A COMPARISON OF FOUR DIFFERENT ADULT DIETS FOR THE ROUTINE LABORATORY REARING OF *AEDES AEGYPTI* (L.)[1]

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INTRODUCTION. The method for maintaining egg production in laboratory colonies of the yellow fever mosquito usually consists of making available to the females, at various intervals, some species of live host. While this method is quite successful, the fact remains that expense and time is involved in maintaining stock colonies of the host.

Attempting to avoid use of the live host, Lea *et al.* (1955) and Knierim *et al.* (1955) began investigations of other proteinaceous materials, supplied on gauze covered cotton pads. The first method employed whole citrated beef blood, and later, mixtures of nine parts citrated beef blood and one part honey. Prior to this, MacGregor and Lee (1929) and McLintock (1952) had used the blood soaked pad method. These investigators added either sugar or honey to the citrated blood.

Since 1955, the stock mosquitoes in this laboratory have been maintained quite successfully on a diet of citrated, hemolyzed beef blood, containing 10 percent sucrose. The method is simply to visit a slaughterhouse with one-gallon jugs in which have been placed approximately 250 ml. of 5 percent sodium citrate. Have these jugs filled with beef blood, and later, at the laboratory, add 10 percent sucrose, and place the blood in conveniently sized containers in the deep freeze. The treated blood is then thawed and used as necessary. The addition of sucrose or honey or other sugars is necessary as a phagostimulant if the blood is frozen because freezing ruptures the red corpuscles. Without whole red cells the blood is not fed upon unless it is sweetened.

Subsequently, Lea *et al.*, (1955, 1956) found that the mosquitoes would produce viable eggs if fed on milk-soaked pads, as well as on a variety of other proteinaceous materials such as powdered egg albumen, proteose-peptone, and the enzymatic digests of soybean meal, yeast, casein, and lactalbumen. Dimond *et al.*, (1955, 1956) showed that the mosquito would produce viable eggs on a synthetic diet composed of 12 amino acids, inorganic salts, and sugars.

Some of these diets would be more convenient to use, both for stock colonies as well as for particular investigational problems, provided that adequate viable egg production results, and that the offspring of subsequent generations are adequate egg producers and are normal in other respects such as longevity. Often the question has been asked "just how good are these diets?"

This study concerns the practical laboratory efficiency of three of the most promising non live host diets when compared with the use of a live host.

The four diets tested were: live host (laboratory mouse); citrated, hemolyzed beef blood containing 10 percent sucrose; a liquid mixture of the 12 essential amino acids, inorganic salts, and 10 percent sugars (Dimond *et al.* (1956); and a 10 percent egg albumen (Nutritional Biochemicals Corp.) plus 10 percent sucrose solution.

Items of particular considerations were: (1) number of days beyond the initial meal to the appearance of first eggs; (2) growth rate of the larvae resulting from eggs laid by adults receiving the various diets; (3) total and total viable egg produc-

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tion; and (4) longevity of the adults and longevity and productivity of the offspring of adults fed the different diets.

MATERIALS AND METHODS. Stock eggs less than two months old, stored at 26–27° C. and 50–60 percent relative humidity were hatched. The larvae were fed Gaines Homogenized Dog Meal, and reared according to the method of Burcham *et al.*, (1956). Upon emergence of 90 percent of the pupae, the mosquitoes were inactivated in the cold room, whereupon 50 males and 50 females were placed in each experimental cage (12" by 8" by 9"). Two replications were used in each case. Ten percent sucrose solutions were placed in the cages to provide food for the young adults.

Two days after the experimental cages were set up, all received the initial feeding. All continued to be fed every other day until egg production ceased and, in some cases, until all of the insects were dead. All diets, other than the live host, were presented by pouring the solution, warmed to about 50° C., onto a gauze-covered cotton pad, and the pad was then placed in a small aluminum cup in the cage. The mice were placed securely in small screen cages, placed in the cages with the mosquitoes, and left for two hours. All live host feeding was done in the afternoon.

New cups containing oviposition strips were added daily or every other day, aged for four days, counted, and in some instances, hatched.

Representative groups of eggs collected from the first generation were then hatched in definite groups according to the days when the eggs were laid. In other words, eggs were hatched for second generation studies from those laid in (A) the first five days of the egg laying period, (B) from 6–10 days, (C) 11–15 days, and (D) 16–20 days. In addition, viability data were recorded for the rest of the egg-laying period, but not enough viable eggs were present from the two non-blood diets to make up an equivalent test. This method was used in order to determine whether failure of a diet might occur at some point during the egg-laying period. A similar arrangement, but on a somewhat reduced scale, was carried out with the third and fourth generations.

RESULTS AND DISCUSSION. The number of days following the initial meal to appearance of first eggs differed, and this difference remained fairly constant throughout the four generations. The average number of days were: live host, 2.2; hemolyzed blood, 3.5; synthetic, 4.5; and egg albumen, 4.5. These differences were about as expected since sugars must be added to all three non-live host diets as a phagostimulant. Sweetened materials go first to the ventral diverticulum instead of to the midgut, as whole blood does, and are apparently pumped to the midgut as the sugars are utilized. Therefore, it is likely that the necessary proteins would not reach the midgut as rapidly or in as great quantity. Also, some extra time may be necessary for additional syntheses with use of the two non-blood diets. In addition, the time tended to increase about one day in adults reared from eggs laid late (after two weeks) in the egg-laying period of the preceding generation, if the diet was synthetic or egg albumen.

Growth rate of the larvae during all parts of the second, third and fourth generations indicated little change. The overall averages in days for four groups of the second generation, plus an early group of the third and fourth generations were: live host, 10.3; hemolyzed blood, 10.4; synthetic, 10.6; and egg albumen, 10.8. More important, the times did not tend to increase or decrease in the generations subsequent to the first generation.

A marked larval mortality (50–60 percent) was recorded when using eggs laid late in the egg-laying period of the preceding generation with the two non-blood diets. This occurred in eggs laid after three weeks of egg-laying.

Egg productivity varied enormously depending upon the diet. Lea *et al.* (1956) found the same. Therefore, the purpose of this portion of the work was to evaluate (1) viable egg production in 40 days, and

(2) the longevity and productivity of the offspring resulting from eggs laid at various intervals during the egg-laying period of the preceding generation.

The results of one of the tests where complete data were recorded for total and total viable egg production are shown in Fig. 1. Forty days of egg-laying was chosen because egg production was practically completed by that time, with the exception of the live host fed insects. Also, it is uncommon to keep a stock cage operating beyond this point.

For the 40 days the overall percentages of viable eggs from the four diets were as follows: live host, 97.0; hemolyzed blood, 94.5; synthetic, 81.5; and egg albumen, 60.6.

It may be noted from Fig. 1, that the percentages or numbers of viable eggs

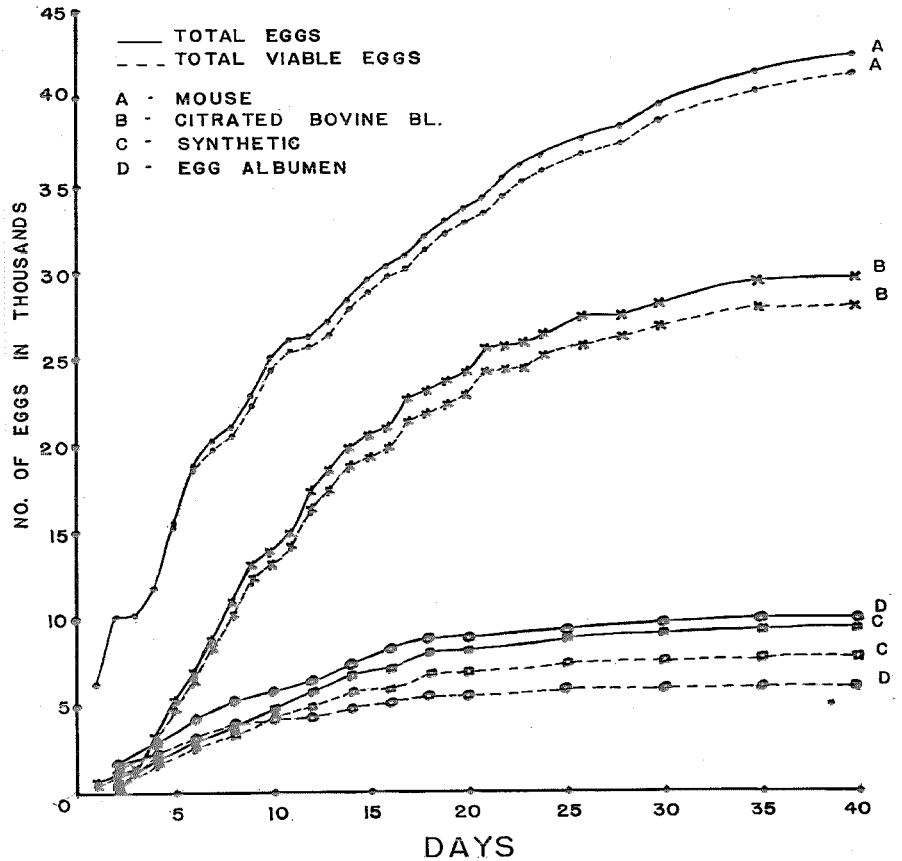

FIG. 1.—Comparison of total and total viable egg production in 40 days using four different adult diets. Results are for 100 females with each diet.

from the insect fed on egg albumen were very low after the first week of egg laying. The same was true of the synthetic diet beyond two weeks of egg deposition. The two blood diets produced a very high percentage of viable eggs throughout the 40 days. In all cases, the eggs were subjected to hatching within a day or two after being aged, so the percentages should be maximum.

The data recorded in Fig. 1 were representative of what occurred relative to egg deposition throughout the four generations. The one important difference, again, was with the synthetic and albumen fed insects. If eggs laid two weeks beyond commencement of the egg laying period were hatched, the resulting adults were only about half as productive as normal, and longevity was reduced by approximately one-third.

Adult female longevity averaged about the same on the three non-live host diets, but about 12 percent greater with the live host diet. This was true through all four generations. The "average days lived" figures for the females whose egg depositions are given in Fig. 1, were 46 days for the live host fed, and about 41 days for the other three groups.

CONCLUSIONS. In the general laboratory rearing of our strain of *Aedes aegypti*, feeding on a live mouse definitely resulted in a greater total deposition of viable eggs than with the other diets tested. In addition, the females lived somewhat longer. With the hemolyzed blood, almost as high percentages of the eggs were viable as with the live host. This was not true of the other diets.

Production of viable eggs was very low beyond two weeks of egg deposition with the synthetic diet and one week with the egg albumen. On the other hand, the numbers of eggs produced in a stock colony are almost always in extreme excess of those utilized. The hemolyzed blood diet has served us extremely well for the past five years. Although the time of larval development and the number of days beyond initial feeding to deposition of first egg was slightly greater using the non live host diets, this would rarely be of consequence.

In all probability all of the diets tested would be applicable for specific investigational purposes. One such use for the hemolyzed blood, in addition to general stock rearing, has been in the feeding of single pairs of mosquitoes in small cages for genetics research. If one is using a number of single pair cages, the use of many live hosts is a disadvantage. In addition, it has been our experience that single females often do not feed well. Using the combined sugar and blood source conveniently insures feeding and egg deposition in virtually all cases.

Finally, we must point out that these conclusions were based on work with the Ohio State strain of mosquitoes. We have used the non live host diets with other strains, but not always with comparable results.

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