STUDIES ON THE OVERWINTERING BIOLOGY OF NATURAL POPULATIONS OF *ANOPHELES FREEBORNI* AND *CULEX TARSALIS* IN CALIFORNIA

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ABSTRACT. Overwintering *Anopheles freeborni* Aitken adults synthesize lipids as a source of overwintering energy; the field population reaches a maximum lipid content in October, after which there is a steady decline during the overwintering period. Approximately 75 percent of the total lipids are composed of triglycerides. The same pattern occurs in *Culex tarsalis* Coquillett adults except that field populations reach their maximum lipid content in November. The timing of lipid synthesis of *C. tarsalis* adults in Fresno County appears to be the same as for the Sacramento Valley.

INTRODUCTION. The mosquitoes *Anopheles freeborni* Aitken and *Culex tarsalis* Coquillett overwinter as inseminated, adult females. Previous studies where the field populations were sampled in mid-October, December, and February indicated that *A. freeborni* adults had reached their maximum lipid content by mid-October, but *C. tarsalis* adults showed a much higher lipid content in December (Schaefer and Washino, 1969). Since the latter work was based on only three sampling times, it was important to determine the lipid content of field populations of both species at frequent intervals throughout the overwintering period; further studies with *C. tarsalis* adults showed that field populations in the Sacramento Valley attained their maximum lipid content in early November and that over 75 percent of the total lipids were triglycerides (Schaefer and Washino, 1970). Evidence exists that *A. freeborni* adults may develop fat bodies as early as August in the Sacramento Valley (Washino, 1970).

Additional studies, described in this paper, were made to determine the timing of lipid synthesis, the lipid class distribution, and fatty acid content of the major lipid classes of field populations of *A. freeborni* adults. Also, since all of the previous adult sampling had been done in the Sacramento Valley, it was of interest to determine the timing of lipid synthesis in field populations of *C. tarsalis* in Fresno County (San Joaquin Valley).

MATERIALS AND METHODS. *A. freeborni* adults were collected in the Sacramento Valley areas of Sutter County, California, at two locations: (1) beneath a bridge (Tarke Bridge), and (2) from the porch of a farmhouse (Dewitt Farm); sampling at each location was initiated in September 1969 and was continued weekly as long as empty gono-inactive females could be obtained. Samples were weighed and then held at −20° C until analyzed. Lipid content was determined as previously described (Schaefer and Washino, 1969); lipid classes were separated by column chromatography as described by Carroll (1961), and the amounts were determined gravimetrically. The fatty acids of the major lipid classes were esterified and analyzed by gas-liquid chromatography in the manner previously described (Schaefer and Washino, 1970).

Adults of *C. tarsalis* were collected at two locations in Fresno County, California, during the 1968–69 and 1969–70 overwintering periods. One sampling location was a stable (Ralls' farm) on the floor of the San Joaquin Valley (elevation 330 feet) and the other was large culverts in Trenton Canyon (elevation 2,000 feet), which is in the foothills and approximately 30 miles east of Fresno. Sampling of *C. tarsalis* populations at the latter locations was not initiated until late September because, until then, few non-gravid females can be found.

RESULTS AND DISCUSSION. The lipid con-

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tent of *A. freeborni* adults increases during September and the maximum level is reached by mid-October (Table 1); during the remainder of the overwintering period, the lipid content steadily declines and it reaches the same approximate level in January that was characteristic of early September. The low level lipid content in January coincides with the time when blood feeding is initiated in the overwintering population. *A. freeborni* adults from the Dewitt Farm became too scarce in December and January to continue these observations. However, the data obtained through November show the same pattern as for the Tarke location, where collection was maintained throughout the study period.

The total lipids extracted from *A. freeborni* adults collected at the Tarke location on October 17 and 24, 1969, were combined (total 27.3 mg), and the separation of lipid classes by column chromatography revealed the results shown in Table 2. About 75 percent of the total lipids were triglycerides, and over 90 percent of the total lipids were in the triglyceride and free fatty acid fractions; these results are almost identical with the lipid class distribution determined for *C. tarsalis* adults, where 77 percent of the total lipids were triglycerides and 15 percent eluted with free fatty acid fraction (Schaefer and Washino, 1970).

Saponification of the *A. freeborni* triglycerides yielded 22.0 mg of fatty acids, which accounted for 91.7 percent of the mass. Esterification of the triglyceride fatty acids with BF_3-methanol yielded 21.9 mg of methyl esters; esterification of the free fatty acid fraction (5.1 mg) yielded only 4.0 mg of methyl esters. The low yield of methyl esters from the free fatty acid fraction suggests that non-fatty acid components were present; however, the yield reported here was higher than that

TABLE 1.—Lipid content of *Anopheles freeborni* adults collected during the 1969–1970 season (20 adults per sample *).

Collection date	Tarke Bridge			Dewitt Farm		
	Wet wt. (mg/adult)	Total lipids (mg/adult)	Lipids (%) (Wet wt.)	Wet wt. (mg/adult)	Total lipids (mg/adult)	Lipids (%) (Wet wt.)
9/4/69	2.33	.255	10.9	2.96	.152	5.1
9/11/69	2.83	.218	7.7	3.20	.237	7.4
9/19/69	3.90	.529	13.5	3.62	.279	7.7
9/26/69	2.98	.461	15.4	3.10	.336	10.8
10/3/69	3.17	.560	17.6	2.89	.450	15.6
10/17/69	3.95	.696	17.6	3.90	.667	17.1
10/24/69	3.88	.704	18.1	3.30	.423	12.7
10/31/69	2.80	.405	14.5	3.96	.634	16.0
11/7/69	3.55	.550	15.5	3.42	.446	13.0
11/14/69	3.69	.588	16.0	3.32	.473	14.2
11/19/69	3.20	.487	15.2	2.79	.417	14.9
11/21/69	——	——	——	3.27	.464	14.2
11/28/69	3.30	.485	14.7			
12/4/69	3.20	.437	13.6			
12/11/69	3.29	.452	13.7			
12/19/69	2.99	.386	12.9			
12/23/69	3.12	.381	12.2			
12/30/69	2.56	.317	12.4			
1/6/70	2.75	.344	12.5			
1/17/70	2.12	.175	8.2			
1/24/70	2.56	.193	7.5			

* Except for the Dewitt Farm samples of 11/19/69 and 11/21/69 which contained 9 and 11 adults respectively.

TABLE 2.—Lipid class analysis of total lipids from *Anopheles freeborni* adults collected in October 1969.

Fraction	Lipid class	Eluant	Lipids (mg)	% of total
1	Hydrocarbons	n-hexane	0.45	1.42
2	Sterol esters	5% ether in hexane	0.58	1.83
3	Triglycerides	15% ether in hexane	23.92	75.67
4	Sterols	25% ether in hexane	0.55	1.74
5	Diglycerides	50% ether in hexane	0.51	1.61
6	Monoglycerides	2% methanol in ether	0.47	1.49
7	Free fatty acids	4% acetic acid in ether	5.13	16.23

for the same fraction from *C. tarsalis* adults (Schaefer and Washino, 1970).

Gas-chromatographic analysis of the fatty acids from the triglyceride and free fatty acid fractions of the *A. freeborni* lipid classes described above are shown in Table 3. The triglyceride fatty acids are similar in composition to that previously reported for the total lipids (Schaefer and Washino, 1969), except that unknown compounds are largely absent; most of the unknown compounds previously found in the total lipids separate with the free fatty acid fraction. It is very interesting that these results, as for the lipid class separations, are very similar to those found for *C. tarsalis* adults (Schaefer and Washino, 1970).

TABLE 3.—Percentage composition of the fatty acids from the triglyceride and free fatty acid fractions of *Anopheles freeborni* lipids.

Compound [1]	Triglyceride fraction	Free fatty acid fraction	Relative retention time
C 12:0	0.8	0.3	0.19
C 12:1	tr.*	tr.	0.24
Unknown No. 1	N.D.**	6.4	0.29
C 14:0	1.3	0.3	0.34
C 14:1	2.1	1.0	0.42
C 16:0	15.1	9.7	0.58
C 16:1	53.2	31.0	0.72
C 18:0	1.2	3.5	1.00
C 18:1	25.5	24.2	1.23
C 18:2 plus Unknown No. 2	0.8	6.4	1.62
C 18:3	N.D.	7.1	2.29
Unknown No. 3	tr.	1.9	2.69
Unknown No. 4	N.D.	2.3	4.08
Unknown No. 5	N.D.	1.3	5.00
Unknown No. 6	N.D.	4.5	5.71

[1] The first number refers to the number of carbons; the second refers to the number of double bonds.

* tr. is trace (less than 0.2%).

** N.D. is not detected.

Overwintering *C. tarsalis* adults could not be found at either of the sampling locations in Fresno County until late October during either the 1968–69 or 1969–70 seasons. Although the lipid content values are somewhat variable (Tables 4 and 5), presumably due to the low number of adults that could be collected on many of the given dates, a general trend is apparent. Lipid content is approximately maximum by the dates on which the first samples could be found at either location. This suggests that the adults originated from populations which matured in other areas and then dispersed. The latter explanation is consistent with the report of Kliewer *et al.* (1969), "Field data from a 5-year study indicate that there is a movement of *Culex tarsalis* Coquillett into field areas adjacent to the San Joaquin Valley in Fresno County in fall, and that overwintering of females takes place there"; while we are in agreement with this conclusion, we have been able to collect *C. tarsalis* adults at locations of the floor of the San Joaquin Valley throughout the overwintering period. For example, we have made regular collections at Ralls' farm (east side of the San Joaquin Valley) as well as from under bridges at locations 40 to 50 miles west of Fresno throughout two overwintering periods. Thus, *C. tarsalis* females overwinter on the floor of the San Joaquin Valley as well as in the foothill locations in Fresno County.

Thus, it appears that adult *C. tarsalis* females synthesize lipids at approximately the same time in the San Joaquin Valley as previously found for populations in the

TABLE 4.—The lipid content of *Culex tarsalis* adults collected in Trenton Canyon during two overwintering periods.

1968–1969					1969–1970				
Collection date	No.	Fresh wt. (mg/adult)	Total lipids (mg/adult)	Lipids (%) (fresh wt.)	Collection date	No.	Fresh wt. (mg/adult)	Total lipids (mg/adult)	Lipids (%) (fresh wt.)
11/8/68	5	2.77	.58	20.9	10/27/69	7	1.48	.314	21.2
11/18/68	8	3.09	.55	18.2	11/4/69	5	2.12	.400	18.8
11/25/68	18	2.49	.47	18.9	11/10/69	6	2.46	.592	24.0
12/2/68	12	2.54	.48	18.6	11/17/69	5	2.40	.490	20.4
12/16/68	7	2.14	.36	16.7	11/24/69	15	2.22	.407	18.3
12/23/68	7	2.31	.37	16.0	12/1/69	29	2.52	.438	17.4
12/30/68	7	2.24	.37	16.3	12/8/69	48	2.51	.411	16.3
12/30/68	8	2.05	.35	17.1	12/22/69	17	2.46	.332	13.5
1/6/69	4	2.00	.30	15.0	12/29/69	33	1.93	.303	15.7
1/13/69	4	2.26	.30	13.2	1/5/70	22	2.13	.276	13.0
					1/12/70	12	2.12	.292	13.7
					1/19/70	7	1.90	.243	12.8
					2/2/70	6	1.75	.266	15.2

TABLE 5.—The lipid content of *Culex tarsalis* adults collected at Ralls' Farm during two overwintering periods.

1968–1969					1969–1970				
Collection date	No.	Fresh wt. (mg/adult)	Total lipids (mg/adult)	Lipids (%) (fresh wt.)	Collection date	No.	Fresh wt. (mg/adult)	Total lipids (mg/adult)	Lipids (%) (fresh wt.)
11/18/68	7	3.20	.48	15.2	10/27/69	5	2.15	.413	19.1
11/25/68	8	2.56	.51	20.0	11/10/69	16	2.80	.539	18.6
12/2/68	13	2.66	.47	17.6	11/17/69	6	3.18	.443	14.0
12/9/68	9	2.64	.38	14.3	11/24/69	11	2.72	.512	18.7
12/16/68	19	2.80	.43	15.6	12/1/69	5	2.51	.520	20.6
12/23/68	9	2.67	.41	15.4	12/8/69	9	3.14	.462	14.6
12/30/68	21	2.47	.28	11.8	12/15/69	15	3.03	.458	15.1
1/6/69	9	2.57	.33	13.0	12/22/69	15	3.10	.521	16.8
1/13/69	5	3.00	.34	11.4	12/29/69	5	2.67	.560	21.0
					1/5/70	7	2.51	.467	18.6
					1/12/70	14	2.86	.418	14.6
					1/19/70	5	2.62	.331	12.7
					2/2/70	4	2.16	.303	14.0

Sacramento Valley and that *A. freeborni* adults synthesize lipids earlier than *C. tarsalis* (Schaefer and Washino, 1969, 1970). The synthesis of triglycerides by adults is apparently a general phenomenon in mosquitoes, which is under neurosecretory control (Lea and Van Handel, 1970; Van Handel and Lea, 1970).

Since blood feeding by adult *A. freeborni* and *C. tarsalis* is minimal during the fall and winter (Washino, 1970; Bellamy and Reeves, 1963), the source and composition of food which adult females utilize for the synthesis of overwintering energy stores (triglycerides) is of considerable interest and is presently under investigation.

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PRELIMINARY REPORT ON ARBOVIRUS ISOLATIONS FROM SOUTH DAKOTA MOSQUITOES COLLECTED DURING THE SUMMER OF 1969[1]

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There has been no epidemiologic study in South Dakota involving arbovirus isolation attempts from mosquitoes. The mosquitoes of South Dakota were first studied intensively by the United States Public Health Service in 1949 and 1950 (mimeo reports, 1951, 1952); virus isolations were not attempted from mosquitoes collected, but population indices were determined. This study was mainly conducted in the James and Missouri River Basins. A more recent mosquito survey in South Dakota was conducted by Gerhardt in 1964 (Gerhardt, 1966). The first arbovirus antibody surveys in South Dakota were conducted by Eckland in 1941 (personal communication). More recently, 1961, Hess *et al.* (1963) found antibodies to St. Louis

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