SYNTHESIS OF AMINO ACIDS IN *AEDES AEGYPTI* L.[1]

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INTRODUCTION. Insect nutrition has received increasing attention in recent years (Trager, 1953; Lipke and Fraenkel, 1956). At the present time, the specific nutrient requirements are known for a number of insects (Albritton, 1954). Mosquitoes have been particularly interesting subjects for nutritional studies, since the larval and adult stages require different nutrients, and have entirely different feeding habits.

An aseptic method of rearing mosquitoes on a completely chemically-defined diet has permitted further advances in the study of amino acids in mosquito nutrition (Singh and Brown, 1957). Also, techniques such as paper chromatography have provided information concerning the free amino acids which naturally occur in mosquitoes (Micks and Ellis, 1951, 1952; Micks, 1956). Little is known, however, regarding the extent to which mosquitoes are capable of synthesizing the non-essential amino acids. It was of interest therefore to combine the two above-mentioned methods to obtain new information concerning this point. The results of this work are presented herein.

MATERIALS AND METHODS. *Aedes aegypti* larvae were raised aseptically on a chemical diet employed by Singh and Brown (1957). The amino acid mixture used in this diet was composed of glycine, alanine, valine, leucine, isoleucine, proline, hydroxyproline, phenylalanine, serine, tyrosine, histidine, arginine, tryptophane, threonine, methionine, cystine and lysine (Table 1). Each of the amino acids (alanine, glycine, serine, tyrosine, systine and proline) which had been found non-essential for *A. aegypti* larvae (Singh and Brown, 1957) was omitted singly from the diet. Two-dimensional chromatograms of fourth-stage larvae from this strain were compared with chromatograms of the regular laboratory-reared strain of *A. aegypti* to determine if those amino acids omitted from the diet were present on the chromatograms. Since the Rf values of all the non-essential amino acids had been previously determined from chromatograms of the regular strain, the appearance of any one of these when omitted from the chemical diet constituted evidence of synthesis.

Fourth-stage larvae were collected and placed in boiling distilled water for two minutes to coagulate the protein. The excess water was removed by touching the specimens to a piece of blotting paper. The larvae were mashed on chromatographic paper with a glass rod with a flattened end. A stream of hot air was

TABLE 1.—Composition of the mixture of amino acids employed in the chemically defined diet.

Amino Acid	Concentration g/1
Glycine	0.25
L-Alanine	1.00
L-Valine	1.20
L-Leucine	1.00
L-Isoleucine	1.12
L-Proline	0.75
L-Hydroxyproline	0.18
L-Phenylalanine	1.00
L-Serine	1.40
L-Tyrosine	0.50
L-Histidine (mono HCl)	0.25
L-Arginine (mono HCl)	0.39
L-Tryptophane	0.36
L-Threonine	0.75
L-Methionine	0.70
L-Cystine	0.13
L-Lysine (mono HCl)	0.66
Total	11.64

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used to dry the paper following the application of each specimen. Ten larvae were used for a single chromatogram. Two-dimensional chromatograms were prepared using Whatman No. 3 mm filter paper cut into 11″ x 11″ sheets. Cellophane tape was used to fasten the chromatograms into cylinders. Propanol-diethylamine-water (85:4:15) was used as a first solvent and butanol-acetic acid water (4:1:1) was employed as the second solvent. Developed chromatograms were dried by hanging them in a cabinet equipped with a small exhaust fan. They were then sprayed with 0.2 percent ninhydrin in water-saturated butanol using a nasal atomizer and dried again.

Colored spots representing free amino acids were cut from chromatograms of both strains of *A. aegypti*. These were then run through a Photovolt transmission densitometer for the purpose of obtaining density curves. The comparative densities of seventeen amino acids in the two strains were then calculated with a planimeter, and the values expressed as area in square centimeters.

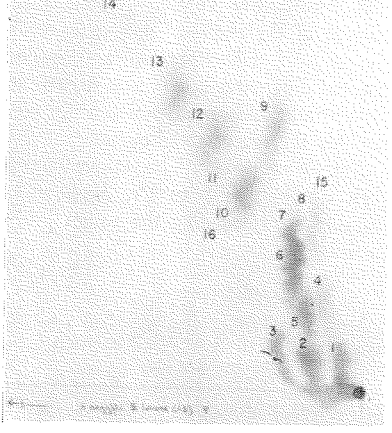

FIG. 1.—Two-dimensional chromatogram of *Aedes aegypti* fourth-stage larvae (diet strain). Propanol-diethylamine-water was used as the first solvent and butanol-acetic acid-water as the second. The numbered spots represent the following amino acids: 1, cystine; 2, arginine and aspartic acid; 3, glutamic acid; 4, lysine; 5, glutamine; 6, glycine; 7, serine; 8, histidine; 9, threonine; 10, alanine; 11, proline (not visible because of yellow color); 12, tyrosine; 13, methionine and valine; 14, leucine and isoleucine; 15, taurine (not visible); and 16, β-alanine (not visible).

RESULTS. Each of the non-essential amino acids, omitted singly from the amino acid mixture of the diet strain appeared on the chromatograms of the fourth stage larvae. Furthermore, aspartic acid, glutamic acid, β-alanine, taurine and glutamine, which were not included in the diet of this strain, were also present on the chromatograms (Figure 1). A comparison of chromatograms of the two strains also revealed striking differences in concentrations of certain amino acids (Table 2). The laboratory strain contained significantly larger amounts of several of the non-essential amino acids. The concentration of tyrosine, for example, was approximately twelve times higher in this strain, which also possessed about twice as much cystine, lysine, histidine, arginine + aspartic acid, glutamic

TABLE 2.—A quantitative comparison of free amino acids in fourth-stage larvae of two strains of *A. aegypti* [1]

Amino Acid	CD Strain [2]	Laboratory Strain
Proline	0.7	1.4
Alanine	6.4	11.2
Threonine	4.8	4.4
Glutamine	5.6	7.0
Isoleucine + Leucine [3]	2.6	2.9
Methionine + Valine [3]	2.6	3.0
Tyrosine	2.4	23.1
Cystine	7.5	15.0
Lysine	2.4	5.7
Histidine	5.3	10.8
Arginine + Aspartic acid [3]	7.4	16.8
Glutamic acid	4.2	9.1
Glycine	4.2	5.7
Serine	4.4	2.8

[1] Values given refer to area in cm^2.

[2] Aseptically reared on a chemically-defined diet.

[3] Amino acids represented by a combined value since the spots were not completely separated on the chromatograms.

acid, proline and alanine as the diet strain. On the other hand, the essential amino acids were present in comparable amounts in the two strains.

DISCUSSION. It is obvious from the results that *A. aegypti* fourth-stage larvae are able to synthesize most, if not all, of the non-essential amino acids. Their presence in larvae reared aseptically on the special chemical diet indicates that they are produced by some mechanism such as transamination or deamination during amino acid metabolism.

It is of particular interest that glutamic acid was not included in the special diet and yet appeared on all chromatograms. There is evidence from other insects that glutamic acid may be a product of transamination. Kilby and Neville (1956) and Barron and Tahmisian (1948) have demonstrated the formation of glutamic acid by transaminases in locust and cockroach (*Periplaneta americana*) tissues respectively. The presence of glutamate/alanine was reported by these investigators in both insects and Kilby and Neville also demonstrated glutamate/aspartate in the locust. It seems likely that mosquitoes may also synthesize alanine, aspartic acid and glutamic acid by means of such transaminases.

Other evidence of amino acid synthesis has been provided by Auclair (1949), working with cockroaches. He found that ingested alanine caused an increase in glutamine and glutamic acid, while an increase in glutamine, alanine and α-ketoglutaric acid raised the glutamic acid content. He also observed that asparagine fed to the insects increased the amounts of aspartic acid and glutamine in the hemolymph.

It can be seen from the results that although *A. aegypti* larvae are able to synthesize serine and glycine, they are not capable of synthesizing sufficient proline since they do not grow well on a proline-deficient diet. Likewise, cystine can be synthesized but not in sufficient quantity, because when it is omitted from the diet development continues only as far as the fourth instar and pupation does not occur. Tyrosine, although not required by *A. aegypti* larvae in the diet, evidently is converted from phenylalanine.

It is interesting that most of the non-essential amino acids are present in larger amounts in the laboratory strain. This difference between the two strains may also be reflected in the length of the life cycle which in the regular laboratory strain is approximately ten days, as compared with eighteen days in the diet strain. These findings suggest that the larvae grown on the chemical diet under aseptic conditions may not receive optimal quantities of these amino acids.

SUMMARY. *Aedes aegypti* larvae were reared on a completely chemically-defined diet under aseptic conditions. Non-essential amino acids (alanine, glycine, serine, tyrosine, cystine and proline) when omitted individually from the diet were present on two-dimensional chromatograms of larvae of this strain, indicating amino acid synthesis.

A quantitative chromatographic comparison of the chemical diet strain with the regular laboratory strain revealed marked differences between the two as to concentrations of certain amino acids, particularly the non-essential ones.

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References

ALBRITTON, E. C. 1954. Standard values in nutrition and metabolism. W. B. Saunders Company, Philadelphia and London.

AUCLAIR, J. L. 1949. Qualitative studies on the metabolism of amino acids in insects. Ph.D. Thesis, Cornell University, Ithaca, N. Y.

BARRON, E. S. G. and TAHMISIAN, T. N. 1948. The metabolism of cockroach muscle (*Periplaneta americana*). J. Cell. Comp. Physiol. 32:57–76.

KILBY, B. A. and NEVILLE, E. 1956. Amino acid metabolism in locust tissues. Biochem. Biophys. Acta. 19:389–390.

LIPKE, H. and FRAENKEL, G. 1956. Insect nutrition. Ann. Rev. Ent. 1:17–44.

MICKS, D. W. and ELLIS, J. P. 1951. Free amino acids in adult mosquitoes. Proc. Soc. Expt. Biol. & Med. 78:69–72.

MICKS, D. W. and ELLIS, J. P. 1952. Free

amino acids in the developmental stages of the mosquito. Proc. Soc. Expt. Biol. & Med. 79:191–193.

MICKS, D. W. 1956. Paper chromatography in insect taxonomy. Ann. Ent. Soc. Amer. 49:576–581.

SINGH, K. R. P. and BROWN, A. W. A. 1957. Nutritional requirements of *Aedes aegypti* L. J. Insect. Physiol. 1: In Press.

TRAGER, W. 1954. Insect nutrition—in insect physiology, edited by K. D. Roeder, pp. 350–386. John Wiley and Sons, Inc., New York, N. Y.