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THE EFFECT OF PHOSPHORUS^{32} ON THE FECUNDITY OF *AEDES AEGYPTI* (L.) AND ITS USE IN DETERMINING BLOOD MEAL VOLUMES

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INTRODUCTION. The measurement of quantity of blood ingested by blood-feeding insects has been the subject of study for some time, most of the studies employing a gravimetric technique. Christophers (1960), in his monograph on *Aedes aegypti,* has summarized many of the problems associated with such studies, concluding that fluid loss from the mosquito during and immediately following engorgement was a major source of error. Boorman (1960) used isotope-labelled blood to avoid some of the problems associated with gravimetric methods, particularly those concerned with the time at which mosquitoes were weighed following feeding.

The availability of the isotope phosphorus^{32} in this laboratory enabled us to apply Boorman's technique (with modifications) on simuliids (Bennett 1963) and to study *Aedes aegypti* in a similar manner. The work reported herein is a comparison of the results obtained by a gravimetric method and by use of isotope-labelled blood. In the course of these studies, casual observation suggested that mosquitoes fed on isotope-labelled blood laid fewer eggs than expected. This aspect was further studied and the results are reported herein.

MATERIALS AND METHODS. The mosquitoes used in this study were obtained from a colony of *Aedes aegypti* maintained in this laboratory. Mosquitoes used in any one experiment were taken from one rearing cage only, and were 5 to 6 days of age when used. Following feeding, engorged mosquitoes were maintained in small cages and provided with a sugar and water diet. The mosquitoes laid their eggs on pieces of wet filter paper placed in small crystallizing dishes. The host animals used throughout were domestic ducks and chicks obtained commercially.

Phosphorus^{32}, in the form of phosphoric acid ($H_3P^{32}O_4$), was obtained from the

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Atomic Energy Commission of Canada. The administration of the isotope to young ducklings and the radiation levels in the mosquitoes, their eggs, and in the blood of the ducks, were determined by the techniques and equipment described previously (Bennett, 1963). The viability of the eggs was determined by keeping all eggs for 4 days from time of laying and then counting the number of larvae hatching from known numbers of eggs placed in water for 24 hours.

RESULTS. The weight of 100, 5-day old, unfed, mated mosquitoes from the same cage was determined by killing and weighing them on a "Grammatic" balance, accurate to 0.001 mg.; however, the figures were rounded to the second decimal. The unfed mosquitoes averaged 1.23 mgs. in weight. Other mosquitoes, from the same rearing and of the same age, were allowed to feed to repletion on domestic chicks. As the mosquitoes left the chicks, they were immediately killed and weighed in groups of five to ten. The time interval from the end of feeding until each group was weighed varied from 10 to 15 minutes. One hundred engorged mosquitoes averaged 2.85 mgs. each. The weight of a cubic millimetre of chick blood was determined by weighing nine samples of blood (140–500 mm^3) in a closed vessel. These volumes of blood ranged from a weight of 206.4 mgs. for a sample of 200 mm^3 to 503.6 mgs. for a sample of 500 mm^3; the averaged results for the nine samples was 1.03 mgs./mm^3 of blood. Using this figure, it was calculated that each mosquito ingested 1.6 mm^3 of blood or 1.36 times its weight. This figure is low compared to that of Jeffrey (1956) but is within the range reported by Christophers (1960) and is almost the same as that reported by Colless *et al.* (1960). If a correction factor of 1 mg. per mosquito is added to account for fluid loss (as indicated by Christophers (1960) and Boorman (1960)), the average meal ingested by these mosquitoes would be approximately 2.60 mm^3, a figure in keeping with that reported by Boorman.

Boorman (1960), using blood cells labelled with Ce^{144}, determined that the average meal of *A. aegypti* was 4.21 (4.0–4.5) mm^3, a figure approximately two and one half times larger than that obtained by our uncorrected gravimetric method. When the opportunity arose, the isotope P^{32} was used in this laboratory to repeat Boorman's technique. The technique employed herein differed from Boorman's in the use of P^{32} rather than Ce^{144} and in the use of a duck instead of a mouse. In addition, the P^{32} was administered 3–10 days prior to the feeding of the mosquitoes, while Boorman administered the isotope approximately 10 minutes before the feeding of the mosquitoes.

Boorman suggested two possible sources of error in this technique. (1) The fluid excreted while the mosquitoes fed might contain isotope. (2) Some of the relatively soft beta emissions might be absorbed by the integument of the insect and not be recorded. The influence of these potential sources of error was ascertained in preliminary experiments.

(1) Fecal drops from mosquitoes feeding on isotope-labelled ducks were tested for the presence of isotope, but none was detected. In addition, samples of blood from labelled ducks were centrifuged and the radiation levels in the cell and serum fractions determined. In six different samples, 90 percent of the radiation was in the cell fraction. Therefore, any loss of isotope in the fecal drops of the mosquito (assuming these drops to represent the serum fraction) would only amount to 10 percent of the total isotope. But no isotope was detected in the fecal drops of the feeding mosquito. Possibly the excreted fluid is not derived from the recently ingested blood meal but is already present in the digestive tract. The incoming blood may merely expel the fluid already present. Otherwise, the mosquito has an unusually rapid and efficient method of extracting fluid from the meal.

(2) Possible absorption of the beta emissions by the integument of the mosquito was tested by measuring the radiation from each of 20 mosquitoes recently fed

on a labelled duck. The blood-filled stomachs were then dissected free from each of these 20 mosquitoes and the radiation levels of the mosquitoes and their stomachs were again determined. The average radiation from the nondissected mosquitoes was 1194 counts/100 seconds while the average for the same mosquitoes plus their stomachs that were dissected free was 1180 counts/100 seconds; the difference was not considered to be significant.

Four groups of mosquitoes were fed on ducks containing different levels of isotope. The calculated volumes (Table 1) hours following their P^{32}-labelled meal. Subsequent radiation levels were determined by direct counts on samples from the remainder of the group. Only the first blood meal contained isotope, all other meals were from non-labelled ducks. In subsequent meals, both labelled and non-labelled mosquitoes fed on the same host. Eggs laid by these mosquitoes were found to be radioactive throughout the course of the experiments, the radiation levels progressively decreasing at each egg laying by about 50 percent.

The results are presented in Table 2; in the column under P^{32}-labelled mosqui-

TABLE 1.—Volume of blood ingested by five-day old *A. aegypti* as determined by use of duck blood labelled with P^{32}.

Experiment	Level of P^{32} in blood counts/100 secs. mm.^{3}	No. of mosq.	P^{32} level in mosquitoes counts/100 secs./mosq. Average	Range	Average meal mm.^{3}
1	99	4	190	133–272	1.92(1.34–2.75)
2	296	25	662	493–1110	2.34(0.99–3.76)
3	213	15	842	366–1109	3.91(1.87–5.15)
4	1407	25	4060	2678–10033	3.19(1.89–7.25)
Weighted average for the four experiments					2.97

ingested by the individuals within a group varied widely, as did the average meal ingested by each of the four groups. The average meal ingested by all four groups was 2.97 mm^3, a figure considerably higher than that obtained by the uncorrected gravimetric method. It is, however, considerably smaller than obtained by Boorman (1960) viz., 4.21 mm^3.

EFFECT OF P^{32}-LABELLED BLOOD ON FECUNDITY. Terzian and Stahler (1958), using gamma irradiation from a cobalt source, obtained partial or complete inhibition of egg-laying in *Aedes aegypti*. Observations on the P^{32}-labelled mosquitoes mentioned previously indicate a lower egg production than normal and further studies were made to determine the effect of beta irradiation on the fecundity of these mosquitoes.

The initial radiation levels in the mosquitoes were determined by direct counts made on a sample from the group 1 to 2 toes, the numbers of mosquitoes used to determine the radiation level are given separately from the numbers laying eggs, thus the figures in the two columns do not agree. The results indicate that fewer eggs were laid by the labelled mosquitoes. The greatest difference between the control and labelled mosquitoes is seen in the number of eggs laid immediately following the ingestion of the labelled blood. The inhibitory effect persisted through the subsequent feedings on unlabelled blood; in one experiment, through four such feedings (Table 2).

The viability of the eggs laid by the isotope-labelled mosquitoes was compared to that of the eggs laid by the control mosquitoes. On the basis of six lots from each group, 75–85 percent of the control eggs hatched, and 74–84 percent of the isotope-labelled eggs hatched. Obviously, the presence of the isotope did not affect the viability of the eggs. Presumably the

TABLE 2.—Comparison of the number of eggs produced by P^{32}-labelled and non-labelled *Aedes aegypti*. Figures in parentheses indicate number of mosquitoes involved. (Labelled and non-labelled mosquitoes fed on the same duck at each feeding.)

Days from initial feeding	P^{32} labelled mosquitoes				Non-labelled mosquitoes	
	Average P^{32} level counts/100 seconds		Average number of eggs		Average number of eggs	
0	1900	(15)	..	..	..	..
5	955	(5)	15	(15)	..	..
0	847	(15)	..	..	..	..
4	415	(10)	15.6	(30)	62	(62)
8	194	(10)	24.5	(20)	78	(60)
0	4235	(20)	..	..	..	..
4	1826	(15)	24	(147)	62	(62)
8	1080	(10)	37	(120)	78	(60)
0	668	(20)	..	..	..	..
4	290	(15)	12	(65)	25	(46)
8	190	(15)	50	(45)	63	(37)
15	113	(8)	48	(18)	68	(21)
20	62	(7)	15	(7)	50	(15)

spermatozoa in the seminal receptacles were not affected by the P^{32} at the levels present in the mosquito. Larvae from the radioactive eggs were not reared to test the possibility that further stages in the life cycle were affected by the isotope.

The lowered egg production by mosquitoes with an initial radiation level of 40 counts/second/mosquito was substantially the same as that shown by mosquitoes with an initial radiation level of 6 counts/second/mosquito. Minimum levels of radiation to produce inhibition of egg laying were not determined. Mortality among both labelled and non-labelled mosquitoes over the test period was about 7 percent, indicating that radiation at these levels did not kill the mosquitoes.

DISCUSSION. The variability of the results reported by other workers as well as those obtained in this study raises the question as to whether an average size of blood meal will be obtained for this species, as factors such as the strain of *A. aegypti* used and the conditions of larval rearing (Colless, 1960) markedly influence the size of the adults. Clearly, however, fluid loss during and immediately after feeding can add considerable error to any gravimetric determination of the size of the blood meal. The use of isotope labelled blood, with its potential for total radiation count (regardless of fluid elimination) reduces the variability introduced by the gravimetric methods.

The data (Table 2) indicate that the mosquitoes labelled with phosphorus^{32} lay fewer eggs than the controls. The greatest differential between the two groups occurs after the ingestion of the labelled blood meal. At this time, the radiation level is the greatest. However, other factors may also influence the lowered egg production at this time. The blood of labelled ducks was not comparable to that of the controls. The packed red cell volume of the labelled ducks was one-half to one-third that of the non-labelled birds. In addition, the blood of the labelled ducks failed to clot. Other differences undoubtedly occurred. The lower number of red cells indicates that the labelled blood did not have as much nutrient material as the non-labelled blood and this alone could lead to a lowered egg production. However, the continued inhibition of egg production when labelled mosquitoes were fed on non-labelled blood indicates that the presence of the isotope has some degree of permanent inhibition on egg laying, regardless of nutrient quality of the meal. Somewhat surprisingly, the viability of the eggs was not affected by the isotope, although this stage is usually considered to be highly susceptible to irradiation.

SUMMARY. The volume of blood ingested by *Aedes aegypti* was determined by a gravimetric method and found to be 1.63 mm^3/mosquito. The volume of blood ingested, determined by a method involving the use of P^{32}, was 3.0 mm^3. It was found that the fecundity of mosquitoes fed on P^{32}-labelled blood was lower than that of mosquitoes fed on non-labelled blood. The lowered fecundity persisted when radioactive mosquitoes were fed on non-labelled blood. The viability of radioactive eggs (at the level of radiation used) was not affected by the presence of the isotope.

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