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.

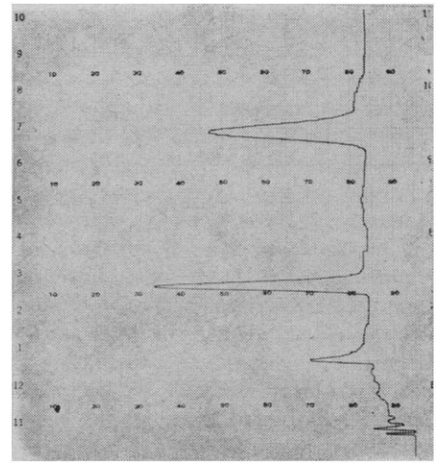

Fig. 1. Typical gas-liquid chromatogram of fatty acid methyl esters recovered after HCl hydrolysis of solvent-extracted human lipoprotein. Ethylene glycol adipate column. The peaks are, respectively, the methyl esters of myristic, palmitic, and stearic acids.

Only insignificant amounts of unsaturated acids were present in either case. The approximate quantities of fatty acids found were in the range of 0.02 μmoles of total fatty acid per 10 mg of protein (Table 1). For quantitative comparison, a standard of authentic *N*-stearoyl alanine was similarly hydrolyzed and chromatographed.

The human lipoprotein extracted with formic acid and ether has also been digested with a mixture of trypsin and chymotrypsin; the resulting digest was acidified to *p*H 2, and the polypeptides were adsorbed on Celite. After drying, the Celite was packed in a column and eluted with petroleum ether, ethanol, and water. It was found that about 90 percent of the bound fatty acids were recovered in the 50-percent ethanol eluate. This peptide fraction was then chromatographed on Dowex 50-W resin (pyridinium salt), and those recovered peptides which contained fatty acids were subjected to partition by countercurrent distribution. With this technique, a major fraction has been partially resolved which, upon acid hydrolysis, yields amino acids plus stearic and palmitic acids.

The α-lipoproteins from rat plasma have similarly been subjected to enzymatic digestion after thorough extraction as described, and partial purification of a peptide fraction containing fatty acid has been achieved. In this case the partial isolation of the acylated peptide has been facilitated by the use of palmitate-C^{14}-labeled lipoproteins. These proteins have been prepared by using a liver perfusion technique with which Marsh and Whereat have demonstrated net synthesis of plasma lipoproteins (*3*). After perfusion of a rat liver with an emulsion of palmitic acid-1-C^{14} in whole blood, it was possible to obtain palmitic acid–labeled plasma lipoproteins. The C^{14} was recovered as palmitate upon alkaline hydrolysis of the lipoprotein following prior extraction by the formic acid and ether.

The results of these studies indicate that fatty acids are bound to the protein moiety of the plasma lipoproteins far more firmly than would be expected with hydrophobic or electrostatic type interactions. In all probability, these fatty acids are covalently bonded to the protein.

Phosphate analyses have been performed on the solvent-extracted human lipoprotein and on the peptides at various stages of purification. At each stage, the fractions containing fatty acid have also been found to contain phosphate. The results of several analyses on the extracted protein and on the most highly purified peptide fractions obtained by partition are given in Table 2. Whether the phosphate is concerned with the fatty acid binding remains to be investigated.

Finally, the physiological significance of these fatty acids which appear to be covalently bonded to the protein portion of the lipoproteins remains unknown. Their influence on the structure of the protein and its ability to bind lipids remains to be established. It is also uncertain whether the small quantities of protein-bound fatty acid reflect losses due to our chemical procedures, or whether they represent the actual amount of covalently linked lipid in the intact lipoprotein molecule.

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Rhesus and Crab-Eating Macaques: Intergradation in Thailand

Abstract. *Conspecificity of the rhesus macaque,* Macaca mulatta mulatta *(Zimmermann, 1780), and the crab-eating macaque* Macaca mulatta fascicularis *(Raffles [1821]), is established by a geographically intermediate series of three specimens transitional in tail length and coat color.*

The crab-eating macaques and rhesus macaques currently are regarded as separate species (*1*) and the nominal species, *Macaca fascicularis* (Raffles [1821]) (*2*) and *Macaca mulatta* (Zimmermann, 1780) (*3*), respectively, frequently are assigned to different subgenera. Specimens collected in Thailand, however, in an area geographically intermediate between the contiguous ranges of these two macaques, show that they intergrade morphologically and, therefore, are races of a single species. The distinctive differences between these monkeys are tail length and color pattern of the back. In the crab-eating macaque the tail is longer than the extended hind leg and averages about

Table 1. Body proportions of crab-eating macaques, rhesus macaques and geographical intermediates; measurements taken in the flesh by collectors.

Location	Specimen No.*	Sex and age	Length (mm)		
			Head and body	Tail	Ratio
Crab-eating macaques					
Burma					
Pakchan R. near Maliwun	A 54972	♂ Juvenile	394	469	1.19
Thailand					
Ko Khram Yai	U 236618	♂ Adult	445	515	1.15
Ko Khram Yai	U 236619	♀ Adult	410	480	1.17
Ko Khram Yai	U 236620	♀ Juvenile	435	430	0.99
Ko Khram Yai	U 236621	♂ Juvenile	320	430	1.34
Ko Kut	U 201552	♂ Adult	419	483	1.11
Geographical intermediates					
Thailand					
B. Umphang, 85 km, E.	A 54679	♀ Adult	460	350	0.76
B. Umphang, 64 km, E.	A 54677	♀ Adult	400	385	0.96
B. Umphang, 45 km, S.E.	A 54816	♂ Young adult	490	270	0.55
Rhesus macaques					
Burma					
Popa Hill	A 163610	♀ Old	450	210	0.47
Popa Hill	A 163611	♀ Old	473	180	0.38
Popa Hill	A 163612	♀ Adult	505	195	0.39
Popa Hill	A 163613	♂ Adult	553	210	0.38
Popa Hill	A 163614	♀ Juvenile	495	230	0.47
Popa Hill	A 163615	♀ Juvenile	461	203	0.44

* Abbreviations: A, American Museum of Natural History catalog number; U, U.S. National Museum catalog number.