure riboflavin gave considerably slower growth. The rôle of yeast nucleic acid is difficult to evaluate. Although it could be substituted for the barium precipitate fraction in the presence of the barium filtrate, it had no effect on the growth obtained in the presence of flavin complex or riboflavin plus vitamin B_6, pantothenic acid, and glutathione.

At present, one can safely conclude that *Aedes aegypti* larvae require, as accessory growth factors, thiamin, riboflavin, pantothenic acid, and vitamin B_6; probably glutathione and nicotinic amide, and certainly other as yet unknown substances present in yeast and in liver extract. Since pantothenic acid consists of β-alanine in amide linkage with a hydroxy acid (11), one may account for the observed slight favorable effects of β-alanine by assuming that some individual larvae have very limited powers of synthesizing pantothenic acid if they are supplied with β-alanine, as is the case with certain strains of bacteria (23, 24).

Older work on the growth factor requirements of insects has been previously noted (2). The flies *Lucilia sericata* (17) and *Drosophila melanogaster* (18) and the beetle *Dermestes vulpinus* (19) require cholesterol. Both *L. sericata* (20) and *D. melanogaster* (21) require thiamin, and the latter also requires riboflavin (21). Tatum (22) has recently found that *Drosophila* larvae required, in the presence of all the known vitamins, three additional factors present in yeast autolysate. Two of these could be separated from each other by precipitation with barium hydroxide in alcoholic solution,

a separation which suggests that they may be the same as the barium precipitate and filtrate fractions required by *Aedes aegypti* and that one of them may be pantothenic acid. Tatum (22) also showed that nicotinic acid is essential for the growth of *Drosophila* larvae.

As more work is done, it becomes increasingly obvious that insects require the same growth factors of the vitamin B complex group as do vertebrates. This is not surprising, since numerous species and strains of bacteria and yeast also require the same substances for growth (5, 23–26). Other species and strains of bacteria and other microorganisms can synthesize one or more of these factors (23, 24, 27). Microorganisms of this latter group under natural conditions supply mosquito larvae with their essential growth factors.

SUMMARY

The larvae of *Aedes aegypti* grew normally under sterile conditions in a medium consisting of killed yeast, flavin complex, or riboflavin, and two fractions derived from liver extract and designated as the barium filtrate and barium precipitate. The latter fraction could be replaced by yeast nucleic acid or by glutathione.

The larvae also grew at an almost optimal rate in a medium consisting of killed yeast, flavin-purine complex, vitamin B_6, pantothenic acid, and glutathione. All of these constituents except the glutathione were absolutely essential. Replacement of the flavin-purine complex by pure riboflavin resulted in slower growth, but nevertheless some larvae reached the adult stage.

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