THE EFFECTS OF BLOOD SOURCE AND QUANTITY ON PRODUCTION OF EGGS BY *CULEX SALINARIUS* COQUILLETT (DIPTERA:CULICIDAE)[1]

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Abstract. A minimum amount of blood was necessary to initiate the development of eggs in *Culex salinarius* Coquillett, and egg production increased steadily in proportion to the amount of blood ingested until a maximum number of eggs developed; thereafter additional ingestion of blood did not effect production of eggs. Also, the source of the blood meal had an important effect on the magnitude of production but did not alter the relationship that existed between the quantity of blood ingested and production; thus 1 mg. of blood from a chicken resulted in 123 eggs, from a guinea pig in 62.7 eggs, from a human in 54.1 eggs, and from a turtle in 122.4 eggs. As body weight and body size (wing length) increased the number of öocytes also increased but did not significantly affect the number of eggs produced as a result of a blood meal on a chicken, a guinea pig, a human or a turtle; therefore size is an unimportant factor compared with quantity and source of blood.

Introduction. The role of blood in the production of eggs by mosquitoes has been investigated for almost 50 years. Although some 30 odd species have now been reported to be autogenous (Chapman, 1962; Smith and Brust, 1970; and Grimstead *et al.*, 1969), most are known to require a blood meal prior to the production of eggs. However, the influence of the amount of blood ingested, the effect of body weight, and the effect of blood from different sources is not well understood.

Data in a study by Roy (1936) indicated that the number of eggs developed in *Aedes aegypti* (L.) increased with the weight of blood ingested and that a small amount of blood produced a small number of eggs; however, the fecundity of *A. aegypti* was not dependent on its size. Barlow (1955) had opposite results with *Aedes hexodontus* Dyar: the fecundity (actual number of eggs laid) was dependent on the weight of the mosquito rather than on the weight of the blood consumed. He therefore concluded that fecundity was influenced by the weight of the female as long as a certain amount of blood was ingested. Subsequently, Colless and Chellapah (1960) working with *A. aegypti* and Ikeshoji (WHO/Vector Control/135.65, FRU/Report No. 11) working with *Culex pipiens fatigans* Wiedemann, reported that both body weight and blood intake affected the production of eggs. As a result, Ikeshoji (op. cit.) and Barlow (1955) both concluded that a larger mosquito had a tendency to ingest more blood and could therefore produce a larger number of eggs, and Ikeshoji suggested that the increase in egg production was caused by the larger number of öocytes found in larger mosquitoes.

Also, Woke (1937a) and Mathis, as reported by Woke (1937a), both working with *A. aegypti* and Halcrow (1956) working with *Anopheles gambiae* Giles, studied the effect of blood source on egg production. Woke (1937a) found that the blood of man produced fewer eggs than the blood of other hosts, but Mathis and Halcrow reported the reverse. Later, Woke *et al.* (1956) were unable to establish a statistically significant correlation between body weight and number of eggs but suggested that a gradient existed between these two variables.

In addition, Roubaud and Mezger, as reported by Woke (1937a), and Ikeshoji (WHO/Vector Control/133.65, FRU/Report No. 9) fed species of *Culex* blood

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from different sources and found that the qualitative effect of blood had a significant effect on the production of eggs. However, Roy (1931) concluded that the number of eggs laid by *Anopheles stephensi* Liston was not affected by the source of blood except that blood of man produced fewer eggs.

A study was therefore made in 1969–1970 at Gulf Coast Marsh and Rice Field Mosquito Investigations Laboratory at Lake Charles, Louisiana to clarify the influence of the various factors on the production of eggs by *Culex salinarius* Coquillett.

Materials and Methods. The mosquitoes used in the study were taken from a colony originating from larvae collected in southern Louisiana in 1966 and maintained in the laboratory since that time by the methods of Chapman and Barr (1969). Those used for the tests were reared in the usual manner and maintained in an insectary at a temperature of 27±3° C. and 70 to 80 percent relative humidity. After the adults emerged, the mosquitoes were provided with water and raisins for 5 days. Then 6 hours before they were to be weighed, the water and raisins were removed, and the mosquitoes were moved to the weighing room where the relative humidity was lower. Each mosquito was removed from the cage, placed in an individual shell vial, anesthetized with carbon dioxide (CO_2), weighed to an accuracy of 0.1 mg, and returned to a vial.

When a guinea pig, chicken, or man was used as a blood source the vial containing the mosquito was held against the host. The amount of blood ingested was controlled by interrupting the feeding process at various intervals. Then the mosquitoes were anesthetized again, weighed, and returned to the vials. The weight of the ingested blood was calculated from the difference in the weight of the mosquito pre- and post-ingestion.

Due to the reluctancy of *C. salinarius* to feed on the turtle and the difficulty of holding the vial containing the mosquito against the turtle so a blood meal could be obtained, several mosquitoes of the same weight were placed together in a cage (12 x 8 x 8 inches) with the turtle. After 12 hours, the blooded mosquitoes were removed, anesthetized, weighed individually and replaced in vials. This method did not permit an accurate estimate of the amount of blood ingested because of the delay from engorgement to weighing (time lapse could have been as much as 12 hours), so mosquitoes that had been fed on a chicken and been weighed immediately were weighed 12 hours later, and a correlation coefficient was calculated. Once a regression line was plotted from these data, it could be used to estimate the amount of blood ingested from the turtle. Some degree of error was still inherent due to possibility of different rates of digestion and elimination.

When the mosquitoes had ingested the blood and the amount had been determined, they were held individually in shell vials for 7 to 10 days to allow development of eggs. Each mosquito was then killed, and the fully formed eggs were dissected and stored for future counting.

The possible relationship between body size and fecundity was investigated by weighing 7- to 10-day-old mosquitoes and measuring the wing from the proximal end of the auxiliary cell to the distal margin of the wing; then the ovaries were dissected, and the öocytes were counted.

Correlation coefficients were calculated for the relationship between the following: (1) quantity of blood ingested and production of eggs (for that segment of the graph, Fig. 1, between the points marked with an asterisk), (2) body weight and production of eggs, (3) body weight and the number of öocytes, and (4) wing length and the number of öocytes.

Results. The results are based on observations of 136 mosquitoes that ingested various amounts of blood from four sources. Thirty other mosquitoes were used in the determination of the number of öocytes in relation to body weight and wing length.

BLOOD QUANTITY AND EGG PRODUCTION. Although four sources of blood were used, the statistical analyses were designed to show the effect of amounts of blood rather than source of blood on the production of eggs. Thus, the comparisons between sources are actually comparisons between tests and not the sources of the blood.

The effect of blood on the production of eggs is summarized in Table 1. The contrast between the first and second test was the most dramatic: less blood was ingested from the chicken than from the human, but 68.7 more eggs were produced with chicken blood. The correlation coefficients for all tests with the chicken, guinea pig, and human were significant at the 0.001 level, which indicated that as the amount of blood ingested increased, the number of eggs also increased, regardless of the source of blood. The results with the turtle were not as significant, but the correlation coefficient was close to the 95 percent (0.05) level. The slight decrease in the probability is most likely explained by the method used to determine the amount of the ingested blood. If it had been possible to conduct all four tests under identical conditions, the correlation coefficient for test 4 would probably not have differed appreciably from those for the other three.

BODY WEIGHT AND EGG PRODUCTION. When body weight was considered in relation to egg production, the weights used were the weights of the mosquitoes before feeding. Again, the comparisons between sources of blood are comparisons between tests. The results are shown in Table 2. The mosquitoes that fed on the turtle had an average body weight 0.2 mg. less than the average for mosquitoes that fed on the chicken, but they produced almost 29 percent more eggs. Correlation coefficients were determined from individual body weights before feeding and the number of eggs produced. The negative correlation coefficients for test 2 (human) and test 3 (guinea pig) indicated that as the body weight increased, the egg production decreased; the positive correlation coefficients for the first test (chicken) and the last test (turtle) indicated that as body weight increased, the egg production also increased. However, a single factor analysis of variance calculated for the body weight of mosquitoes used in the four tests was not significant at the 0.1 level, which indicated that the observed differences between body weights were not significant.

Since the weight of blood ingested was not a factor in the comparison of body weight to egg production, the differences in the methods used in the fourth test (turtle) and the other three, should not be important. Also, since the statistical analyses were designed to determine only the effect of body weight on the production of eggs, the quantity and source of the blood ingested should again be of no importance. However, these two variables do seem to have been responsible for the erratic correlation coefficients that were obtained.

RELATION OF BODY WEIGHT AND WING LENGTH TO ÖOCYTE NUMBER. Table 3 shows that as the body weight increased, the number of öocytes increased (values were determined by constructing a linear graph of body weight and wing length to öocyte number). For each increase in body weight, there was a progressively

TABLE 1.—Amount of blood ingested and production of eggs by *C. salinarius* fed blood from four sources.

Blood source	Number mosquitoes used	Mean weight of blood ingested (mg.)	Mean number eggs laid	Level of significance
Chicken	42	0.9	108.0	<0.001
Human	30	1.1	39.3	$<.001$
Guinea pig	39	1.4	93.4	$<.001$
Turtle	25	1.7	138.8	$<.1; >.05$

Table 2.—Body weight and production of eggs by *C. salinarius* fed on blood from four sources.

Blood source	Number mosquitoes used	Mean weight of unfed mosquitoes (mg.)	Mean number eggs laid	Correlation coefficient	Level of significance
Chicken	42	2.6	108.0	0.466	<0.01; >0.001
Human	30	2.5	39.3	—.014	> .1
Guinea pig	39	1.9	93.4	—.039	> .1
Turtle	25	2.4	138.8	.348	< .1; > .05

larger increase in the number of öocytes: an increase in body weight from 1.6 mg. to 1.9 mg. resulted in an increase of only one öocyte; and increase from 3.7 mg. to 4.0 mg. resulted in an increase of 13 öocytes. Therefore, as body weight increased, the number of öocytes also increased, but the actual increase in numbers of öocytes was small. For example, as body weight increased 150 percent (from 1.6 mg. to 4.0 mg) the number of öocytes increased less than 29 percent (from 224 to 288). Although the correlation between body weight and wing length was significant at the 0.001 level, the relationship between wing length and öocyte number was more meaningful: as the wing length increased 17 percent, the number of öocytes increased 29 percent.

Blood Source and Egg Production. Figure 1 shows the effect of blood source on egg production. A minimum amount of blood (0.2 mg.) was required to initiate egg production when *C. salinarius* fed on the chicken, and this amount produced a minimum of 8.2 eggs. Then the egg production increased in proportion to the amount of blood ingested to 1.8 mg. after which an increase in amount of blood ingested had little effect (Figure 1). A blood meal of 1.0 mg. from the chicken produced 123.3 eggs.

Table 3.—Body weight and wing length and the calculated number of öocytes present in the ovaries of *C. salinarius.*

Body weight (mg.)	Number of öocytes	Wing length (units)[a]
1.6	224	36.8
1.9	225	36.9
2.2	229	37.3
2.5	235	37.9
2.8	243	38.9
3.1	252	39.7
3.4	263	40.8
3.7	275	42.0
4.0	288	43.2

[a] 1 unit = 0.082 mm.

When the mosquitoes were fed on the guinea pig, the minimum amount of blood (0.4 mg.) produced 6.0 eggs; thus, 0.2 mg. more blood produced 2.2 fewer eggs than the minimum from the chicken. Also, the maximum amount of blood that had any significant effect on egg production was 2.9 mg., which was 1.1 mg. more than the maximum amount that affected egg production when the mosquitoes fed on the chicken. A mean number of 62.7 eggs was produced per 1.0 mg. of blood from the guinea pig.

When the mosquitoes were fed on the human, 0.4 mg. of blood was the minimum needed for production of eggs (13.4). Egg production ceased to increase after the ingestion of 1.2 mg. of blood from the human, which was 0.6 mg. less than the maximum for the chicken and 1.7 mg. less than the maximum for the guinea pig. A maximum of 55.8 eggs were produced after feeding on human blood compared with 173.6 after feeding on chicken blood and 158.7 on guinea pig blood. Thus, about 211 and 184 percent more eggs were produced when the mosquitoes fed on the chicken and guinea pig, respectively, than when they fed on the human, and 1 mg. of human blood produced 54.1 eggs compared with 123.3 for chicken blood and 62.7 for guinea pig blood. Then, on a milligram of blood, chicken blood produced 127.9 percent more eggs and guinea pig blood 15.9 percent more eggs than human blood.

The amount of blood ingested from

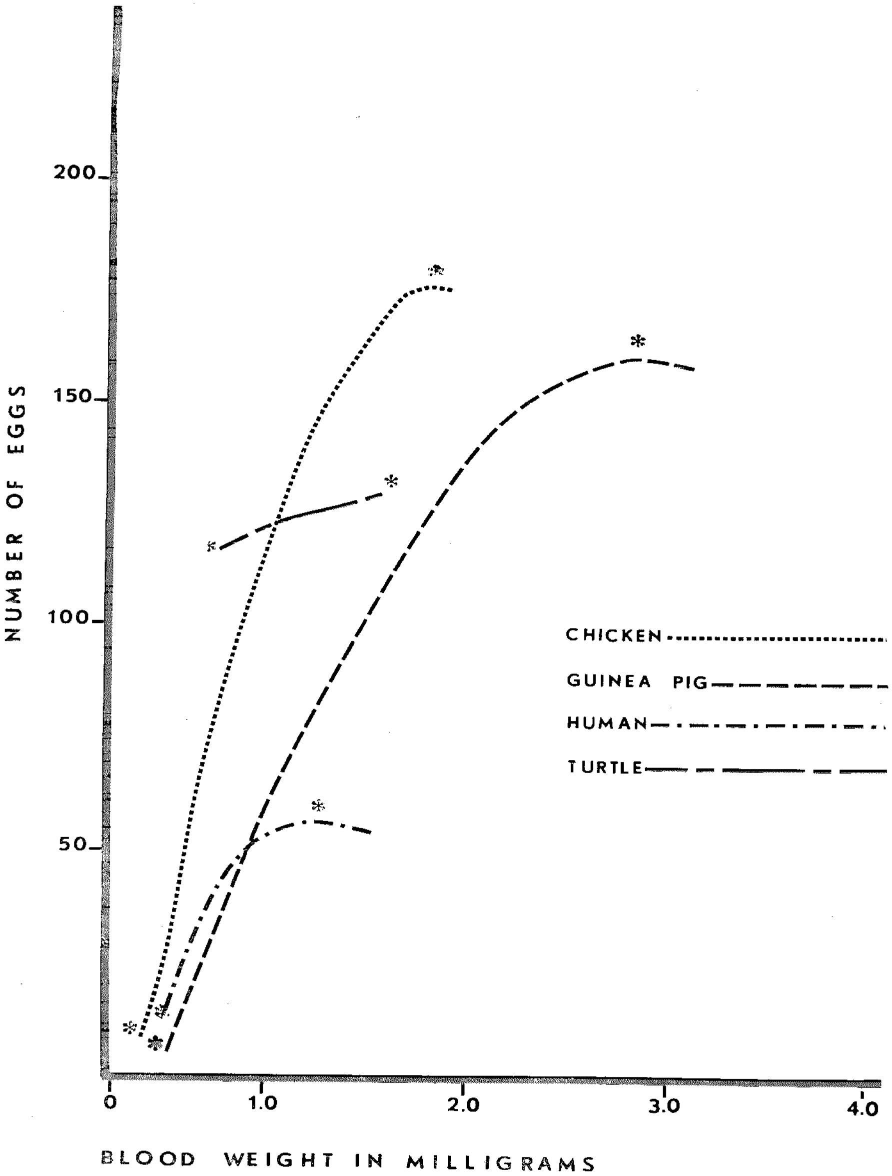

FIG. 1.—Amount of blood ingested and the number of eggs produced by *C. salinarius* fed blood from four sources.

the turtle could not be controlled, so the minimum amount necessary for egg production was not determined. However, the data in Figure 1 indicate a sharp decrease in the rate of egg production after 1.1 mg. of blood were ingested, and 1.0 mg. of blood produced 122.4 eggs. This value was much higher than comparable values for guinea pig and human blood but differed little from those for chicken blood.

Discussion. Body weight and body size (wing length) were related to fecundity in *C. salinarius* because as they increased, the number of öocytes also increased; however, the increase in the number of öocytes was small compared with the increase in body weight.

The importance of blood source to egg production was indicated by the following: 1 mg. of blood from the chicken produced 123.3 eggs, from the guinea pig 62.7 eggs, from the human 54.1 eggs, and from the turtle 122.4 eggs. Woke (1937b) reported that *Culex pipiens* L. produced 39.7 eggs per 1.0 mg. of blood when fed on a human and 82.2 when fed on a canary. He also found that when *A. aegypti* ingested 1.0 mg. of blood from man, guinea pig, turtle, and canary, the number of eggs produced, respectively, was 34.0, 55.3, 50.2, and 48.8 (Woke 1937a). Thus, in both of Woke's studies, the number of eggs produced per milligram of blood ingested and the differences in the numbers of eggs produced due to source were considerably less than those observed from *C. salinarius* in the present study.

Body weight did not have a significant effect on egg production in three of the four tests though the correlation coefficient for the fourth test (chicken) was significant at the 0.01 level. However, such a correlation coefficient does not necessarily express a valid relationship since quantity of blood and source were ignored. Also, the effect of quantity and source overshadowed the small increase in number of öocytes in the larger mosquitoes.

Barlow (1955) stated that the fecundity (egg production) of *A. hexodontus* collected in the field "seemed to have been correlated with both weight of female and weight of blood meal." However, he also found that the weight of blood ingested decreased with the date of capture though neither the weight of the mosquitoes nor the fecundity decreased during the period. He therefore concluded that "fecundity was dependent on the weight of the female rather than on the weight of blood consumed."

Ikeshoji (WHO/Vector Control/135.65, FRU/Report No. 11) reported that body weight had a significant effect on egg production when *C. p. fatigans* were fed on a chicken, but when he tested the quantitive effects of blood from different sources on the egg production (WHO/Vector Control/135.65, FRU/Report No. 9), he failed to report the effect of body weight on egg production.

If the results of the present research with *C. salinarius* had been based on the results obtained with only the chicken as the blood source they would have agreed with those reported by Barlow (1955) and by Ikeshoji (WHO/Vector Control/135.65, FRU/Report No. 11). However, the correlation observed with this blood source was obviously invalid when the mosquitoes were fed blood from other sources.

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THE RESIDUAL TOXICITY OF SIX INSECTICIDES TO MOSQUITOES IN WINDOW TRAP HUTS AT MAGUGU, TANZANIA, AND AT TAVETA, KENYA

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INTRODUCTION

Since 1960 the Tropical Pesticides Research Institute has been one of the centres carrying out Stage IV experimental hut screening of new compounds against wild mosquitoes as part of the World Health Organization's Evaluation Scheme for Insecticides. The progress of this scheme, which was started primarily to evaluate alternative insecticides for use against resistant insect vectors, has recently been reviewed (Wright, 1971).

This paper reports the screening of three new organophosphate insecticides: OMS–1170 (also known as phoxim or Bayer 77488), OMS–1197 (also known as chlorphoxim or Bayer 78182), and OMS–1211 (also known as Iodofenphos or Ciba C9491); and three new carbamates: OMS–708 (also known as Mobam or MCA600), OMS–1028 (also known as Bayer 38799), and OMS–1202 (also known as U.C. 8454). Results of a trial of lindane are also included as a standard. The work was carried out during four seasons between March 1967 and July 1970.

STUDY AREAS. The trials were carried out at two outstations of the Tropical Pesticides Research Institute at Magugu, Tanzania, and Taveta, Kenya. The Taveta area has been described by Smith and Draper (1959) and the Magugu area by Smith (1964a). Both stations are in the East African Savannah Zone, and both have an annual rainfall of 600–700 mm, falling mainly in two seasons, from late October to early December, and mid-March to mid-May.

Anopheles gambiae species A and B

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