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.

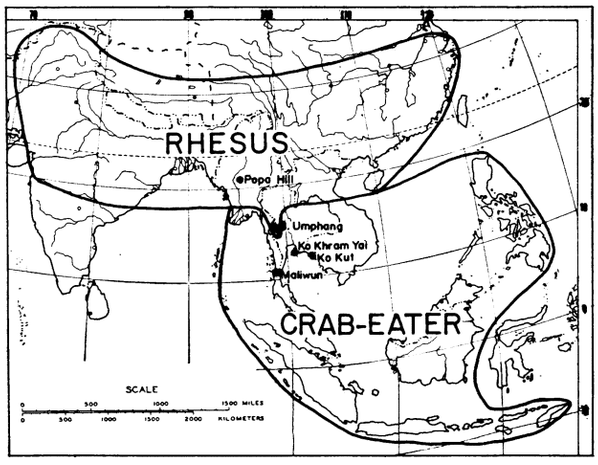

Fig. 1 (left above). Approximate limits of distribution of rhesus macaques and crab-eating macaques; places named on map are reference points for localities listed in Table 1.

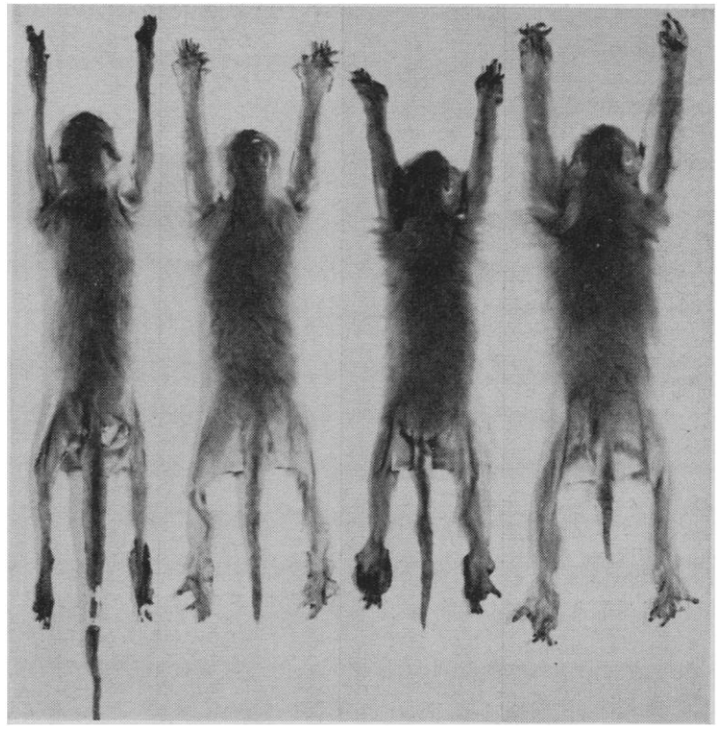

Fig. 2 (right above). Crab-eating macaque (extreme left) from Ban Sadein, 16 km northwest of Maliwun, Burma, compared with macaques collected (left to right) 53 miles (85 km) east, 40 miles (64 km) east, and 28 miles (45 km) southeast of B. Umphang, Thailand; specimen at extreme right closely approaches rhesus macaque in tail length and color pattern of back.

110 percent of the combined length of head and trunk; the upper back and lower back are more or less uniformly colored, ranging from pale brown to dark brown. In the rhesus macaque the tail is shorter than the extended hind leg, averaging about 40 percent of the combined length of head and trunk, and the back tends to be bipartite, grayish-brown anteriorly and tawny on the rump. The geographical range of the crab-eating macaque includes the Indo-Chinese and Malay Peninsulas, Sumatra, Java, Borneo, several small East Indian islands, and the Philippine Islands. The range of the rhesus macaque is north of the range of the crab-eating macaque; it occupies a broad band centered between 20°N and 30°N and extending from eastern Afghanistan and Pakistan eastward to the China Sea. Along the Dawna Range, which is a southward extension of the Tibetan highlands into the Indo-Chinese Peninsula, the range of the rhesus macaque interdigitates with the range of the crab-eating macaque (Fig. 1).

In 1924, in this area of interdigitating ranges near B. Umphang, a mountain village in western Thailand, A. S. Vernay collected three macaque specimens (*4*) that bridge the morphological gap between *fascicularis* and *mulatta*. The tail of an adult female (AMNH 54679) collected 53 miles (85 km) east of B. Umphang [altitude, 800 ft (240 m)] is 76 percent as long as the head and trunk; the back is almost uniformly ochraceous (individual hairs grayish-brown basally, pale yellowish distally) with a barely perceptible brightening of the tone posteriorly. The tail of another adult female (AMNH 54677), collected 40 miles (64 km) east of B. Umphang [altitude, 1000 ft (300 m)], is 96 percent as long as the head and trunk, and the back is ochraceous anteriorly, becoming distinctly tawny posteriorly (tips of hairs gold instead of yellowish). A young adult male (AMNH 54816) collected 28 miles (45 km) southeast of B. Umphang [altitude, 1750 ft (530 m)] hardly differs from typical *mulatta*; the tail is 55 percent as long as the head and trunk, and the back is bipartite, grayish-brown anteriorly (hairs lacking pale tips) and clearly defined tawny posteriorly. As a series, these three geographically intermediate specimens are almost perfectly transitional in tail length and coat color between typical *fascicularis* and *mulatta* (Fig. 2 and Table 1).

Strong independent confirmation of the conspecificity of *mulatta* and *fascicularis* is provided by reports that rhesus and crab-eating macaques are reciprocally interfertile in captivity and that hybrids also are fertile in both sexes (*5*). My own study (now in progress) of the penis bones of macaques likewise indicates that *mulatta* and *fascicularis* form a natural group with respect to this key taxonomic character. Skull characters of macaques have not yet been adequately studied for taxonomic purposes; preliminary study reveals no sharp line of distinction between the skulls of *mulatta* and *fascicularis*. According to rules of zoological nomenclature (*6*), the expanded species including rhesus and crab-eating macaques takes the name of the oldest named component, *mulatta*; the correct name of the rhesus macaque therefore is *Macaca mulatta mulatta* (Zimmermann, 1780) and that of the crab-eating macaque is *Macaca mulatta fascicularis* (Raffles [1821]). Disposition of subspecific names available for described forms of rhesus and crab-eating macaques must await comprehensive revision of the genus. Preliminary study indicates that, like *fascicularis*, some other currently recognized species probably are also conspecific with *Macaca mulatta;* other named forms of macaques, *nemestrina* and *assamensis*, for example, may intergrade to constitute another enlarged species.

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7. I thank officials of the American Museum of Natural History and the U.S. National Museum for permission to study collections in their charge. I am also grateful to officials of the Chicago Natural History Museum for special research facilities made available to me, and to Martin Ptacek for taking the photographs.

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Calciphylaxis: Passive Transfer

Abstract. *Rats were sensitized by mouth with dihydrotachysterol and subsequently challenged by a subcutaneous injection of ferric dextran. Mineralization at the injection site is barely detectable 17 hours after challenge. If, at this time, the challenged skin is transplanted onto a normal recipient, mineralization continues in the nonsensitized host. Sensitization is indispensable only for the initiation of the calciphylactic response and, once "triggered," the mechanism for this type of mineralization is transferable.*

Calciphylaxis is a biologic mechanism through which mineralization can be elicited selectively in limited areas of the body by the administration of "challengers" (for example, iron compounds) during a "critical period" after "sensitization" (for example, with parathyroid hormone or vitamin-D derivatives) (*1*). To gain more insight into the mechanism of this response, experiments were designed to determine whether a calciphylactically challenged skin region will continue to undergo progressive mineralization once transferred from a sensitized donor to a nonsensitized host.

Ninety female Sprague-Dawley rats (*2*) with a mean initial body weight of 100 g (range, 95 to 105 g) were subdivided into nine equal groups (40 donors, 40 recipients, and 10 controls) and treated as indicated in Table 1. As a calciphylactic sensitizer, 1 mg of dihydrotachysterol (DHT) (*3*) in 0.5 ml of corn oil was administered by stomach tube on the 1st day. Ferric dextran, "Fe-Dex" (*4*) was diluted to contain 500 μg of elementary iron in 3 ml of water. Twenty-four hours after administering DHT, the subcutaneous tissue under the shaved back of each rat in groups 2 to 5 was infiltrated with this amount, over an area approximately 3 cm in diameter. Seventeen hours later, the Fe-Dex-treated area was excised, a small portion of it was taken for histologic study, and the rest was transplanted either into untreated recipients or into hosts which had been sensitized with DHT 24 hours prior to transplantation, in the same manner as the donors. In order to minimize the possibility of infection and external trauma, the skin flaps were introduced through a small incision underneath the dorsal skin of the host and deposited flat against the back. The operation was performed essentially according to a previously described technique (*5*), but only the cutaneous wound of the host was sutured.

The animals were killed with chloroform on the 6th day. The biopsy specimens taken at the time of transplantation, and the transplants removed at autopsy were inspected with a dissecting loupe and fixed in alcohol-formol (four parts of absolute alcohol and one part of 10 percent formalin) for subsequent staining of paraffin-embedded sections by the von Kóssa technique for the demonstration of calcium phosphate. The intensity of calcification was expressed in terms of an arbitrary scale of 0 to 3 (*1*).

As indicated in Table 1, skin transplants from untreated (group 1) or merely from Fe-Dex treated (group 2) donors onto untreated recipients, underwent virtually no calcification except for occasional traces of "dystrophic" mineralization of presumably traumatized cutaneous muscle fibers. The Fe-Dex-treated skin of DHT-sensitized donors (group 3) showed either no trace or only histologically visible traces of calcification in the challenged derma at the time of transplantation (as judged by the biopsy specimens); however, these same skin flaps exhibited even macroscopically conspicuous calcium deposits after transplantation into unsensitized recipients. Mineralization was most intense along the cut edges of the

Table 1. Passive transfer of calciphylaxis.

Treatment		Calcification in dermis
Donor	Recipient	
Untreated	*Group 1* Untreated	**0**
Fe-Dex	*Group 2* Untreated	**0**
DHT + Fe-Dex	*Group 3* Untreated	**1.8**
DHT + Fe-Dex	*Group 4* DHT	**2.8**
DHT + Fe-Dex	*Group 5* (Skin left *in situ*)	**3.0**

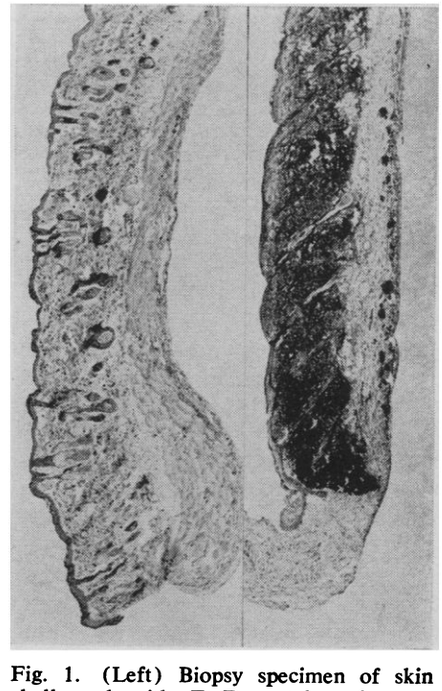

Fig. 1. (Left) Biopsy specimen of skin challenged with Fe-Dex, taken from a DHT-sensitized donor just prior to transplantation. No calcification is visible in dermis. (Right) Specimen from same skin flap removed from an untreated host at autopsy. Calcification (black areas) visible throughout the dermis, but most conspicuous at cut margin. A few fibers in the cutaneous muscle are also calcified (both sections by the von Kóssa method, × 35).