there are at least two distinct hemolymph proteins in cecropia pupae, which are lipoproteins. By cutting these bands from the gels and refluxing them in an ethanol : ether mixture to release the lipid moiety, we could demonstrate that a major portion of the radioactive diglyceride in the hemolymph is situated in these bands. This suggests that lipoprotein complexes with diglyceride as the lipid moiety, are the means by which cecropia, and perhaps other insects, transport lipids. This would be analogous to the situation in mammals where triglycerides and unesterified fatty acids are probably transported in combination with plasma proteins.

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Swamp Mosquito, Culiseta melanura: Occurrence in an Urban Habitat

Abstract. *Adult* Culiseta melanura *were collected in Boston during three consecutive years (1961–63). Breeding sites were found along the bank of an impounded stream and in a small water collection in a concrete-lined pit.*

The reported restriction of *Culiseta melanura* (Coq.) to fresh-water swamps (*1, 2*) is significant because of the apparent potential of this mosquito as a vector of eastern equine encephalitis. Heretofore it has not been regarded as an important vector (*3*) because it has not been found near centers of human population. However, during the course of studying mosquito populations in Boston, Massachusetts, numerous *C. melanura* adults were captured.

The adults were collected in a dormitory of the Harvard Medical School, in the immediate vicinity of which are many institutional buildings. A small impounded stream, the Muddy River, is located about 450 meters away. Although its banks are cleaned periodically, eroded pockets and rodent holes are present along its margins. The nearest fresh-water swamp is at least 11 kilometers distant.

Adult mosquitoes (*C. melanura, C. minnesotae* Barr, *Culex restuans* Theobald, *C. salinarius* Coq., and *C. pipiens* L.) were collected at several sites, but *C. melanura* was found only in a utility tunnel beneath the building near an open window. A 1.8 × 0.9 m air shaft, containing approximately 10 cm of water rose 6 m from the window to the street. Mosquito larvae were generally present in this water, in a nearby catch basin, and in a variety of temporary water collections. Collections of resting adult mosquitoes were made at least once each week for 4 years. Samples of larvae were also taken weekly from the air shaft and catch basin, and, less regularly, from the other sites. Specimens were identified, and, when possible, succeeding stages were reared in the laboratory. Thus, identification of wild-caught females was frequently supplemented by study of larvae and males.

Sixteen adult *C. melanura* were collected in the utility tunnel during the course of the study (Table 1). Their abundance appeared to vary from year to year, but, in general, they were collected singly during the warm months. The capture of males and of gravid females suggested that a breeding source was nearby.

A systematic search for *C. melanura* larvae was conducted during the 15-month period ending October 1963 (*4*). Although more than 3000 larvae were captured (*Culex pipiens, restuans,* and *territans* Walker), no *C. melanura* were found until the end of August 1963. At this time, two egg rafts, which gave rise to *C. melanura* larvae, were collected from a pool that had previously been sampled 11 times. This permanent site (0.9 m × 1.2 m × 10 cm) was in an eroded portion of the bank of the Muddy River. The pool was sheltered by thick brush and, where the eggs were found, had undercut the bank. No larvae of this species were found in the pool in subsequent collections. One month later, however, 43 *C. melanura* larvae were discovered in the breeding site in the air shaft.

In the study area *C. melanura* were apparently well adapted to the man-made environment in that they were present during each of several years. The absence of prior records concerning these mosquitoes in urban areas may be due to their apparently elusive habits. Adults were recovered from the deep underground site but not in one at street level; the larvae seemed unusually sensitive to disturbance, and because they remained submerged for long periods, were difficult to collect. These difficulties are compounded by the morphologic similarities between *C. melanura* and *Culex pipiens,* which may result in *Culiseta melanura* being overlooked when *Culex pipiens* is abundant.

The breeding of *Culiseta melanura* appeared to be discontinuous, and adult abundance seemed to vary from time to time. Indeed, the distribution of this species is usually described as being focal (*1, 2, 5*). Nevertheless, it is conceivable that relatively dense populations of this mosquito could develop in the vicinity of human habitations under favorable conditions.

The possible role of *C. melanura* as

Table 1. Adult *Culiseta melanura* collected during 4 years, in a utility tunnel.

Date	Sex	Ovarian state
	1960	
—	—	—
	1961	
4 Sept.	F	Resting
16 Sept.	F	Resting
	1962	
18 June	F	Gravid
14 July	F	Gravid
31 July	F	Resting
6 Aug.	F	Resting
23 Aug.	F	Resting
24 Aug.	F	Gravid
29 Aug.	F	Gravid
11 Sept.	M	
	1963	
15 Aug.	F	Resting
27 Aug.	F	Resting
6 Sept.	F	Resting
1 Oct.	F	Resting
1 Oct.	F	Resting
1 Oct.	M	

a vector of eastern equine encephalitis to man requires further consideration. Trap collections frequently indicate that this mosquito is numerous and its rate of infection with virus may exceed 2 percent (*2*). Although female *C. melanura* appear to prefer avian hosts as their blood source, they will also feed on a variety of mammals, including man (*5, 6*). That this mosquito has now been found in an urban center suggests that it may be more important than previously assumed in the transmission of the virus of eastern equine encephalitis to man (*7*).

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Structure of Lipoproteins: Covalently Bound Fatty Acids

Abstract. *Plasma lipoproteins, extensively extracted with organic solvents, contain small quantities of firmly bound long-chain fatty acids. Upon enzymatic digestion of the protein and partial purification of the resulting peptides, fatty acids are found associated with a peptide fraction which contains organic phosphate. The nature of the linkage between the fatty acid and peptide is unknown.*

It is well known that most of the lipid component of the lipoproteins is readily separable from the protein and that the mode of attachment consists primarily of electrostatic and hydrophobic bonds. However, there is generally a residual amount of lipid which is difficult, if not impossible, to remove by solvent action or by physical means. This study was concerned with the nature of this residual, firmly bound lipid and its mode of attachment to the protein.

High-density (> 1.063 and < 1.21) plasma lipoprotein was isolated from rat serum by ultracentrifugation with the methods of Rodbell (*1*) and Havel *et al.* (*2*) as modified by Marsh and Whereat (*3*). This high-density lipoprotein exhibited a single peak which floated in a medium adjusted to a density of 1.21 in the analytical ultracentrifuge and migrated with the mobility of an α-globulin in free-boundary electrophoresis.

Similarly, a mixture containing both high- and low-density lipoproteins was isolated from human plasma by a modification of the method of DeLalla and Gofman (*4*). Human ACD plasma was adjusted to a density of 1.21 with solid KBr and centrifuged at 100,000*g* for 16 hours. The upper 20 percent of the solution was removed from the tube, diluted with solvent, and recentrifuged. This step was repeated a second time. The human lipoprotein mixture contained several peaks which moved with varying flotation rates in a medium at a density of 1.21 in the ultracentrifuge. No sedimenting peaks were present. In free-boundary electrophoresis several components were found to be present with mobilities of α- and β-globulins.

The recovered lipoproteins were dialyzed against water, dried, extracted twice with ether, and freed of remaining adsorbed lipid by dissolving the lipoprotein in concentrated formic acid at 0°C, followed by precipitation of the protein with ethyl ether. In control studies with albumin and lipovitellin this procedure has been shown to remove adsorbed phosphatides and fatty acids effectively. The formic acid–ether extraction was performed four times. Thereafter, a sample of extracted protein (approximately 10 mg) was further extracted in a Soxhlet apparatus for an additional 8 hours with an azeotropic mixture of chloroform and methanol. The solvent used during the final hour of extraction was collected and evaporated; the residue was saponified, and the fatty acids were extracted into petroleum ether after acidification. The fatty acids were methylated and assayed by gas-liquid chromatography. It was found that less than 0.001 μmoles of fatty acid per 10 mg of protein were removed by the solvent during the final hour of extraction.

The extracted protein was dried over P_2O_5 and then hydrolyzed with 6*N* HCl at 105°C for 16 hours. A mixture of saturated fatty acids was recovered by extraction with petroleum ether and their methyl esters were assayed by gas-liquid chromatography. The high-density rat lipoprotein yielded palmitic and stearic acids in a ratio of approximately 1 : 2. Similar results were obtained by methanolysis with the procedure of James (*5*). From the human lipoprotein mixture, variable amounts of myristic, palmitic, and stearic acids were recovered after hydrolysis (Fig. 1).

Table 1. Fatty acid content of solvent-extracted lipoprotein for six samples. Micromoles of fatty acids per 10 mg of dry protein.

Myristic	Palmitic	Stearic	Total
	Rat high density lipoprotein		
0.0*	0.0053	0.0093	0.015
.0	.0050	.0083	.013
.0	.010	.024	.034
	Human lipoproteins		
0.0091	0.010	0.015	0.034
.0034	.020	.018	.041
.0*	.010	.022	.032

* The average of duplicate analyses for each acid. The other determinations represent single analyses of individual preparations.

Table 2. Moles of phosphate per mole of total fatty acids, determined on two separate samples of human lipoprotein after formic acid–ether extraction followed by extraction with chloroform-methanol and on three peptide fractions obtained from the protein. The peptides were isolated by partition in two different solvent systems. Phosphate was determined by the method of Bartlett (*6*).

Solvent-extracted protein preparation No.	Phosphate per mole of fatty acid
5	1.0*
6	1.9*
Peptide fractions	
1	0.91
2	0.92
3	1.3

* These determinations represent 0.040 and 0.061 μmole of phosphate per 10 mg of protein, respectively.

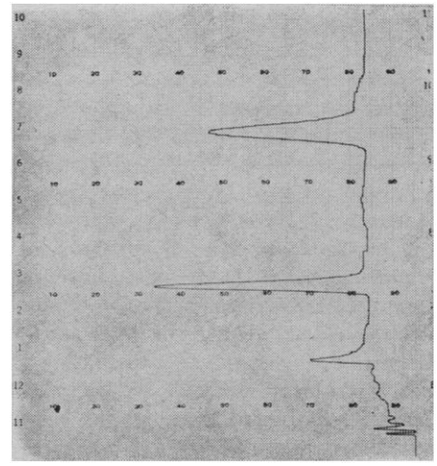

Fig. 1. Typical gas-liquid chromatogram of fatty acid methyl esters recovered after HCl hydrolysis of solvent-extracted human lipoprotein. Ethylene glycol adipate column. The peaks are, respectively, the methyl esters of myristic, palmitic, and stearic acids.