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FLIGHT PERFORMANCE AND FUEL UTILIZATION AS A FUNCTION OF AGE IN FEMALE *Aedes taeniorhynchus*

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ABSTRACT

Sugar-fed female *Aedes taeniorhynchus* were flown 4.5 hr on a flight mill twice a week for the eight weeks of their life span and analysed for flight performance (speed of flight and distance flown) and for utilization of haemolymph sugars, glycogen and triglycerides.

Flight performance remained at peak performance level for the first 5 weeks of adult life, showing a decline starting with the sixth week. Females flew vigorously for 1 to 2 hr and then slowed down gradually towards the end of the flight period. Glycogen was the only energy reserve utilized during sustained tethered flight. It was nearly exhausted in flown females throughout the eight weeks of their life span. Different amounts of glycogen were utilized during efficient sustained flight, e.g., 30—40 cal/hr per g during the second to the fifth weeks when flight was maximum as compared to 14—24 cal/hr per g during the sixth to eighth weeks when flight was considerably slowed down. Females maintained on sugar *ad lib.* achieved maximum reserves of glycogen (0.8 cal/female) and triglycerides (5.5 cal/female) during the second week and they maintained the same levels until the sixth week of their life span. The significance of these results to dispersal in mosquitoes during their life span is discussed.

INTRODUCTION

The effect of age on flight performance in mosquitoes was first demonstrated with female *Aedes aegypti* and *Culex tarsalis* in tethered flight (Rowley and Graham, 1968; Rowley, 1970a). Performance was maximal during the first 14 days of adult life in both species; it decreased substantially at the beginning of the third week in both species, and again, sharply in the fourth week in *A. aegypti,* but not as much in *C. tarsalis.* Our recent studies of spontaneous flight performance of *Aedes taeniorhynchus* females suggested that age did not affect flight performance to so great an extent since sugar-fed females showed consistent sustained flight activity with a bimodal circadian rhythm for four weeks of study (Nayar and Sauerman, 1971a).

Both *A. aegypti* and *C. tarsalis* apparently fly at an almost constant speed while tethered on a flight mill during the six weeks of daily flights, which lasted from 126 to 336 minutes in *A. aegypti* and 235 to 358 minutes in *C. tarsalis* (Rowley and Graham, 1968; Rowley, 1970a). However, Rowley (1970b, p. 1843) later modified his initial conclusions saying, “Obviously, flight speed decreases with duration in exhaustive flight.” Our observations on the tethered flight of female *A. taeniorhynchus* and *Aedes sollicitans* showed that these mosquitoes flew vigorously for 1 to 2 hours and then slowed down, but they were able to fly for several more hours (Nayar and Van Handel, 1971). Therefore, quantitative energy utilization was determined during the first hour, the period of maximum flight capacity and least variability.

Rowley and Graham (1968) and Rowley (1970a, 1970b) flew sugar-fed females in all their studies, yet these investigators did not take into account the contribution of energy from sugar present in the crop during flight. They attributed all energy required for flight to the glycogen reserves only. However, Nayar and Van Handel (1971) and Nayar and Sauerman (1971b) by feeding radioactive glucose and other metabolizable sugars and flying immediately female *A. taeniorhynchus* tethered on a flight mill, demonstrated that when glucose or other metabolizable sugars are available, they were utilized before any use was made of accumulated glycogen reserves.

The present study was initiated to get a better understanding of flight performance and utilization of energy reserves in aging female *A. taeniorhynchus.*

MATERIALS AND METHODS

Adults of *A. taeniorhynchus* were obtained from larvae reared at 27°C under LD 12:12 as described by Nayar and Sauerman (1971b). At emergence females were separated from males within 4–6 hr and maintained 200/cage on 10% sucrose solution at 27°C under LD 12:12 as described by Nayar and Sauerman (1971a).

Females were flown tethered on flight mills both in groups of 4 and individually (Nayar and Van Handel, 1971; Nayar and Sauerman, 1971b). Two days before flying any group, about 70 recently sugar-fed females were removed from maintenance cages and then maintained in a plastic cup as described earlier (Nayar, 1968) on water without food under the same temperature and light conditions. This standardized procedure was followed in order to allow the females time to absorb all recently fed sugar and convert it to glycogen and triglyceride reserves, thus eliminating any contribution of energy from freely available sucrose during flight.

Females were flown tethered twice a week, i.e., on Tuesdays and Fridays, for eight weeks. Flights were of 4.5 hr each, instead of to exhaustion. This time interval was selected because earlier studies had shown that an hour or two before females were exhausted their speed became very variable and this could lead to tremendous variations in energy utilization during flight (Nayar and Van Handel, 1971). The data obtained from two flights a week were averaged for the week. At flight starting time 5 groups of 4 females were removed, anesthetized, weighed on a microbalance and preserved in about 0.5 ml of absolute MeOH and labeled as the 'non-flown before' group. Three groups of 4 females each were flown on modified flight mills (Nayar and Van Handel, 1971) and 6 individuals were flown on individual flight mills (Nayar and Sauerman, 1971b). The distance flown on each flight mill was recorded every half hour. Flight was terminated at the end of 4.5 hr and females were preserved in 0.5 ml of MeOH. The three groups of 4 females each were preserved as such, but 6 individuals were grouped into 2 groups of 3 individuals per tube. At the same time 5 groups of 4 females, which had been maintained in plastic cups and were not flown, were also preserved and labeled as the 'non-flown after' group. Therefore, using the same experimental plans as used by Nayar and Van Handel (1970), 20 females 'non-flown before,' 18 females flown and 20 females 'non-flown after,' were analyzed for each flight time for sugars, glycogen and triglycerides by the method of Van Handel (1965a). All results are expressed in cal/mosquito in order to make fat comparable to carbohydrates. One mg of carbohydrate was calculated as 4 cal and 1 mg of triglyceride as 9 ca. Since no significant differences were observed in speed of tethered flight and quantitative energy utilized by 4 mosquitoes as a group and in individuals, all the data were pooled and both individually flown females and females flown in groups of 4 were treated as one. Biological variation was minimized by the highly standardized method of rearing and the performing of experiments week after week, and by using enough samples for replicate analyses.

These experiments were performed for eight weeks' duration, but it was observed that after mean life span of 6 weeks (Nayar and Sauerman, 1971a) was over, the variations increased in all measurements.

RESULTS

a) Speed and distance flown in aging females

Table 1 shows that the first hour speed of tethered flight in female *Aedes taeniorhynchus* rises from 1360 m/hr to a maximum of approximately 1800 m/hr by the third week. Thereafter, there is no change until the sixth week, when substantial decreases in speed appear and continue until by the eighth week the speed is down to 945 m/hr. The females did not fly consistently during the 4.5 hr tests; after 1 to 2 hr of vigorous flight, they slowed down gradually, until by the end of 4 hr their speed was reduced by 30 to 50% relative to the initial first hour speed during the first six weeks. This suggests that females of this species could maintain tethered flight for considerable periods (8 to 12 hr) at a reduced speed until exhausted, at least until they reach their mean life span of about 6 weeks. This also suggests that as the females pass the point of their mean life span their mean speed is considerably reduced, thus showing the effect of senescence.

Table 1 also shows that the total average (of 2 tests per week) distance flown on a flight mill in 4.5 hr increased from 4115 meters in the first week to a maximum of 7040 meters in the third week. The distance flown then decreased slowly by 5180 meters in the sixth week and rapidly thereafter, until the eighth week, when it was only 2740 meters. Thus no indications of a sharp decline in distance flown were observed during the mean life span, i.e., up to 6 weeks, but after that the decrease in distance flown was substantial.

b) Qualitative and quantitative utilization of energy reserves in aging females

Fig. 1 shows that differences in depletion in values of haemolymph sugars and triglyceride reserves during the 8 weeks of investigation were very small in flown and non-flown female *A. taeniorhynchus.* This confirmed the earlier results (Nayar and Van Handel, 1971) that haemolymph sugars and triglyceride reserves present in females do not make a significant contribution to the increased energy demand during flight in mosquitoes. However, during each flight period differences in value of glycogen reserves in flown and non-flown females were quite apparent throughout the 8 weeks of study, suggesting that in the absence of free sugars in the crop, glycogen was the only energy reserve which was utilized appreciably during flight.

Table 2 shows that during the first week of 4.5 hr of sustained tethered flight glycogen utilization is 0.05 cal/1000 m or 18 cal/hr per g and that this increases to 0.06 cal/1000 m or 29 cal/hr per g by the second week, and reaches a maximum of 0.09 cal/1000 m or 40 cal/hr per g by the fourth week; thereafter, there is a decrease and by the sixth week it reaches 0.06 cal/1000 m or 24 cal/hr per g. A further rather substantial decrease occurs during the next two weeks, when glycogen utilization is 0.05–0.06 cal/1000 m or 14 cal/hr per g during the seventh and eighth weeks.

Fig. 1 also shows that when female *A. taeniorhynchus* were maintained on 10% sucrose solution, it took one week of continuous *ad lib* feeding before glycogen and triglyceride reserves approached maximum values, 0.8 cal/female (200 ug/female) for glycogen and 5.5 cal/female (500 ug/female) of triglycerides. The values of haemolymph sugars remained very consistent, between 0.15 to 0.22 cal/female (60 to 88 ug/female) throughout the 8 weeks of life span, but values of all three energy yielding reserves showed considerable fluctuations during the last two weeks (Fig. 1).

DISCUSSION

The tethered flight performance (speed of flight and distance flown during 4.5 hr flight period) of female *A. taeniorhynchus* was little affected by age up to 6 weeks after emergence (the point of mean life span) and confirmed results obtained on earlier sustained spontaneous flight, where no effect was indicated during the 4 weeks of investigation (Nayar and Sauerman, 1971a). However, after 6 weeks, i.e., beyond the point of mean life span, much greater variability occurred both in speed and in distance flown, which could be attributed to the inclusion of some of the females which were in the process of dying. Thus both speed of tethered flight and distance flown during seventh and eighth weeks showed a substantial decrease. This indicates that aging female mosquitoes show a progressive decline in flight performance, and in general conforms with work reported for insects and animals (Comfort, 1964; Clark and Rockstein, 1964). This study also suggests that female *A. taeniorhynchus* are different in their flight behavior and flight performance from other mosquitoes and Diptera which have been investigated,---*A. aegypti* (Rowley and Graham, 1968), *C. tarsalis* (Rowley, 1970a), *Drosophila sp.* (Williams *et al.* 1943; Wigglesworth, 1948) and *Musca domestica* (Rockstein and Bhatnagar, 1966)--- all of which showed pronounced decreases in flight performance by the end of the second week of adult life. The extension of good flight performance to six weeks in female *A. taeniorhynchus* could be related to the migratory and dispersal behavior of this species (Provost, 1952, 1957). In addition to the exodus flight on the night of emergence, these mosquitoes disperse over wide areas through appetential flights during the second through fourth weeks. In contrast to this, all other species investigated, e.g., *A. aegypti, C. tarsalis, Drosophila sp.*, and *M. domestica,* fly and disperse little.

Female *A. taeniorhynchus,* after a vigorous tethered flight of 1 to 2 hr, showed substantial decrease in speed (30 to 65% of the speed during the first hour of flight) during the final hours of the flight period (Table 1). These results are in contradiction to the observations in *A. aegypti* where there was little change in the speed of tethered flight (Rowley and Graham, 1968) and in *C. tarsalis* where there was 3 m/min decrease in speed during 120 min of flight, which was not considered significant (Rowley, 1970b).

The results on energy utilization clearly show that in female *A. taeniorhynchus* in the absence of free sugars in the crop, only glycogen reserves are used for sustained flight, and the energy requirements as calculated from depletion of glycogen during sustained vigorous flight for 4.5 hr from second to fifth weeks, both inclusive, were 0.06 to 0.09 cal/1000 m or 29 to 40 cal/hr per g. Both of these conclusions confirm and support our earlier findings that female *A. taeniorhynchus* use only glycogen as flight fuel, and utilization of carbohydrate for 1 hr of sustained vigorous tethered flight was 0.08 to 0.10 cal/1000 m or 27 to 37 cal/hr per g (Nayar and Van Handel,

Table 1 – Speed (meters/hr) of tethered flight by 36 females every hour for the 4 hours during 8 weeks and average distance flown in 4.5 hours of tethered flight by female *A. taeniorhynchus.*

Age after emergence (Weeks)	1st	2nd	3rd	4th	5th	6th	7th	8th
Flight during hours	Speed (meters/hr ± S.E.)							
1	1360 ± 97	1580 ± 72	1782 ± 74	1790 ± 93	1860 ±116	1367 ± 97	1265 ± 77	945 ± 84
2	1079 ± 89	1408 ± 66	1623 ± 89	1513 ± 90	1534 ± 101	1182 ± 111	936 ±122	717 ± 121
3	782 ± 59	1189 ± 67	1508 ± 84	1248 ± 79	1298 ± 115	982 ± 91	655 ± 86	495 ± 75
4	692 ± 45	914 ± 58	1262 ± 60	1001 ± 77	1045 ± 83	903 ± 111	485 ± 70	336 ± 61
Total av. distance flown in 4.5 hr	4115± 292	5634 ± 293	7040 ± 343	6550 ± 356	6461 ± 435	5184 ± 508	3910 ± 329	2740 ± 324
Percent reduction of the first hr speed after 4 hrs	50	42	30	44	44	34	62	65

Table 2 – Quantitative energy utilization (calculated from loss of glycogen) during flight in aging female *A. taeniorhynchus*.

Age after emergence (During weeks)	Mean wet body weight (mg/female)	Hours flown	Total mean distance flown (m/hr ± S.E.)	Glycogen utilized (cal/female)	Energy utilization/female	
					cal/1000m	cal/hr per g
1st	2.6	4.5	4115 ± 292	0.21	0.05	18
2nd	2.8	4.5	5634 ± 293	0.36	0.06	29
3rd	3.0	4.5	7040 ± 343	0.51	0.07	39
4th	3.2	4.5	6550 ± 356	0.58	0.09	40
5th	2.9	4.5	6461 ± 435	0.41	0.06	31
6th	2.8	4.5	5184 ± 508	0.30	0.06	24
7th	2.8	4.5	3910 ± 329	0.18	0.05	14
8th	2.6	4.5	2740 ± 324	0.16	0.06	14
Mean for forst 6 weeks	2.9	4.5	5830 ± 371	0.40	0.70	30
first						

1971). The slightly lower values of energy utilization during 4.5 hours of sustained tethered flight than during 1 hr of vigorous flight could be due to the variations in flight speed and distance during 4.5 hours of flight. These conclusions therefore do not support the suggestion "A substrate or substrates other than glycogen must be utilized by mosquitoes during extensive flight since they are capable of flying for 3 to 4 hours after depleting their primary source of flight energy." (Rowley, 1970b, p. 1843).

Comparison of Tables 1 and 2 shows that substantially higher utilization of glycogen (0.06 to 0.09 cal/1000 m or 29 to 40 cal/hr per g) occurred during weeks second to fifth, when females flew consistently at a higher speed, i.e., between 1000 and 1800 m/hr during the 4.5 hr duration of flight, while substantially lower utilization of glycogen (0.04–0.06 cal/1000 m or 14 to 24 cal/hr per g) occurred during the first week and from weeks sixth to eighth, when females flew at variable and considerably lower speed, such as 335 to 1360 m/hr, during the 4.5 hr duration of flight. Thus the most conspicuous manifestation of senescence in flight performance is the considerable decrease in both speed as well as distance flown after the point of mean life span.

Rowley and Graham (1968) showed that in aging female *A. aegypti* all available glycogen was utilized in exhaustive flights during the first two weeks of their age, whereas during the third and fourth weeks, glycogen in exhausted mosquitoes increased concomitantly with a reduction in flight ability. The results presented here in Fig. 1 show that glycogen was used proportionately to the flight performance throughout the aging period in female *A. taeniorhynchus.* This would suggest that female *A. aegypti,* which were maintained on sucrose prior to flight, utilized both free sucrose from the crop as well as the stored glycogen reserve for exhaustive flight during the first two weeks, but in third and fourth weeks, due to a decrease in flight performance, they could utilize only sucrose for flight and were exhausted before much of the glycogen was depleted. Thus the older female *A. aegypti* had higher amounts of glycogen left at the time of apparent exhaustion.

In energy-reserve-depleted *A. sollicitans* females fed a single meal of glucose: fructose 1:1 mixture, levels of glycogen reserves reach a maximum in 8–12 hr, independent of the amount of sugar fed, whereas the triglyceride pool grows in proportion to the sugar meal (Van Handel, 1965b). But in female *A. taeniorhynchus* maintained on 10% sucrose fed *ad lib.,* levels of both glycogen and triglyceride reserves reached their maximum after the first week of feeding and then stabilized from the second to fifth weeks before showing a decline in both glycogen and triglyceride levels. It is at this age that flights also start declining in speed and in distance. It is also of interest to note that in *A. aegypti* values of glycogen varied between 70 to 90 ug/female (Rowley and Graham, 1968), whereas in female *A. taeniorhynchus* these values varied between 175 to 220 ug/female between the second and fifth weeks. This greater amount of glycogen, which is apparently species-specific, could explain the longer duration of sustained tethered flights observed in *A. taeniorhynchus.* The maximum duration of tethered flight, 5.6 hr, was observed during second week with *ad lib.* sugar feeding in *A. aegypti* (Rowley and Graham, 1968) as compared to about 7 hr of tethered flight duration observed in unfed 1–day old female *A. taeniorhynchus* (Nayar and Van Handel, 1971).

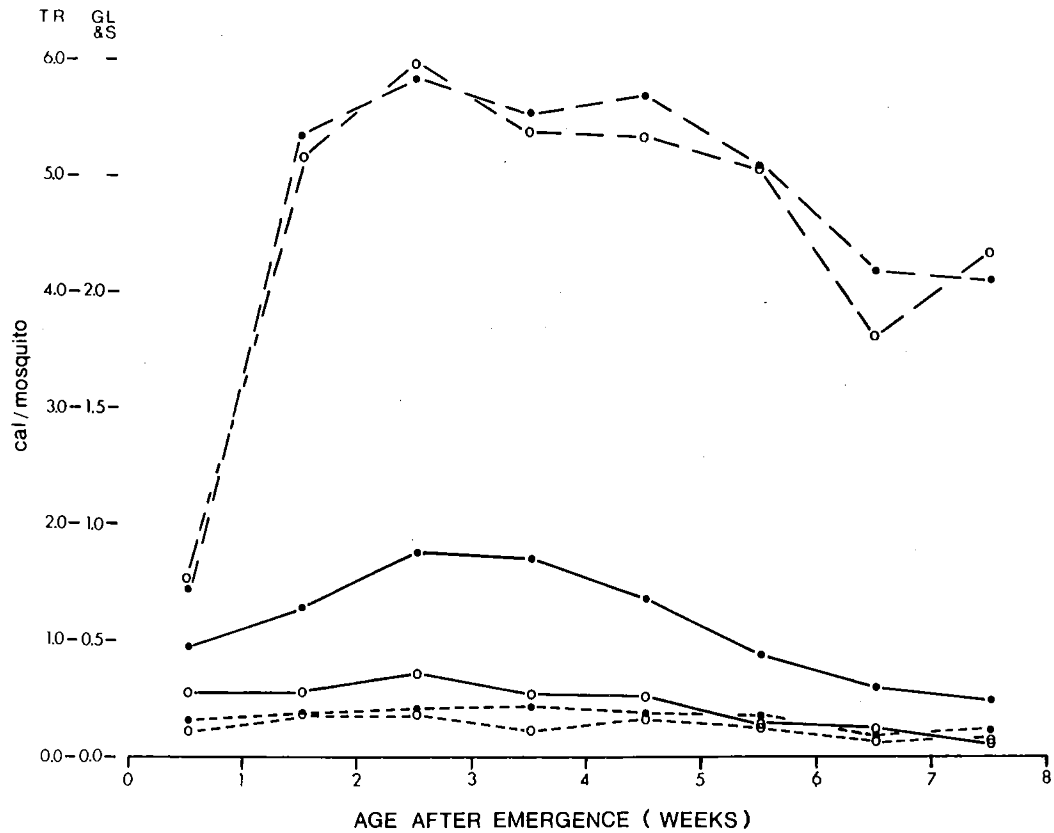

Fig. 1: Fluctuations in levels of triglycerids (Tr.) (broken line), glycogen (Gl.) (solid line) and haemolymph sugars (s.) (dotted line) in flown (o) and non-flown (•) female *A. taeniorhynchus* during 8 weeks of their life span.

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