Scale 5, 10 mm Hg; and Scale 6, 6 mm Hg. The output of this amplifier is then fed into a meter type copper oxide rectifier coupled to a one milliampere direct current Esterline Angus recorder which is capable of giving a direct graphic record.

Remarks. This carrier system is markedly improved over those previously described in that it has a simplified A.C. source for the transducer, a phase sensitive balancing network and greater sensitivity. Operation is uncomplicated by instability and readings as low as 0.05 mm Hg can be recorded.

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Free Amino Acids in the Developmental Stages of the Mosquito.* (19318)

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The relatively few studies on the amino acids of insects have largely dealt with a single developmental stage, usually immature, and the hemolymph has been the principal body fluid subjected to analysis. Consequently, very little is known concerning the changes in amino acid levels that occur during proteolysis throughout the life cycle of an insect. Since the mosquito has a relatively short life span and exhibits very rapid growth, it was felt that this insect would afford both appropriate and interesting material for the study of the variation in free amino acids occurring during metamorphosis.

Materials and methods. The mosquito species employed were *Culex quinquefasciatus* and *Aedes aegypti* which were reared in the laboratory. Egg rafts, 4th-stage larvae, pupae, adult males and females were used. The larvae were fed a standard diet consisting entirely of finely powdered dog chow. The larvae and pupae were washed with several changes of distilled water and the excess removed with blotting paper prior to weighing. Unfed adult male and female mosquitoes were collected 2 to 3 days after emergence and lightly anesthetized with chloroform. Approximately 200 mg of each stage was weighed and homogenized in a tissue grinder with 1 ml of water. The material was centrifuged and the supernatant retained. Protein precipitation was carried out by adding one volume of 95% ethanol to about 5 ml of sample and centrifuging. The supernatant was treated with 3 volumes of chloroform (15 ml) with subsequent centrifugation at a low speed, and the aqueous fraction was removed for amino acid determinations.

The paper chromatographic method(1) was employed for determinations of free amino acids in the mosquito extracts. Whatman filter paper No. 4 was used with water-saturated phenol as the first solvent and 2,4-lutidine (3 parts to 1 part water) as the second. Standard amounts of extracts were delivered to the filter paper for the one-dimensional chromatograms and represented 12.5 mg or approximately 6 mosquitoes, while 40 mg was used for the 2-dimensional runs. The amino acids were revealed by spraying the filter paper with a solution of 0.2% ninhydrin in water-saturated butanol and heating it for approximately 15 minutes at 80°C.

Results. Free amino acids found in the eggs, larvae, pupae and adult mosquitoes are

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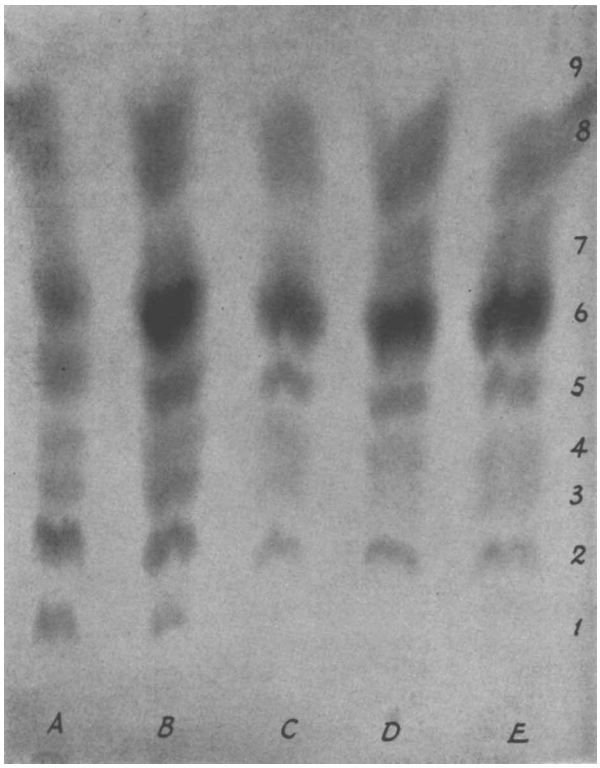

FIG. 1. Chromatogram of extracts of *C. quinquefasciatus*. A, eggs; B, larvae; C, pupae; D, adult females; E, adult males. (1, aspartic acid; 2, glutamic acid; 3, taurine; 4, serine and glycine; 5, threonine and lysine; 6, tyrosine, alanine and B-alanine; 7, valine and arginine; 8, tryptophan, leucines, methionine and histidine; 9, proline).

as follows: alanine, glutamic acid, β-alanine, isoleucine, leucine, taurine, proline, histidine, serine, valine, methionine, threonine, lysine, arginine, aspartic acid, glycine, tyrosine, and tryptophan. The relative concentrations of amino acids in the various stages of *C. quinquefasciatus* are indicated in Fig. 1 by the different intensities of the spots. The larvae appear to have the highest concentration of amino acids followed closely by the adult mosquitoes. The amino acid concentration in the extract of pupae is the lowest of all; however, 2-dimensional chromatograms revealed that this stage has considerably more alanine and tyrosine than any of the others. The eggs contained relatively small quantities of alanine and large amounts of aspartic and glutamic acids. Furthermore, aspartic acid appeared in chromatograms of the eggs and larvae, but not in those of the pupae and adults at the level of concentration used. Likewise, glutamic acid is present in considerably larger amounts in the extracts of the eggs and larvae. A comparison of the 4th-stage larvae, pupae and adults of *A. aegypti* (Fig. 2) in which the pupae were only a few hours short of maturity shows that there is a marked decrease in the intensity of all the amino acid spots in extracts of the latter with the exception of glutamic acid which occurs in rather large quantities. The adult male and female mosquitoes of both species presented essentially the same chromatographic picture.

Discussion. The level of free amino acids in the various developmental stages of the mosquito seems to be rather variable depending in part on the proximity to molting. In preparation for this process, it may be that amino acids of the pupae, at least, are utilized more rapidly than they can be replaced, perhaps due to a lowered metabolic rate in this non-feeding stage. Shortly after metamorphosis, however, the free amino acids seem to be restored rather rapidly to their original

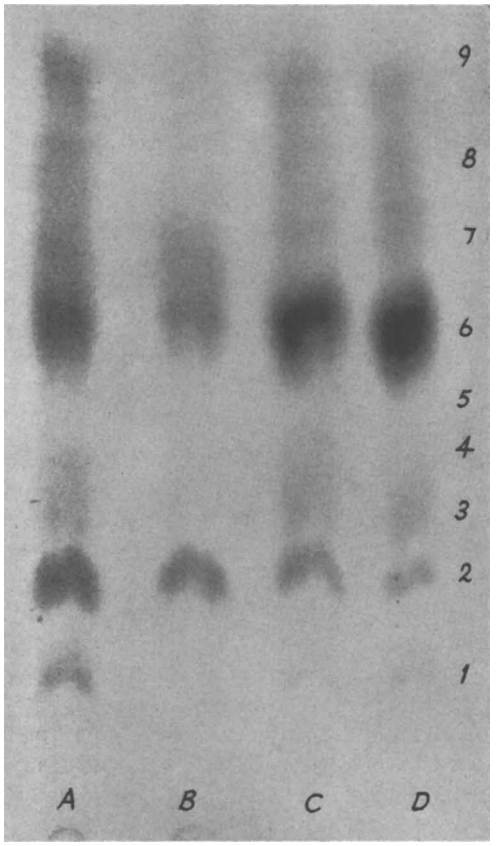

FIG. 2. Chromatograms of extracts of *A. aegypti*. A, larvae; B, pupae; C, adult females; D, adult males. (1-9 as above).

level. It is interesting to note that tyrosine is present in larger amounts in the pupae inasmuch as this amino acid is known to play an important role in the darkening or tanning of the insect integument.

The present observations are partly in accord with those of Agrell(2) who has found that although the amino acid concentration in *Calliphora erythrocephala* is comparatively constant throughout pupal development, an appreciable drop occurs at 60 to 80% of the pupal time. Maluf(3), however, states that the amino acids of the blood of pupae are highest and that this is probably due to proteolysis occurring during histolysis. The significance of his conclusions, however, is open to question since they are apparently based upon determinations of only three species of larvae and four species of pupae belonging to a single order.

Golberg and De Meillon(4) have established that the amino acids essential for mosquito larvae (*A. aegypti*) are glycine, leucine, isoleucine, histidine, arginine, lysine, tryptophan, threonine, phenylalanine and methionine. The only one of these which was not detected in the larvae used in the present study is phenylalanine, however, these authors note that the larvae seem equally capable of utilizing either phenylalanine or tyrosine. Phenylalanine was also not found in the body fluids of adult mosquitoes(1). Although tryptophan was not revealed by paper chromatographic methods in either the larvae or the pupae, its presence was ascertained by the method of Shaw and McFarlane(5).

Summary. (1) The free amino acids of mosquito eggs, larvae (4th-stage), pupae and adults (males and females) were extracted with ethanol and identified by paper chromatography. The following amino acids were found in the various stages studied: alanine, glutamic acid, β-alanine, isoleucine, leucine, taurine, proline, histidine, serine, valine, methionine, threonine, lysine, arginine, aspartic acid, glycine, tyrosine, and tryptophan. (2) Extracts of pupae contained lower concentrations of most amino acids than the other stages, with the greatest drop occurring shortly before emergence of the adults. Other quantitative variations from one stage to another involved only 2 or 3 amino acids.

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