A PRELIMINARY NOTE ON SOME NUTRITIONAL REQUIREMENTS FOR REPRODUCTION IN FEMALE *AEDES AEGYPTI*[1]

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In a previous paper, Lea *et al.* (1955) reported that a high level of egg production resulted if *Aedes aegypti* females were fed either citrated blood or skimmed milk from a cotton pad. It was necessary to mix honey with the blood or milk as an attractant to induce feeding, and the most satisfactory ratio of honey to blood or milk was found to be 1:9. Best results were obtained if the mixture was heated to about 100° F before being absorbed on a cotton pad for feeding.

Because of the excellent results obtained when milk was substituted for the blood-meal, the writers attempted to find other proteinaceous materials which would stimulate oviposition in *Aedes*. It was subsequently found that a number of proteins, both plant and animal, when mixed with honey and presented to the mosquitoes on cotton pads, would meet the nutritional requirements for egg production. Among the materials which proved to be successful were human serum albumin, human gamma-globulin, hemoglobin, egg albumin, pepsin, lipase, trypsin, proteose-peptone, and the enzymatic hydrolysates of lactalbumin, casein, soybean meal, and yeast. These results are fully reported by Lea *et al.* (in press). It might also be mentioned here, that skimmed milk, egg albumin, and proteose-peptone were fed to *Anopheles quadrimaculatus*, and eggs were obtained in all three cases. No further work with this species has been done, however.

The success with the various proteins, and particularly with the protein hydrolysates, led to the hope that oviposition might occur if the mosquitoes were fed a mixture of amino acids, and that it might thus be possible to determine the basic nutritional requirements for reproduction in *Aedes* females.

The importance of the amino acid isoleucine had already been established in studies by the writers. A mixture of 5 g. of beef hemoglobin (Difco) made up to 100 ml. with 10 percent honey water, when fed on a cotton pad daily to a group of 200 female *Aedes*, resulted in the deposition of 16 eggs in 21 days. However, a group of 200 mosquitoes fed the same mixture but with 1.5 g. of DL-isoleucine added, laid over 6,000 eggs in the same period.

These results confirmed the work of Greenberg (1951) who fed *Aedes aegypti* a number of protein materials through a membrane, but could only induce the insects to feed if washed erythrocytes were present in the food preparation. While egg production with a suspension of sheep erythrocytes alone was low, the addition of several proteins raised considerably the number of eggs produced. In testing amino acids, he found that isoleucine, when added to the erythrocyte suspension, also increased egg production, however, the addition of a mixture of 9 other amino acids caused no increase.

It is significant to note that Block and Bolling (1951) have reported that both beef and sheep hemoglobins contain relatively low levels of isoleucine as compared to other hemoglobins and proteins in general. Otherwise hemoglobin appears to contain the normal complement of amino acids.

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It may be suggested from these results that the addition of proteins containing isoleucine or of isoleucine itself to the sheep erythrocytes or beef hemoglobin corrected a deficiency in this amino acid, and that only a medium containing more than a certain minimum level of isoleucine would result in a high level of egg production from *Aedes aegypti*. Orten, *et al.* (1945) have shown that purified beef or human globin fails to support growth in the rat unless isoleucine is added.

The importance of the amino acid tryptophane was also demonstrated in further tests. While an enzymatic hydrolysate of casein (Difco Casitone) produced a relatively large number of eggs, the acid hydrolysate of casein (Difco Casamino Acids, Technical) produced none. Since acid hydrolysis destroys tryptophane, tests were run with the acid digest fortified with this amino acid and abundant oviposition resulted.

TABLE 1

Amino acid composition in grams per 100 ml. of several experimental media. The total egg production from 500 females after nine days is given for each medium.

Amino acids	Medium number					
	1	2	3	4	5	6
L-Arginine	0.50	0.50	0.50	2.50	5.00	.....
L-Alanine	0.60	0.55	....	.....	.....	.....
L-Aspartic acid	1.00	0.60	....	.....	.....	.....
L-Cystine	0.20	0.04	0.20	.....	.....	.....
L-Glutamic acid	1.00	2.33	1.00	.....	.....	.....
Glycine anhydride	0.50	0.05	0.50	.....	.....	4.50
L-Histidine mono HCl	0.70	0.21	0.70	0.20	.....	.....
DL-Isoleucine	1.00	0.63	1.00	0.50	.....	.....
DL-Leucine	2.00	1.00	2.00	0.50	.....	.....
L-Lysine mono HCl	0.90	0.90	0.90	0.50	.....	.....
L-Methionine	0.20	0.34	0.20	1.50	.....	.....
DL-Phenylalanine	0.70	0.50	0.70	0.80	.....	.....
L-Proline	0.50	0.50	....	.....	.....	4.50
DL-Serine	0.20	0.77	....	.....	.....	.....
DL-Threonine	0.80	0.38	0.80	0.50	.....	.....
L-Tryptophane	0.40	0.12	0.40	1.00	1.00	1.00
L-Tyrosine	0.40	0.67	....	.....	.....	.....
DL-Valine	1.00	0.65	1.00	2.00	5.00	.....
Total wt.	12.60	10.74	9.90	10.00	11.00	10.00
Total eggs	2,600	1,600	2,000	29	0	0

In looking over the amino acid composition of several proteins which had successfully produced eggs, the writers noted that 18 acids were present in most of the proteins in fairly large amounts. A solution of these amino acids was made in 10 percent honey water, and this formulation was fed daily on cotton pads to a group of 500 newly emerged female mosquitoes. Several trials were made with the same amino acids but with variations in their concentrations. To date, the most productive mixtures of 18 acids are Media 1 and 2 as given in table 1. Medium 1 contains the amino acids in the proportions which are reported to be present in blood; Medium 2 was prepared on the basis of the amino acid content of casein.

Tests with the above mixtures and similar groups of 18 amino acids have indicated that a combination of levulose and dextrose (5 g. of each in 100 ml. of medium) can be substituted for honey as an attractant, thus eliminating the possibility that the honey may contribute some necessary factors to the mosquitoes.

It also appears that the addition of 0.15 g. per 100 ml. of medium of a commercially available salt mixture to the amino acid mixtures results in a moderate increase in egg production.[2]

Thus far, the effect of adding cholesterol, nucleic acid, and vitamins, and the value of adjusting the pH of the amino acid solution is undetermined.

In addition to the groups of 18 amino acids, several trials have been made with smaller mixtures. A group of 13 amino acids was tried and found to produce significant numbers of eggs (table 1, Medium 3).

From a group of 10 amino acids (table 1, Medium 4) was obtained only a very few eggs, and the significance of these results must remain in doubt until they have been studied further.

Several groups of 3 or 4 amino acids were also tested (table 1, Media 5 and 6). These mixtures contained isoleucine or tryptophane, which had already been shown to be indispensible, or both of these materials, together with enough of the highly soluble amino acids, proline and glycine, to give an approximate total of 10 g. These mixtures were all unproductive.

Work is continuing in attempts to evaluate the importance of each of the amino acids and the roles played by other nutritional factors such as vitamins and minerals. It is hoped that these results will be significant additions to the knowledge of mosquito physiology.

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[2]Salt Mixture W, Nutritional Biochemicals Corporation, Cleveland, Ohio.