EFFECTS OF GROWTH OF *AEDES AEGYPTI* L. LARVAE ON A CHEMICALLY DEFINED MEDIUM *

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INTRODUCTION. Normal development of an insect on a synthetic diet depends not only upon the presence of all the required chemicals but also upon the ability of the insect to utilize them. Since many insects live in the food on which they feed, their normal growth and development is affected both by the dietary constituents *per se,* and the physical environment created by their presence. Furthermore, insects in an aseptic medium are constantly adding metabolites, which in nature might be utilized by other organisms present in the environment of the insect. These metabolic products of insect excretion may produce an imbalance in the physical and chemical nature of the chemically-defined diet with subsequent effects on insect growth and development.

Although considerable care is taken in nutritional work to adjust the pH and other factors of the diet, little attention has been given to the physical environment which the diet provides. Recently, the importance of osmotic pressure in the growth of *A. aegypti* in a chemically-defined diet has been demonstrated (Singh, 1957; Singh and Brown, 1957). Likewise, the role of surface tension in the development of mosquitoes has been emphasized (Singh and Micks, 1957).

In order to elucidate some of the changes which occur in a chemically-defined diet due to the presence of the developing insect, the effects of growth of *Aedes aegypti* larvae on pH, viscosity, surface tension, osmotic pressure and the amino acid level of the diet were investigated.

MATERIALS AND METHODS. *Aedes aegypti* larvae were grown on a chemically-defined medium developed by Singh and Brown (1957). On this diet, *A. aegypti* larvae generally complete the first instar on the fourth day, the second instar on the seventh day, the third instar on the tenth day and pupation occurs on the fourteenth day. Each tube of rearing medium contained 50 mg. of the solid fraction of the diet and 6 ml. of the liquid fraction. Three sets of tubes containing the chemically-defined diet were inoculated with 1, 2, and 4 first instar larvae respectively, while a fourth set, containing no larvae, was kept as a control. All experiments were performed in duplicate. Observations on pH, viscosity, surface tension, osmotic pressure, and amino acid levels of the medium were made on the first, fourth, seventh, tenth and fourteenth days.

Viscosity was measured with an Ostwald viscosimeter in minutes, as the difference between double distilled water and the diet. The pH was recorded with a Beckman glass electrode pH meter. The surface tension readings were taken with a Harkins drop weight apparatus (Harkins and Brown, 1919; Harkins and Harkins, 1929). The osmotic pressure was obtained using the Beckman cryoscopic method and is recorded as freezing point depression. Amino acid levels were measured chromatographically. Chromatograms were made by spotting 0.01 ml. of the medium on 3 mm. Whatman filter paper and were run, developed and read according to the methods previously described (Micks and Gibson, 1957). Total amino acid levels are represented as density curves derived from 0.01 ml. of material and recorded as area per cm.^{2} with a K & E, compensating polar planimeter.

In order to obtain a more accurate comparison, all values were corrected against the values of double distilled water on any

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particular day and against the average mean of the first day's values in each set of experiments.

RESULTS. The growth and development of *A. aegypti* larvae in the defined diet produced definite physical and chemical changes in it. Although there was no appreciable change in the pH throughout the experiment, the viscosity was altered (Figure 1). Tubes with one and two larvae (75.5 dynes/cm.) was higher than the others at four days, and yet was the lowest at 10 days, dropping to approximately 68 dynes/cm. The medium containing one larva was lower than the controls throughout this period of time. There was a subsequent rise in the surface tension of all tubes to the fourteenth day.

The osmotic pressure showed a continuous rise in all tubes during the period

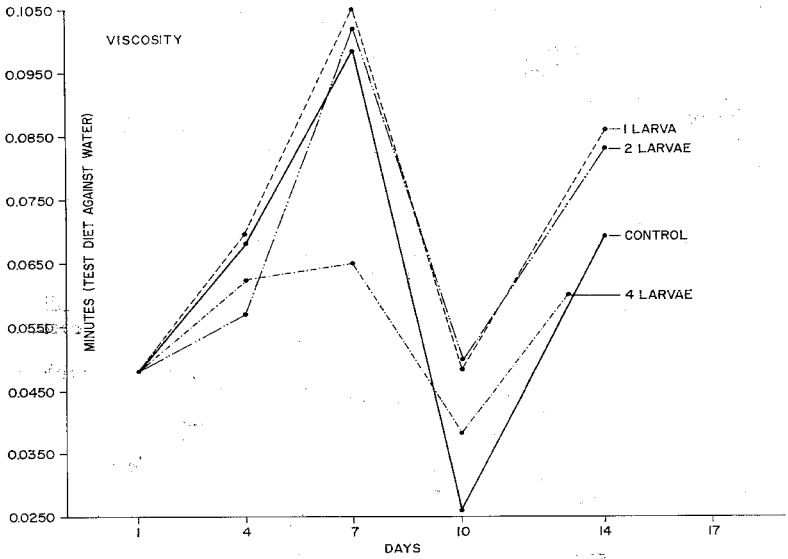

FIG. 1.—A comparison of the viscosity of media containing 1, 2 and 4 larvae with the control.

larvae largely followed the controls until the tenth day at which time the viscosity of the media with the larvae dropped to approximately 0.0500 min. while that of the controls fell to about 0.0250 min. From this point on, the viscosity remained higher in these test groups. On the other hand, it remained lower in the tubes containing four specimens, never exceeding 0.0650 min. at any recorded interval.

The effects of *A. aegypti* larvae on surface tension were essentially the reverse of their effects on viscosity (Figure 2). The surface tension of the diet containing four

of the experiment except between the fourth and seventh days when there was a slight drop in osmotic pressure in media with both two and four specimens (Figure 3). The effect of four larvae per tube on the osmotic pressure of the medium was very evident. Following the marked drop on the seventh day to $\triangle 0.330^\circ$ C., there was a subsequent rise to a maximum of $\triangle 0.390^\circ$ C., as compared with $\triangle 0.490^\circ$ C. in the controls. In general, the osmotic pressure increased with a decrease in the number of specimens.

Amino acid levels increased steadily in

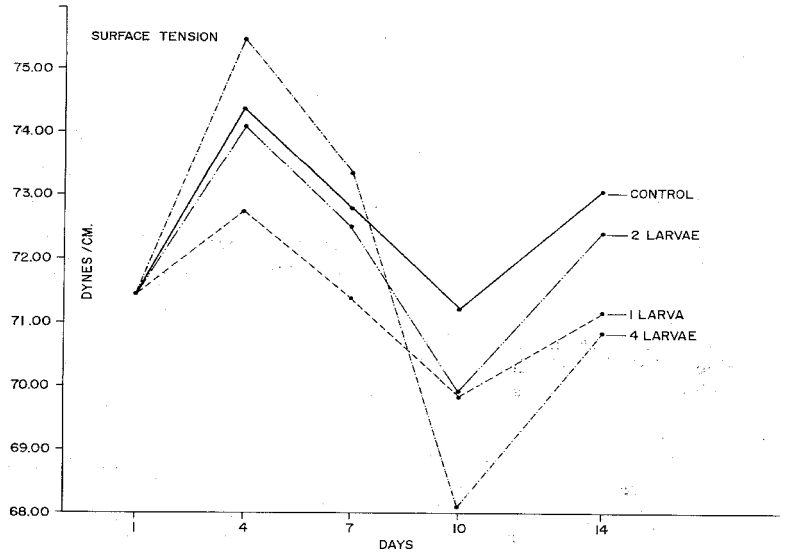

FIG. 2.—A comparison of the surface tension recorded for all test and control media.

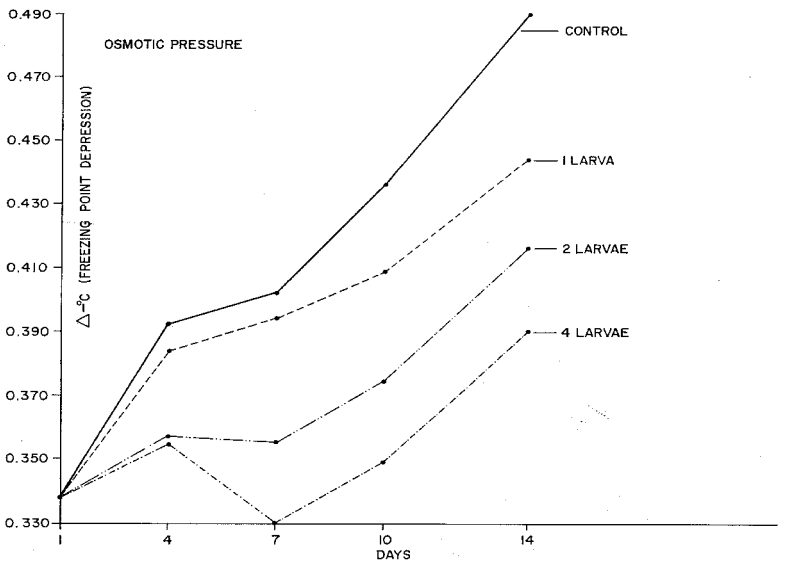

FIG. 3.—Changes in osmotic pressure of the test and control media during a 14-day observation period.

all sets of experiments until the seventh day when the media with four larvae and the controls began to drop (Figure 4). In tubes with one and two specimens, the amino acid levels continued to rise until the tenth day and then decreased. The density curves obtained from media with four larvae per tube were similar to those of the controls except for slightly higher values in the test medium on the seventh and tenth days. Figure 5 shows that the free amino acid content of the media containing a single larva is appreciably higher than that of the controls. This increase occurs in those areas represented by lysine, glycine, serine, histidine, alanine, threonine, methionine valine, leucine and isoleucine.

DISCUSSION. It is apparent that the larval medium is not a static phase in which the insect lives and completes its development with merely an accompanying reduction in the various essential nutrients. On the contrary, this environment undergoes dynamic changes resulting from the interactions between it and the growing larvae. The changes in the control media are difficult to explain but may be due in part to evaporation and certain chemical reactions. The long period required for development of the larvae in the diet may indicate that various essential components become less and less available as growth proceeds.

The fact that media containing the greatest number of larvae (4) exhibited the lowest viscosity is probably due to their greater utilization of the available food. Furthermore, even though the surface tension varied considerably from one experimental group to another, it remained within the normal limits in which most mosquito species develop (Singh and Micks, 1957). The steady increase in osmotic pressure during larval development is probably the most important limiting physical factor. It has been demonstrated by Singh (1957) and Singh and

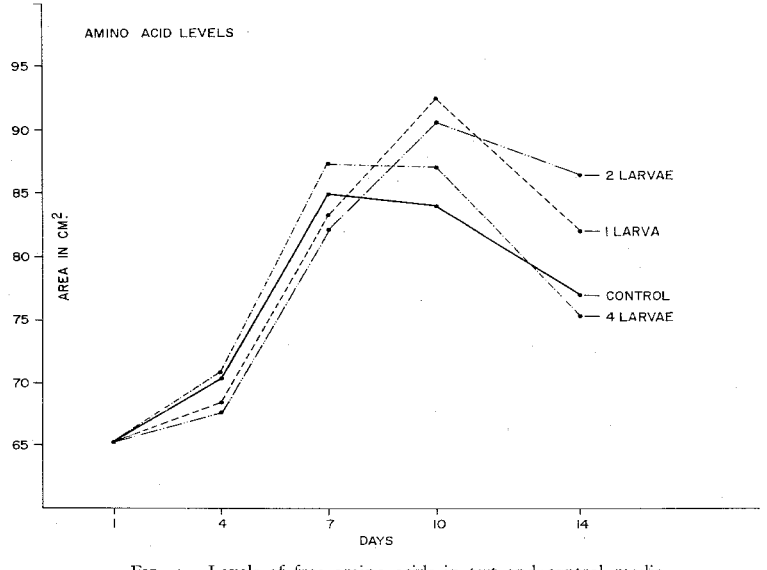

FIG. 4.—Levels of free amino acids in test and control media.

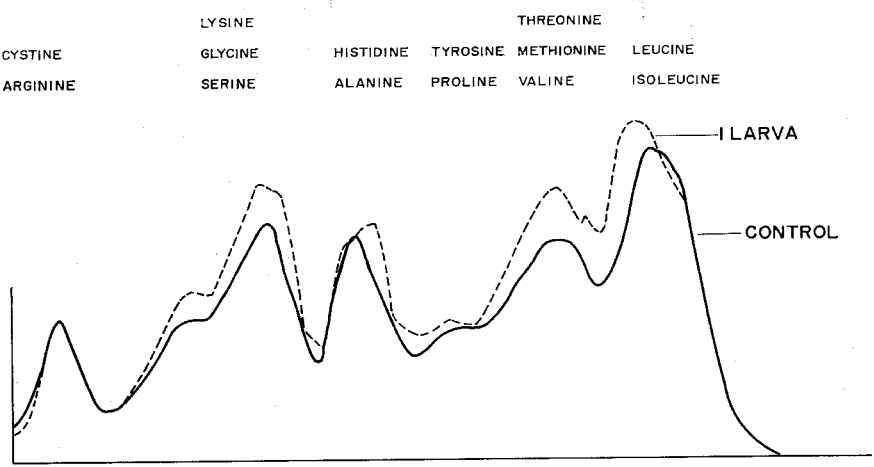

FIG. 5.—Density curves obtained from one-dimensional chromatograms of rearing medium which contained one specimen and the control medium, on the tenth day of the experiment.

Brown (1957) that larvae can survive and utilize dissolved amino acids provided the osmotic pressure of the diet does not exceed $\triangle 0.40°$ C. This observation would seem to indicate that the increase in free amino acids in the media containing only one or two larvae was due in part to the high osmotic pressure. Under these conditions the larvae could not effectively utilize the amino acids. These results provide support for our general observations that larvae show better growth when there are several of them in the medium.

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